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01. Dental Field and Maxillofacial Area

01.01
Keynote: The role of cell–cell communication for the transition from inflammation to bone regeneration

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It is suggested that early interactions between inflammatory cells, mesenchymal stem cells, osteoblasts and osteoclasts are critical for bone regeneration and stability of prostheses. An example is that the inflammatory and regenerative processes seem to be intimately linked. Recent evidence suggests that signals generated by macrophages have both immunomodulatory and regenerative effects. For example, classically activated, in contrast to alternatively activated, macrophages promote the osteogenic differentiation of human mesenchymal stem cells (hMSCs) *in vitro*. This cell–cell communication is mediated both by soluble signals (cytokines and growth factors) and by macrophage-derived, 50–100 nm, microvesicles/exosomes, containing proteins, mRNAs and microRNAs, which are internalized by hMSCs. This indicates that multiple cell–cell and cell–implant/matrix interactions play important roles for the transition from inflammation to osteogenesis. From a bone engineering point of view, recent data on stem cells of embryonic origin will be discussed. The differentiation and mineralization properties *in vitro* of matrix free-growth human embryonic stem cells (MFG-hESCs) and embryonic stem cell-derived mesodermal progenitors (hES-MPs) in relation to hMSCs will be communicated.Valadi, Ekström et al., Nature Cell Biology, 2007, 9, 654; Peppo et al., Tissue Engineering Part A, 2010, 16, 2161; Peppo et al., Tissue Engineering Part A, 2010, 16, 3413; Omar, et al., Biomaterials, 2011, 32, 8190.

01.02
Keynote: Influence of cellular inflammatory response on integration and vascularization of synthetic bone substitute materials

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The present overview focuses on the influence of host cellular inflammatory response on biomaterial vascularization and integration. Various synthetic, xeno- and allogenic bone substitute materials and bio-membranes with or without pre-cultivation with human osteoblasts were implanted into the subcutaneous tissue of small animals, muscle tissue in goats or human bone tissue. The materials differed in chemical composition, size, shape and porosity. The tissue reaction, especially cellular degradation and vascularization of the biomaterials was systematically evaluated by applying standardized histological and novel histomorphometrical techniques. The results showed that changes in size, shape, porosity and chemical composition led to either a mononuclear cellular or a multinucleated giant cell-based tissue response, which influenced biomaterial vascularization. Interestingly, the *in vivo* survival of pre-cultivated osteoblasts on bio-membranes and their propensity for bone matrix production seemed to be independent of the material-specific cellular inflammatory response. The present data underline the fact that changes in biomaterial physico-chemical characteristics can be used to tailor materials for specific applications in tissue engineering, i.e., soft and hard tissue. The results encourage continuation of development of synthetic bone substitutes and membranes. These studies were partly supported by German and EU grants Ancabor and IKTE.

01.03
Poly (L-lactic acid) nano-structured microspheres for dental tissue engineering

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Introduction: The development of dentin-like structures by tissue engineering is one of the current challenges. In this context, we developed novel strategies to prepare poly(L-lactic acid) nanofibrous microspheres (NF-MS) and smooth surface microspheres (SS-MS) as injectable cell carriers, and investigated the potential of dental pulp stem cells (DPSCs) to interact with these carriers for dental tissue regeneration.
Methods: Human DPSCs (5×10^5) were seeded on NF-MS (0.3 mg) and SS-MS (3.0 mg), respectively. The cell-microsphere constructs were cultured for 24 h, 3, 7, and 14 days using supplemented DMEM (2 ml). To determine the cell viability and proliferation, a LIVE/DEAD® and PCNA detection were performed. DMP1-C, DMP1-N, DSPP, and RUN×2 markers were analyzed by using western blot and qPCR.
Results: DPSCs on NF-MS showed a higher cell viability level than on SS-MS. DPSCs maintained adequate cell proliferation levels on both NF-MS and SS-MS at 24 h, 3 and 14 days. DMP1-C terminal expression was positive after 24 h, 3 and 14 days on both NF-MS and SS-MS. However, DMP1-N terminal was exclusively expressed at 24 h on NF-MS and SS-MS. The expression of DSPP on SS-MS was high along 14 days of culture. Finally, RUN×2 was highly expressed on NF-MS after culture for 7 days.
Conclusion: DPSCs cultured on the NF-MS are able to retain high cell viability, proliferation, and physiological functions to induce differentiation into mineralized dental tissues. NIH P30DE2074/FIS PI08–06.

01.04
Research and development of implant-type tissue-engineered cartilage clinically applied for the nasal correction of the cleft lip and palate patients

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Secondary correction of cleft lip-nose presents a formidable challenge in cleft lip and palate surgery. Although, numerous approaches including autologous bone graft, autologous cartilage graft and implantation of artifical materials have been proposed to address the the cleft nose

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deformity, suitable graft materials cannot be obtained from any part of body or the artifical biomaterials. The clinical application of tissue-engineered cartilage with greater firmness and a 3D structure, which we term 'implant-type', is expected to overcome limitation of the present graft materials. Therefore, we developed (i) a proliferation medium for chondrocytes to realize a more than 1000-fold increase in number without using fetal bovine serum, and (ii) a scaffold system that effectively preserves chondrocytes in the engineered tissue and provides the adequate 3D shape to the tissue, and finally, we have established the the implant-type tissue-engineered cartilage, which is made by applying cultured auloglous auricular chondrocytes to the scaffold consisting of poly lactide porous body and atelocollagen hydrogel. After the acquisition of institutional and governmental permission, we began the clinical trial of the implant-type tissue-engineered cartilage for treatment of a nasal deformity in patients with a cleft lip and palate. In the presentation, we will show the techniques of the implant-type tissue-engineered cartilage and its clinical outcome at the early stage.

01.05
Craniofacial osseous critical size defect regeneration is mediated by neural crest specific regulation of BMP-2-signalling

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Osseous critical size defect (CSD) - regeneration in craniofacial bones (CB) has been shown to be accelerated by the use of bone substitute materials (BSM), soft tissue barrier membranes and local BMP-2 gene delivery. Related to the cranial neural crest origin of CB, the osteoproliferative BMP-2 downstream transcription factor Runx-2 mark is expressed exclusively in CB. Msx-1 functionally antagonizes terminal osseous differentiation by repressing Dlx-5 expression. It has not been clarified if Msx-1 is specifically involved in the BMP-2 mediated guided CSD regeneration. The aim of the experimental study was to evaluate the influence of local BMP-2/4 gene transfer on the expression of Msx-1, Dlx-5 and Runx-2 during experimental CSD regeneration in CB of the adult domestic pig.20 adult domestic pigs received cylindric CSD (1 x 1 cm) in the calvarial bone. Defects were allowed to regenerate by either filling with: (i) autologous bone; (ii) BSM (HA/TCP), (iii) BSM (HA/TCP) + BMP-2/4 transfected osteoblasts. All defects were covered by a membrane (polyethylene glycol, persistence 120 days). BMP-2 gene transfer was realised by a fusion protein (liposomal *in vitro* BMP2-transfection with the artificial tag V5). Quantitative histomorphometry (Toluidin-blue staining) and immunohistochemistry (Msx-1, Dlx-5, Runx-2) was perfomed after 4 and 12 weeks. In defects, treated with autologous bone or BMP-2 transfected osteoblast a significant higher regeneration was found.

01.P01
Alveolar augmentation by octacalcium phosphate collagen composites with titanium mesh in canine mandibular bone defects

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Introduction: The present study investigated whether bone regeneration by implantation of octacalcium phosphate and porcine atelocollagen composite (OCP/Col) would be enhanced if mechanical stress to the implanted OCP/Col were alleviated.
Methods: OCP/Col disks were implanted into a mandibular defect of adult beagle dogs that were divided into untreated, OCP/Col, and OCP/Col/Mesh groups. In the OCP/Col/Mesh group, mechanical stress toward the implanted OCP/Col was alleviated by a titanium mesh, and bone regeneration by these three groups was compared after 6 months.
Results: Macroscopically, the alveolus in the OCP/Col/Mesh group was augmented vertically more than other two groups. In morphometric analysis, the bone volume in the OCP/Col/Mesh group was significantly greater than those in other two groups. Histologically, the OCP/Col/Mesh-treated alveolus was augmented by matured bone tissue, and consisted of outer cortical and inner cancellous structure.
Conclusions: Because, the vertical bone regeneration by OCP/Col was successful when the mechanical stress to the implanted OCP/Col was alleviated, OCP/Col should be a useful bone substitute with active structural reconstitution.

01.P02
The effect of ALP expression of gingival fibroblast according to the culture time

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Gingival fibroblasts (GF) are the most abundant cell type in periodontal connective tissues, and have distinct functional activities in the repair of periodontal tissues and in inflammatory periodontal diseases. Human gingival fibroblasts (hGF) can be used as a stem cell for periodontal tissue engineering. This study examined whether *in vitro* the alkaline phosphatase (ALP) expression of hGF can be enhanced according to the cultivating time. hGF was obtained from the excised gingival tissue of an implant patient undergoing second surgery. The tissue was incubated at 37 °C in 5% CO_2 and 95% humidity, and the cultivating media was changed every 2 days. The fifth passage hGF cells were cultured in a medium containing Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 1X antibiotic antimycotic solution. The control hGF was cultured for 1 day. The experimental hGF was cultured for 6 and 15 days. With increasing culture time, the experiment group showed a spindle shape more clearly than the control group and an increase in the number of cells. This study evaluated the ALP expression of hGF using ALP staining. In the experimental group, the hGF showed enhanced positive ALP staining with increasing cultivating time. No cells showed positive ALP staining in the control group. Overall, these results suggest that the ALP expression of hGF can be increased according to the culture time, and that the hGF can be used for periodontal tissue engineering.

01.P03
Tooth regeneration from dental pulp stem cells: An allograft swine model

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A tissue engineering approach for tooth regeneration may provide a promising treatment for tooth loss. In this study, the dental pulps obtained from incisive, canine, premolar, or molar tooth from one-year-

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old pigs were harvested by surgical operation. The dental pulp tissues were first cultured and expanded *in vitro* to obtain dental pulp cells (DPCs), and then DPCs were differentiated to odontoblasts and osteoblasts, respectively. The oral epithelium was also harvested from adult pigs and cultured to obtain the epithelial cells. A three layer structure gelatin-chondroitin-hyaluronan tri-copolymer scaffold was prepared and the epithelial cells, odontoblasts, and osteoblasts, and were seeded into the upper, middle, and lower layer, respectively. This cells/scaffold construct was transplanted to the right and left mandible alveolar socket of pig, and totally eight pigs were included. Radiographic and histologic examinations were used to identify the regenerated tooth at 13.5 months post-implantation. Immunohistochemical staining was used to detect proteins specific to tooth regeneration. The present results showed that seven pigs developed a complete tooth with crown, root, pulp, enamel, dentin, odontoblast, cementum, blood vessels, and periodontal ligaments. This study revealed that DPCs in combination with a scaffold can regenerate a complete tooth; the implanted site may have a critical role for the shape of regenerated tooth.

01.P04
New tissue engineering models for dental tissue engineering

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Introduction: Our previous published research demonstrated that tissue engineering approaches could be used to direct post-natal dental stem cells to generate accurately formed tooth crowns. Current efforts focus generating whole teeth of predetermined size and shape.
Materials and Methods: Co-cultured human dental mesenchymal cell sheets and porcine dental epithelial cell sheets were generated on UpCell Surface thermosensitive tissue culture plates (Thermo Scientific, Rochester, NY). Cultured human DM cell - GelMA constructs (10^6 cells/50 μl, GelMA 5%) and unseeded control GelMA were prepared. Triplicate experimental and control constructs were cultured for 1, 2 and 3 weeks.
Results and Conclusions: GelMA supported the proliferation of human DM cells. Co-cultured human DM/porcine DE cell sheets layered over DM cell seeded GelMA formed organized cell layers and expressed Runx-2 and amelogenin. These results demonstrate the potential use of novel 3D bioengineered tooth constructs as models to study DE/DM cell interactions in tooth development, and to facilitate dental tissue regeneration therapies in humans. Acknowledgements:
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01.P05
Potential role for aligned fibre scaffolds in periodontal tissue engineering

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Periodontal disease is a chronic inflammatory process characterized by destruction and loss of connective and mineralised tissues and current therapeutic techniques may not result in full regeneration of the lost periodontal structures. However, a tissue engineering approach has the potential to fully regenerate the periodontal tissues. The aim of this study was to investigate the effect of fibre alignment of electrospun poly-L-lactic acid (PLLA) mats on the biological response of periodontal ligament fibroblasts (PDLFs). An 8% PLLA polymer solution was electrospun to fabricate aligned scaffolds and random PLLA fibre scaffolds were fabricated as a control. PDLFs were seeded onto the PLLA scaffolds and cultured for varying periods of time with and without mechanical loading. The resultant constructs were analysed by scanning electron microscopy (SEM), confocal microscopy, immunohistochemistry and q-PCR. Alignment of the electrospun PLLA fibres was confirmed by SEM and good attachment and proliferation of PDLFs on both scaffold types was observed. On aligned scaffolds, PDLFs showed a higher level of cellular activity and were elongated in shape and oriented along the long-axis of the fibres. Immunohistochemical and gene expression analyses indicated that fibre alignment influenced the phenotype of periodontal ligament fibroblasts. In conclusion, aligned fibre matrices may be suitable candidates for periodontal tissue engineering.

01.P06
A biphasic scaffold for simultaneous alveolar bone and periodontal ligament regeneration

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This study describes the design of a biphasic scaffold composed of a Fused Deposition Modeling scaffold (bone compartment) and an electrospun membrane (periodontal compartment) for periodontal complex regeneration. This strategy combined osteoblast impregnated in the bone compartment and multiple periodontal ligament (PDL) cell sheets on the electrospun membrane. After 21 days in culture the osteoblasts deposited mineralized matrix in the bone compartment and the cell sheets formed of a PDL-like tissue. The scaffolds were placed onto a dentin block and implanted in a rodent subcutaneous model for 8 weeks. In the bone compartment a more intense alkaline phosphatase staining was observed with higher bone density as shown by μCT, when the scaffold contained osteoblasts. A thin mineralized cementum-like tissue was deposited on the dentin surface for the groups with multiple PDL cell sheets (as observed by H&E and Azan staining). These groups showed a better attachment of PDL-like tissues onto the dentin block compared with the scaffold without cell sheets. Immunohistochemistry revealed the presence of cementum protein 1 (CEMP1), a specific marker for cementum, at the interface between PDL and the dentine blocks. These results demonstrated that the combination of multiple PDL cell sheets and a biphasic scaffold allows the simultaneous delivery of the cells necessary for *in vivo* regeneration of alveolar bone, periodontal ligament and cementum.

01.P07
Adhesion and proliferation of human periodontal ligament cells on biocompatible polymer scaffolds

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Human periodontal ligament (PDL) cells obtained from extracted teeth are expected as a useful cell source for tissue engineering. We have reported that poly(2-methoxyethyl acrylate) (PMEA) shows excellent biocompatibility. Here, we investigated adhesion, proliferation, morphology and secreted proteins of PDL cells on PMEA and other polymers, in order to design a scaffold with appropriate periodontal tissue regeneration. The polymer surfaces were prepared by a spin coater on a polyethylene terephthalate (PET). The surface analysis was confirmed by static water contact angle and X-ray photoelectron spectros-

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copy. After the PDL cells were cultured on PMEA, the cells were fixed and stained by DAPI, phalloidin and anti-vinculin antibody, and were observed by confocal laser scanning microscopy (CLSM).The cell morphology was characterized by scanning electron microscopy (SEM). PDL cells adhered and spread on all tested polymer surfaces except on poly(2-hydroxyethyl methacrylate) and poly(2-methacryloyloxyethyl phosphorylcholine-co-butyl methacrylate), with initial spreading and focal adhesion formation. The number of adhered cells on PMEA showed almost the same that on PET. The result suggests that the PDL cells adhered selectively on PMEA, because it was confirmed blood cells such as platelets did not easily adhere on PMEA. SEM and CLSM revealed that PDL cells produced extracellular matrices on PMEA. PMEA can be expected as an excellent scaffold for periodontal treatment.

01.P08
Craniofacial bone tissue engineering with biomimetic constructs seeded with periodontal ligament progenitor cells

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Principles of successful bone tissue engineering therapies rely closely on the ability of scaffolds to guide progenitor cells to the desired phenotype. Here, we demonstrate a new approach using biomimetic construct composed of hydroxyapatite modified with *in vitro*-derived extracellular matrix (HA-ECM) and seeded with periodontal ligament progenitor cells (PDLCs). The study aimed to investigate the effect of HA-ECM on the osteogenic differentiation of PDLC and the effect of PDLC-seeded HA-ECM on the bone repair. Isolated PDLC, analyzed by flow cytometry and assessed for osteogenic differentiation ability, were maintained on HA-ECM or HA alone and assessed by MTT, alkaline phosphatase (ALP) assays and real-time qPCR at different time intervals after seeding. PDL-seeded constructs with the most prominent *in vitro* performance at day 3 and 14 were implanted into rat calvarial critical size defect and assessed by histomorphometric analysis after 12 weeks. PDL acquired osteoblast-like phenotype on HA-ECM more efficiently, demonstrated by significantly higher ALP, osteogenic cytokine and osteoblast-related gene expression profiles. Treatment with PDL-seeded HA-ECM revealed a dramatic improvement in calvarial bone repair with the highest amount of newly formed bone elicited by cell-seeded constructs generated at day 14. The results highlight PDLC-seeded HA-ECM as a promising tool for craniofacial bone repair.

01.P09
Optimizing cell sources for cleft palate repair

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Introduction: In major defects of the cleft palate there is a need for a suitable bone forming cell to form the hard part of the upper palate. One option is to use human jaw periosteal cells (HJPs), which can be easily obtained from the patient using an oral biopsy. The aim is to compare the osteogenic potential of primary HJPs with an osteogenic MSC cell line (hESMPs) assessed as an alternative cell source.
Materials: HJP biopsies were received from two donors. HJPs and hESMPs were seeded in DMEM and α-MEM, respectively. On day1, samples were divided into four different media conditions; supplemented with varying concentrations of Dexamethasone (Dex), with or without Ascorbic acid-2-phosphate and Beta-glycerolphosphate. Osteogenic differentiation was assessed by measuring alkaline phosphatase activity and calcium deposition by Alizarin red Staining.
Results: The two donor samples yielded cells with differing morphologies, but both were capable of osteogenic differentiation. One had an initial fusiform morphology and required Dex to mineralise. The other had an osteoblast-like morphology and was able to form mineral without Dex. The hESMPs required Dex to stimulate osteogenesis.
Conclusion: Both sets of HJPs showed osteogenic potential but under different conditions. The conditions required for inducing osteogenesis may depend on factors, such as patient variability, patient age and site of extraction. These cells show promise as an autologous cell source for cleft palate repair.

01.P10
Dentin regeneration using a PLGA-HA scaffold seeded with human dental pulp stem cells

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Recently, dentin generation has been demonstrated using dental pulp mesenchymal cells seeded onto a scaffold. The purpose of this study was to examine the performance of PLGA-based scaffolds; 80% and 90% porosity, each with or without hydroxyapatite (HA) Human dental pulp stem cells (hDPSCs) were obtained from molar osteogenic differentiation ability in each lot of hDPSCs was examined *in vitro*. The hard-tissue production in the scaffolds which were seeded with hDPSCs was analyzed by transplanting. At 16 weeks after transplantation, the implants were retrieved and evaluated by micro-CT, histology, and immunohistochemistry *In vitro* osteogenic differentiation ability was shown in all lots of hDPSCs. In the *in vivo* experiment, micro-CT analysis showed calcified tissues in all cases of the HA-added PLGA scaffold implants. Interestingly, the scaffolds with 90% porosity showed more increase in density of calcification than those with 80% porosity. In contrast, a larger amount of calcified tissue was observed in the scaffolds with 80% porosity compared with those with 90% porosity. Histological examination demonstrated calcified tissue formation in the implants of HA-added scaffolds. These results suggest that the HA-added PLGA scaffolds are more suited for promoting the formation of new dentin-like tissue from hDPSCs. The porosity of the scaffold has an impact on the quality and quantity of the newly formed tissue.

01.P11
Morphological evaluation of bone after implantation of bioceramic bone substitutes – hydroxyapatite (HAP), tricalcium phosphate (TCP) and biphasic ceramic (HAP/TCP) in experiment

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Introduction: Hydroxyapatite (HAP), tricalcium phosphate (TCP) and biphasic ceramic (60/40) are frequently used as bone substitute biomaterials for jaw bone augmentation. The aim of our study was to evaluate bone tissue response to different bioceramic bone substitutes in animal experiments.
Materials and Methods: Intraosal implantation of bioceramic granules in size range from 0.5 to 1 mm was performed in rabbit lower jaw. Bone/hybrid biopsies were collected with 3 mm trephine bur after 3 months. Tissues were processed for routine histological examination (haematoxylin and eosin) and the detection of bone morphogenic protein (BMP2/4) and osteocalcin (OC).

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Results: HAP granules, followed by a biphasic ceramic, showed the best integration in bone tissue with remarkable formation of new bone. Around TCP granules formation of connective tissue capsule was observed, but without significant signs of inflammation.
Conclusion: Implantation of HAP bioceramic granules, more than TCP or HAP/TCP granules promotes bone regeneration with pronounced expression of OC and BMP2. Acknowledgments:

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01.P12
Human dental pulp stem cell sheet for bone tissue engineering

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Introduction: Cell sheet technology is considered to be useful for tissue engineering of various tissues and organs including bone. We present here a cell sheet fabricated by human pulp stem cells for bone tissue engineering.
Materials and Methods: Dental pulp was obtained from the third molars of mandible of 19–22-year-old patients. Dental pulp cells isolated by enzymatic treatment were positive for the stem cell marker, STRO-1. Cultured cell spontaneously formed sheet structures in several weeks. Character of the cell sheets were analyzed by immunohistochemistry and reverse transcriptase-polymerase chain reaction (RT-PCR). In addition, these cell sheets were transplanted into bone defects and on bone surface of mice, and the bone formation was histologically observed.
Results: Cell sheets cultured in osteogenic media were positive for alizalin red S staining, indicating their osteogenic/ odontogenic potential. The cell sheets were immunohistochemically positive for osteocalcin (OCN), bone sialoprotein (BSP) and dentin sialophosphoprotein (DSPP). RT-PCR analysis showed the cell sheets expressed alkaline phosphatase (ALP), Runx-2, collagen type I (Col I), OCN, BSP and DSPP. *In vivo* transplantation experiment showed that dentin- or bone-like tissues were formed within the bone defects. Conclusion: The cell sheets fabricated by dental pulp stem cells may be useful as a bone tissue engineering technology.

01.P13
Amelogenin peptide induces osteogenesis in hMSCs via PKC and ERK1/2

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Amelogenin (Amel) assembles as nanospheres *in vivo*, forming an extracellular matrix (ECM) complex that releases peptides via proteolytic cleavage. It is the main constituent of enamel matrix derivative (EMD), a decellularized porcine fetal tooth germ ECM protein complex, used clinically as Emdogain® to promote bone/periodontal regeneration and wound healing. It is not clear if the active portion of EMD is Amel or proteolytically cleaved peptides. We examined if human mesenchymal stem cells (MSCs) are sensitive to a phosphorylated tyrosine-rich Amel peptide (FC), and if FC stimulates MSC osteogenesis. MSCs were treated with rhAmel or FC for 9, 30, or 90 min and phosphorylation of 35 proteins measured by phospho-kinase proteosome array, which showed that PKC, ERK1/2 and β-catenin were highly phosphorylated by FC. This was confirmed by measuring PKC activity and levels of phospho-ERK1/2 and β-catenin: rhAmel and FC increased PKC at 9 min; FC induced higher phosho-ERK1/2 at 30 min than rhAmel; phospho-β-catenin was highest in FC treated cells. rhAmel and FC induced expression and production of osteogenic markers (alkaline phosphatase, OCN, OPG, COL1, BMP2, VEGFA) (FC>rhAmel). FC induces osteogenic differentiation of MSCs, suggesting that it may be the active bone regeneration component of Amel. FC mediates osteogenic differentiation through activation of PKC and ERK1/2 and degradation of β-catenin. This suggests that FC can be used as an ECM for bone and periodontal regeneration.

01.P14
Engineering of bone using porous calcium phosphate cement and bone marrow stromal cells for maxillary sinus augmentation with simultaneous implant placement in goats

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The aim of this study is to explore the effects of maxillary sinus floor elevation and simultaneous dental implantation with a tissue engineered bone complex of CPC scaffolds combined with BMSCs. Eighteen bilateral maxillary sinus of nine goats were randomly allocated into three groups, and the CPC/BMSCs complex were used to elevate maxillary sinus floor with a simultaneous implant placement and the effects were compared with those treated with CPC alone or autogenous bone. After a healing period of 3 months, sequential triad-color fluorescence labeling, Micro-CT, as well as histological and histomorphometric analyses indicated that the tissue engineered BMSCs/CPC complex could promote earlier bone formation and mineralization, and maximally maintain the volume and height of augmented maxillary sinus. By comparison, CPC alone or autogenous bone achieved less bone formation and later mineralization. Besides, the average bone-implant contact (BIC) value reflecting the osteointegration was (35.63% ± 9.42%) in BMSCs/CPC group, significantly higher than (22.47% ± 4.28%) in CPC alone group or (28.26% ± 8.03%) in autogenous bone group. In conclusion, CPC serves as a potential substrate for BMSCs for the maxillary sinus floor augmentation and simultaneous implantation. The tissue-engineered bone might enhance the stability of implants and thus be of great significance to achieve improved quality to restore the oral function in clinic.

01.P15
A calcium phosphate coated biphasic scaffold for periodontal complex regeneration

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Periodontitis is a common infectious disease that results in the degradation of the supporting tissues around teeth, which if left untreated can lead to tooth loss in the later stages of the disease. We have developed a biphasic scaffold for simultaneous regeneration of the alveolar bone and the periodontal ligament (PDL). This study represents a step forward into the optimization of this scaffold by utilizing a fused deposition modeling scaffold coated by a calcium phosphate layer for the bone compartment whereas the periodontal compartment consisted of a melt electrospun scaffold onto which PDL cell sheets were placed. Both the *in vitro* and *in vivo* performances of this biphasic scaffold were evaluated and it was observed that the alkaline phosphatase activity

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was significantly higher for the CaP coated samples and that the coated scaffolds displayed higher mineralization volume than the non-coated group. The scaffolds were placed onto a dentin block and implanted in a rodent subcutaneous model for 8 weeks, and higher bone formation was observed for the coated scaffolds. Histological analysis revealed that the large pore size of the periodontal compartment permitted the vascularisation of the cell sheets and a PDL-like tissue was observed at the dentine interface. This work demonstrates that the combination of cell sheet technology together with an osteoinductive biphasic scaffold could be utilized to address the limitations of current periodontal regeneration techniques.

01.P16
Towards a non-invasive method for phenotyping differentiating mesenchymal stem cells

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The ability of mesenchymal stem cells (MSCs) to differentiate along multiple lineages lends great promise for future regenerative therapies. Many scientific challenges however have to be addressed before such therapies can be translated for patient benefit. One challenge is the need for a non-invasive method for stem cell phenotyping prior to their use in a medical procedure. The aim of this study was to investigate the possible use of NMR spectroscopy as a non-invasive analytical tool to monitor the proliferation and subsequent differentiation of human dental pulp stem cells (HDPSCs) along an osteogenic pathway, both as monolayers and following seeding on to 3D scaffolds. HDPSCs were cultured in basal or osteoinductive medium either as monolayers or on electrospun collagen or hydroxyapatite wollastonite scaffolds. NMR spectra were obtained at several time points over a 6 week period. Spectra were processed as a time series, filtered and clustered according to each metabolite profile in order to identify markers responsible for cell phenotype. Specific patterns were identified coincident with the start of the differentiation process. Alongside spectral analysis, histological staining and biochemical assays were carried out to confirm cell differentiation. This preliminary data suggests that spectroscopy may provide a useful non-invasive method for MSC phenotyping to accelerate the route to cell based therapies and potentially act as a quality control tool at pre-implantation.

01.P17
Suitability of AlgOss 100 for the combined release of BMP-2 and VEGF

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Angiogenesis and osteogenesis are closely related processes sharing some essential mediators like VEGF (Vascular Endothelial Growth Factor) and BMP-2 (Bone Morphogenetic Protein-2). During formation of new bone VEGF expression is found in the early phases whereas BMP-2 is expressed continuously. Therefore sequential or coincidental release of these growth factors may result in enhanced bone formation. Scaffolds loaded with different types of growth factors will presumably usher a new area of so called multimodal biomaterials. We previously demonstrated that BMP-2 can be immobilized in high-amounts (up to 10 mg/g) on the phycogenic bone replacement material AlgOSs100 [1]. The bound BMP-2 is released in a highly bioactive form (K0.5 ~ 2.4 nM). Here we analyze the suitability of AlgOss 100 for the controlled binding and release of VEGF. Self-prepared VEGF (K0.5 ~ 1.8 pM) could be immobilized in an amount of ca. 80 μg/g AlgOss 100. The protein was released according to a two-phase exponential decay: an initial burst phase (half-life ca. 1 day) followed by a prolonged second phase (half-life ca. 23 days). Due to the high-affinity binding of VEGF to its receptor the amount of protein released is entirely sufficient to trigger a biological response. These highly promising results are the basis for a next set of experiments analyzing the combined loading and release of BMP-2 and VEGF on AlgOss 100.[1] Zurlinden, K. et al. (2010) Biomed Tech 55 (Suppl. 1), 28–30

01.P18
In vitro dental stem cell response to hypoxia mimicking bioactive glasses

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A main limitation for regeneration of large bone and dental defects is the lack of neo-vascularization within the tissue. Development of smart materials that favour angiogenesis is gaining interest for tissue engineering applications [1]. In this study, cobalt ions which promote the expression and release of pro-angiogenic proteins by mimicking hypoxic conditions [2], were incorporated into bioactive glasses (BG), to produce a series of cobalt-containing BG (CoBGs) at 0%, 1%, 2% and 4% calcium substitution [3]. Ion release was achieved by CoBGs incubation in DMEM medium leading to partial dissolution of the glasses. The wide range of cobalt concentrations released allowed us to explore the effect of the substitution percentage on the cell response. Human dental stem cells from permanent (DPSC) and deciduous (SHED) teeth were isolated and the cell viability and proliferation upon ion release from the CoBGs was assessed using alamarBlue®, MTT and BrdU assays. The incubation of conditioned media with DPSC and SHED showed a dose-dependent effect on cell metabolic activity and proliferation. Our results suggest that CoBGs may have the potential to be used as scaffolds for dental repair. Further investigations are currently being performed to determine whether CoBGs can favour the production and release of pro-angiogenic molecules by dental stem cells.

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01.P19
Characterization of the mesenchymal stem cells harvested from a new source, human supernumerary teeth

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Post-natal stem cells in the human dental pulp have been used as a promising source of multipotent mesenchymal stem cells (MSCs). Recently, dental pulp of supernumerary teeth was suggested to be another potent source for MSCs. Since most of the supernumerary teeth are surgically removed, they may be a suitable source of MSCs. The objective of this study is to compare the growth rate, gene expression, immunological phenotype, osteogenic and adipogenic differentiation potentials of MSCs harvested from the dental pulp of permanent, deciduous, and supernumerary teeth. Dental pulp was isolated from mesiodens supernumerary teeth (n = 5) extracted at the Dental

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Hospital of Nihon University School of Dentistry. Expressions of embryonic stem cell markers were examined. The antigen profiles of cultured cells were analyzed by detecting the expression of the stem cell surface markers. In addition, their potential for directional differentiation into osteogenic and/or adipogenic cell lineages was assessed. All cultures of the supernumerary tooth-derived dental pulp mesenchymal cells contained the cells which expressed cell markers such as CD73, CD105 and CD146, and successfully differentiated into osteogenic cell lineages; i.e., the cells displayed MSC properties, while their marker expression varied among samples. In conclusion, the property of dental pulp-derived mesenchymal cells from supernumerary tooth was comparable to that of cells from both permanent and deciduous tooth pulp.

01.P20
Adipose-derived stromal cells as a source in periodontal regeneration

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Periodontal regeneration has been a challenge in periodontics. Mesenchymal stem cells in periodontium have been reported to contribute to reconstruction of the lost periodontium. An alternative source of adult mesenchymal stem cells is found in adipose tissue. The purpose of this study was to examine whether adipose-derived stromal/stem cells (ASCs) may promote periodontal regeneration in surgical defect. Subcutaneous fat tissue from F344 rat was minced and digested in collagenase solution. Crude stromal cells after filtration and centrifugation were cultured and expanded at 37 °C in 5% CO_2 for 7 days. A surgical defect of approximately $2 \times 3 \times 1$ mm was made in the mandibular body using a bur driven with saline irrigation under anesthesia. In the defect, periodontal tissue including cementum, alveolar bone and periodontal ligament were removed from the buccal surfaces of the first and second molar roots. Cultured ASCs seeded into PLGA scaffolds (GC Dental Products Company) were transplanted in the defect. Micro CT analysis and histological examinations at 5 weeks after transplantation revealed an enhanced regeneration of periodontal tissue in the defect with ASCs in PLGA, but not in the defect with PLGA alone. These findings suggest that ASCs infiltrated into PLGA could be a suitable promoter for the periodontal tissue regeneration *in vivo*.

01.P21
Development, characterization and '*in vivo*' biological assessment of sol-gel coatings for biomedical application

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The success of the connection between an oral implant and the bone substrate depends on either biological factors of the patient or implant characteristics. The main reason for failure of dental implants is the wrong anchor with the bone and the fact that osseointegration is a long process. One way to avoid these problems is the development of coatings to improve the bioactivity of metallic implants. This will allow creating direct and strong linkages with the bone. In the present work, an organic-inorganic hybrid coating is obtained via sol-gel from different molar ratios of silane compounds: MTMOS and TEOS. Since substrate hydrophobicity influences on the attachment and spreading of cells, TEOS is used to increase the hydrophilicity. The effect of coatings composition in the degradation kinetics, drug release and cellular and tissue biological behaviour were studied. The behaviour of the cells in contact with the coatings was evaluated through *in vitro* assays using ASCM cells. For the *in vivo* biological evaluation, MTMOS/TEOS 70 : 30 coated Titanium blasted implants were implanted in a proximal tibial metaphysis of NZ rabbits, during 1, 2, 4 and 8 weeks. *In vitro* results showed that coatings with high TEOS content constitute a better cellular substrate for the ASCM cells proliferation and differentiation and an improvement in the calcified extracellular matrix formation. The osseointegration and osseoinduction abilities of coated implants were improved at 1 and 2 weeks.

01.P22
Biodegradable occlusive membranes for oral and periodontal surgery

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Occlusive membranes are used in oral and periodontal surgery to prevent the ingrowth of soft tissue into areas where slowly regenerating tissues, like alveolar bone and periodontal tissue, need time and space for an adequate regeneration. We developed four types of biodegradable occlusive membranes by electrospinning solutions of polycaprolactone incorporating different percentages of HAp in relation to PCL: 0%, 10%, 20% and 30% HAp. The membranes were characterized regarding their morphology, mechanical properties, hydrophilicity, degradation rate, bioactivity, cytotoxicity and cellular adhesion. Tensile tests revealed that the incorporation of HAp weakens the membrane but does not compromise nor its mechanical properties nor its integrity for the intended purpose. Contact angle measurements revealed the effectiveness of the treatment with NaOH in rendering the membranes hydrophilic. The membranes showed no evidence of degradation up to 4 weeks in a medium that mimics the oral environment. Simulated body fluid immersion tests confirmed that the presence of HAp improves the bioactivity of the membrane, favoring apatite formation. Cytotoxicity and cellular adhesion tests with SaOs-2 cells revealed no cytotoxicity and greater relative cell viability compared to the control, proving the ability of these membranes to interact with the biological environment and protect and aid the regeneration of bone and periodontal tissue.

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01.P23
Tissue non-specific alkaline phosphatase expression by human dental pulp cells: A selectable marker for mineralisation?

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Recently, tissue non-specific alkaline phosphatase (TNAP) was used to identify a sub-population of bone marrow mesenchymal stem cells (BMMSCs) with high mineralising potential. We aimed to study TNAP expression by human dental pulp cells (HDPCs) under various conditions with a variety of MSC markers and to use FACS to determine TNAP + HDPCs mineralisation potential. HDPCs from third molar dental pulps were culture expanded or directly analysed by flow cytometry. HDPCs and BMMSCs were cultured at densities of 1×10^3–1×10^5 cells/cm^2 and time periods of 1–21 days before expression of TNAP and other MSC markers was determined. HDPCs, FACS sorted for TNAP, were cultured in osteogenic media (28 days) before histology, SEM imaging and EDX analysis. Both primary and cultured HDPCs expressed TNAP. Analysis of expanded HDPCs revealed TNAP co-expression with MSC markers such as CD29, CD44 and CD166. Increased cell density enhanced HDPCs TNAP expression. Following osteogenic induction, FACS sorted TNAP + HDPCs were shown by histology, SEM and EDX to have more mineral deposits compared to TNAP- and unsorted HDPCs. These data confirm that TNAP is expressed by HDPCs together with other MSC markers and show that cell density affects TNAP expression levels. Sorting for TNAP + cells gives a subpopulation of HDPCs which are pro-mineralising compared to TNAP- and unsorted HDPCs. We conclude that TNAP is a potentially useful marker for HDPC selection in osteoinductive regenerative therapies.

01.P24
Dental pulp regeneration: The role of preameloblast -conditioned medium on human dental pulp stem cells

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Objectives: Regeneration therapy of pulp using human dental pulp stem cells (hDPSCs) has been emerged to maintain the tooth vitality against the conventional endodontic therapy. Preameloblast-conditioned medium (PA-CM) from murine apical bud cells induces the odontogenic differentiation of hDPSCs and promotes dentin formation. The present study aims to evaluate the effect of PA-CM with hDPSCs on pulp regeneration *in vivo*.
Methods: hDPSCs were seeded in the pulp cavity of 5 mm-thick human tooth slice with or without PA-CM treatment, and then they were transplanted into immunocompromised mice subcutaneously.
Results: In the control group, skeletal muscle with pulp-like tissue was regenerated in the pulp cavity. Reparative dentin-like structure with the entrapped cells was lined on the existing dentin wall. However, in the PA-CM treated group, only pulp-like tissue was generated without muscle and reparative dentin-like structure. Moreover, human odontoblast-like cells exhibited palisade arrangement around the pulp and typical odontoblast processes elongated into the dentinal tubule.
Conclusion: These findings suggested that PA-CM induced vascularized pulp-like tissue regeneration of hDPSCs.

01.P25
Axially vascularized bone substitutes, a novel model introducing regenerative medicine to reconstruction after cancer surgery

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Introduction: The creation of axially vascularized bone substitutes (AVBS) has been successfully demonstrated in several animal models. One prototypical indication is bone replacement after cancer surgery where the challenges include large defects with deficient vascularity due to eventual irradiation.
Methods: Based on our previous experiments we extended the creation of an arterio-venous loop to generate vascularized bone substitutes to a new defect model in the goat mandible. We are presenting a novel model of inducing axial vascularization of biphasic calcium phosphate scaffolds to regenerate bone in critical size defects using local vascular axes (Head and Neck vessels) avoiding any need for further tissue transfer. Through our controlled comparative study we evaluated the model through qualitative and quantitative analysis including new techniques of angiography and complex 3D reconstruction imaging, mechanical loading, and histological studies.
Results: We were able to show the possibility to generate an *in vivo*, functionally competent, axially vascularized bone substitute in goat mandibular defects harnessing the regenerative capacity of the living organism and avoiding any significant donor site morbidity.
Conclusion: From our findings we conclude that this novel model may well offer new perspectives for reconstruction of bone defects following cancer surgery and irradiation and will benefit from the significant reduction of donor site morbidity.

01.P26
Proliferation and differentiation of human periodontal ligament cells on biocompatible polymers

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Human periodontal ligament (PDL) cells on extracted third molars are discarded as medical wastes. PDL cells have an ability of differentiation capacity same as mesenchymal stem cells derived from bone marrow. PDL cells could be differentiated into three types of cells: osteoblast, fibroblast and adipocyte. Therefore, human PDL cells are expected as a promising source of mesenchymal stem cells. It is necessary to culture PDL cells on a biocompatible scaffold for the purpose of regenerating tissue. Here, we cultured PDL cells on biocompatible polymers for example (poly(2-methoxyethyl acrylate)) and cell adhesion, proliferation, focal adhesion kinase (FAK) and phosphorylated FAK(pFAK) were investigated. Also, PDL cells were evaluated by the protein expression level of STRO-1 known as mesenchymal stem cell maker. PDL cells cultured on the biocompatible polymer showed high initial adhesion and proliferation, and produced a large amount of pFAK. These results indicate that the signaling between adsorbed proteins and the integrin are highly activated on the biocompatible polymers, and that PDL cells could be used in tissue engineering of bone regeneration through activating bone differentiation gene without using medicine. Finally, these experiments were approved by the Ethics Committee of Yamagata University.

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01.P27
BMP-2/-6 enhance the proliferation of human periodontal ligament stem cells via stem cell factor (SCF)/c-kit signal

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Stem cell factor (SCF)/c-kit signaling activates cell proliferation, differentiation, and apoptosis in several biological systems. Although the c-kit expression in mesenchymal stem cells is low, some researchers suggest the importance of c-kit positive population for regenerative medicine. Therefore, the purposes of this study were investigating the existence of c-kit positive fraction and the role of SCF/c-kit signaling in human periodontal ligament stem cells (hPDLSCs). The effects of rhBMP-2 or -6 on the expression of c-kit in hPDLSCs were also examined. FACS analysis showed c-kit positive populations (0.65 ± 0.3%, $n = 7$) were found in hPDLSCs. Real-time PCR also confirmed the gene expression of c-kit. The colony forming ability (CFA) of c-kit positive hPDLSCs was significantly higher than that of the unsorted population. The proliferative activity of hPDLSCs was increased after stimulated with rhSCF. Addition of rhBMP-2 or -6 remarkably enhanced the expression of c-kit in hPDLSCs demonstrated by real-time PCR and FACS analysis. These results suggest that c-kit positive fraction existed in hPDLSCs, and SCF/c-kit signal transduction along with BMP signaling involved in the proliferation of hPDLSCs.

01.P28
Morphogenesis around HAp bioceramic implants loaded with autologous mesenchymal cells after implantation in subcutaneous tissue of rabbits

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Two options for graft substitutes are used: (i) hydroxyapatite (HAp) alone, (ii) HAp loaded with mesenchymal cells for replacement of lost bone tissue in maxillofacial and orthopedic surgery. Histomorphological evaluation of connecting tissue around HAp and HAp with mesenchymal cells was used. Bone with bone marrow samples were obtained from iliac crest of 10 California male rabbits. Mesenchymal cells were isolated using enzymatic digestion with collagenase type XI and seeded for expansion in DMEM/10% FBS. HAp tablets loaded with autologous mesenchymal cells were implanted in subcutaneous tissue of right scapular region. HAp tablets without cells were implanted on the left side. Six month post implantations, samples were collected and histological protocol using staining with hem/eoz and Machson trichrome was performed. Two layer capsule formation was observed around HAp tablets loaded with cells more intensive as in HAp alone samples. Inner part of formed capsule consisted predominantly of mononuclear cells/plasmocytes and macrophages. External layer of capsule contained mostly connective tissue with separate microvascularised in-grows through internal capsule into HAp tablet. An active formation of collagen was observed, whereas no signs of inflammation were detected. Two layered capsule around synthetic HAp tablets loaded with autologous cells is similar to the periosteum structure and may serve as part of bone induction capacity of bioceramics loaded with cells.

01.P29
Selection of dental pulp stem cells populations as a cell source for use in tissue engineering of the skin and oral mucosa

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Introduction: Bioengineered tissues such as skin and oral mucosa could be generated from alternative cell sources like human dental pulp stem cells (HDPSC). In these cases, cell viability should always be determined to ensure adequate cell functions. The aim of this study is to determine the most appropriate HDPSC cell passage for use in TE.
Materials and Methods: Ten consecutive passages of HDPSC were obtained by enzymatic digestion of pulp tissue. To determine cell viability, trypan blue staining and EPXMA were used in 50 specimens per passage was performed on the central area of the cell nucleus.
Results: Trypan blue exclusion analysis suggests that the lowest cell viability level corresponds to cell passage P2, with subsequent increase and a maximum at P9. By EPXMA, the lowest cell viability was found at P3 and maximum at P9. An increase of the intracellular chlorine concentration was found at P3 and P9, suggesting that a process of necrosis and cell senescence could be associated to these passages, respectively.
Conclusion: EPXMA allows the accurate determination of cell viability and the identification of mechanisms underlying cell death. According to our results, P9 would be the best moment for this stem cell source to be used as substrate for TE of the skin and oral mucosa.
Acknowledgements: Supported by grants FIS PI11-2668 from Instituto de Salud Carlos III and SAS PI273/2010 from Junta de Andalucia.

01.P30
In vivo evaluation of chitosan/TCP/alginate self-setting biomaterial for dental application

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The aim of this study was to formulate the biodegradable, injectable biomaterial acting as a bone substitute for dental application. The injectable system was obtained by agglomeration of chitosan/βTCP microspheres and relied on alginate gel formation in the presence of calcium ions released from polymer granules. The system consisted of two phases: solid – CH/ βTCP beads and liquid one – alginate, acting as a microsphere carrier which tends to gel in presence of Ca^{2+} released from the beads. Mass relation between CH/TCP and alginate was 1 : 2 and self-setting time of the biomaterial was influenced by cross-linker concentration used. The biomaterial was tested *in vivo* on rat model and was compared with commercially available product. The materials were implanted into experimentally created bone defects

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(d = 7 mm) in rats skull. There was histological evaluation and immunohistochemical staining (Sialoprotein, Osterix Sp7) done after 4 and 12 weeks from the surgery. The histological studies showed that in case of CH/TCP/Alg biomaterial there was newly formed bone tissue observed around CH/TCP/Alg biomaterial just after 4 weeks from the surgery and trabecular bone formation after 3 months from the implantation. In contrast, commercially available product consisting of TCP granules coated with PLA layer showed inflammatory response and lack of bone tissue formation neither after 4 nor 12 weeks from the implantation.

01.P31
Bone regenerative potential after HAP implantation in to maxillary sinus using immunohistochemistry

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Introduction: Bone morphological and immunohistochemical investigation were used to evaluate bone quality before implantation and after bone regeneration.
Materials and methods: Twenty patients underwent two stage sinus lift operations with HAP (RTU) and with Bioss (Geistlich®). 2 mm bone core biopsies were taken before and 6 months after bone augmentation. We examined hematoxilin/eosin staining for the review pictures, bone proteins - osteopontin (OP), osteocalcin (OC), osteoprotegerin (OPG), cellular stress indicator - heat shock proteins (HSP); growth factors: TGFβ, BMP2/4, antimicrobial protein - defensin β2 (DF).
Results: All patients showed pronounced expression of OC and OP which are bone mineralization and regeneration markers. Defensin β2 and HSP were low pronounced. Analyzing apoptosis, TGFβ, BMP 2/4 high variability were observed. Expression of OP showed strong tendency to elevate to the statistical significant difference after bone augmentation.
Conclusions: Marked expression of osteopontin showed patient bone remodeling and mineralization potential after bone grafting. Increased expression of osteoprotegerin after bone grafting indicated the increased activity of bone cells. This work has been supported by the European Social Fund within the project 'Multidisciplinary Research in Biomaterials Technology of New Scientist Group', No.2009/0199/1DP/1.1.1.2.0/09/APIA/VIAA/090, (PVS ID 1380).

01.P32
Artificial skin and oral mucosa generation using an alternative MSC source

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Introduction: Human umbilical cord Wharton's jelly stem cells (HWJSC) previously demonstrated to have high differentiation potential. However, differentiation to epithelial cells has been poorly studied. In this work, we have studied the differentiation potential of HWJSC into epidermal tissues of oral mucosa and skin.
Methods: Primary cell cultures of HWJSC, oral mucosa and skin fibroblasts were obtained from human tissue biopsies. Then, fibrin-agarose artificial stromas were generated with skin and oral mucosa fibroblasts within, and HWJSC were cultured on top. Immunofluorescence analysis was performed at 10, 20 and 30 days to determine the expression of cytokeratins, PANCK, filaggrin and involucrin.
Results: Our results showed that HWJSC were able to transdifferentiate to epidermal tissues corresponding to oral mucosa, with positive expression of PANCK and CK1 after 10 days of culture and of filaggrin and involucrin after 30 days. The analysis of the skin substitutes showed positive expression of CK1, involucrin and filaggrin after 10 days, and PANCK after 20 days of development.
Conclusion: The results suggest that HWJSC possess the potential to transdifferentiate ex vivo to epidermal tissues on 3D fibrin agarose scaffolds.
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01.P33
Tooth socket restoration with the application of bone morphogenetic protein-2-binding heparan sulfate

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Dental implants are indicated as impacted teeth replacements, which often result in a compromised and extended alveolar ridge bone (ARB). This requires an autograft from the third molar region to the future implant site. An alternative is therefore necessary as this procedure is associated with graft rejection/resorption and site morbidity. This study aimed to engineer an bone-graft substitute to accelerate ARB healing for the anchorage of prosthetic teeth. Since bone morphogenetic protein 2 (BMP-2) relies on heparan sulfate for its stability and bioactivity, we evaluated the *in vivo* efficacy of a HS variant (HS3) that we tuned to maximally activate endogenous BMP-2 delivered with a composite polycaprolactone-tricalcium phosphate (PCL-TCP) biomaterial. For each of the 10 male micro-pigs employed, teeth were extracted and a large lateral buccal-ridge bone defect created on each side of the mandible. Defects were treated (n = 3/grp) with PCL-TCP scaffolds alone, 30 μg HS3 or 10 μg BMP-2. X-rays analysis at 6 months showed similar bone formation in the BMP-2 and HS3-treated groups that was further verified by μCT. PCL-TCP alone displayed an inferior bone forming capacity. In both the HS3 and BMP-2 groups, new bone infiltrated the defect from the lower host alveolar bone and sides. Resin histology on all groups corroborated the μCT data. HS3 was able to stimulate robust bone formation in an ARB defect model such that the placement of a prosthetic tooth implant might be possible.

01.P34
Development of mandibular reconstruction device made of titanium fiber scaffold

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Introduction: Mandibular reconstruction after tumor ablative surgery is essential for returning QOL of oral cancer survivors. But bone reconstruction surgery puts a severe strain on the patient. To date, the author reported that titanium fiber mesh scaffold had favor biocompatibility and high bone formation ability that was enhanced by hydroxyapatite coating with molecular precursor method. In the present study, the author described the development of new mandibular reconstruction device with the titanium fiber mesh scaffold.
Materials and Methods: Adult female Japanese white rabbits were used in the present study. Mandibular reconstruction materials made of titanium fiber mesh scaffold were made with or without hydroxiapatite

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coating. Hydroxiapatite coating was performed with molecular precursor method. Under general anesthesia mandibular bone was resected and a part of the bone was completely removed. The 10 mm length mandibular defect was reconstructed by the titanium fiber mesh scaffold. The material was fixed with titanium plate. 21 weeks after surgery, the five coating and five non-coating animals were sacrificed and the reconstructed part was evaluated.
Results and discussion: In hydroxyaatite coating titanium fiber mesh scaffold, newly formed bone were seen in and out of the materials, and the defect was completely restored with newly formed bone, suggesting that titanium fiber mesh was powerful materials for mandibular reconstruction device by hydroxyapatite coating.

01.P35
Evaluation of bone regeneration using stem cells from pulp tissue of human deciduous teeth seeded onto PLGA scaffolds

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Bioengineering involves the discovery of new tissue production methods. Aiming to evaluate the use of nanotechnology with stem cells (SCs) for bone formation in rats, scaffolds from poly (lactic-co-glycolic acid) (PLGA) were produced by electrospinning technique. SCs from five samples of human deciduous teeth (SCDT) in a process of rhizolysis were seeded onto culture plates (control) and scaffolds (test) for adhesion and cell viability assays. To evaluate their ability to promote bone formation, critical skull defects (8 mm in diameter) were induced in 15 rats. They were divided into three groups (n = 5): I–only scaffolds; II–scaffolds/SCDT; III–scaffolds/SCDT, kept for 13 days in osteogenic medium. Sixty days postoperatively, the caps were removed for histometric analysis of the amount of new formed bone. Approval was met by the Ethics Committee of the Federal University of Rio Grande do Sul (n° 19273). Cellular adhesion and viability in all groups was observed to be similar throughout the experiment, without statistical difference. Histological examination demonstrated that group III showed 17% ± 4.31% of new bone formation with statistical difference, compared to groups I and II, respectively (10.7% ± 3.22% and 9.39% ± 2.55%). It is concluded that the PLGA scaffolds produce favorable results in relation to interaction with the SCDT. The use of PLGA and cells in association with osteogenic medium results in promoting bone formation.
Financial support: CNPq, CAPES, FAPERGS and SCRI

01.P36
Study of the interface between gingival fibroblasts and dental implants

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Dental implants are a widely used as an alternative to some conventional dental treatments. However successful use of dental implants and related prosthetic components depends on the biocompatibility and bioadhesiveness at the implant-tissue interface. Therefore, it is important to clarify the mechanisms by which gingival fibroblasts and osteoblasts attach to artificial surfaces as the implant is in contact with soft tissue and bone. These knowledges are important both from fundamental and practical point of view. The aim of this work was to evaluate factors, which determine attachment of soft tissue cells to the surface of dental prosthetic materials. For this reason, the primary dental fibroblast lines from human gingival subepithelial tissues were obtained and characterized. Cell adhesion to the prosthetic components made of zirconium oxide, titanium, metal alloys, and ceramic prosthetic components as well as differently laser modified titanium samples was investigated. Optimal sample surface processing methods were selected and their surface topography was evaluated by atomic power microscopy. Surface roughness indexes were determined as well. Expression of the adhesion molecules in cells grown on modified titanium samples was evaluated.

01.P37
Lgr5 mediated integrin signaling promotes the proliferation of dental epithelial progenitor cells

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Although previous studies suggest the importance of dental epithelial cells for tooth regeneration, the mechanisms regulating the maintenance and proliferation of dental epithelial cells are not well understood. Rodent incisors are a good model for studying dental epithelial cells since they grow through their lives and epithelial stem cells are maintained in their proximal end called 'cervical loop'. The committing cells in the cervical loop become dental epithelial progenitor cells called 'pre-ameloblasts', and differentiate into ameloblasts forming enamel.In this study, we observed that the lower incisors of null mutant for CD61 (also known as integrin beta 3, $CD61^{-/-}$) were significantly shorter than those of wild-type at 8 week-old. The expressions of Fgfr2, Lgr5, and Notch1, which are involved in the proliferation of pre-ameloblasts and the maintenance of dental epithelial stem cells, were reduced in the 2 week-old $CD61^{-/-}$ cervical loop. In a pre-ameloblastic cell line ameloblast-lineage cell (ALC), suppression of CD61 by the transfection of siRNA (siCD61) reduced the proliferation. The mRNA expressions of Lgr5 and Notch1 in ALC cells were reduced by siCD61. The suppression of Lgr5 by siLgr5 inhibited the proliferation of ALC cells. These results suggest that the integrin signaling is responsible for proper growth of lower incisor and promotes proliferation of dental epithelial progenitor cells through Lgr5 signal.

01.P38
Pituitary tumor transforming gene 1 (PTTG 1) regulates migration of human oral squamous cell carcinoma by regulation of epithelial to mesenchymal transition

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Human pituitary tumor-transforming gene 1 (PTTG1), which is a newly identified proto-oncogene, overexpress in many nonendocrine-related tumors as well as endocrine-related cancers. Also, it has been

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known to involve in progression of tumor and metastasis. Epithelial to mesenchymal transition (EMT) is a crucial step in tumor development and metastasis. However, the function and the regulation mechanism of PTTG1 on migration of oral squamous cell carcinoma (SCC) remain still unclear. Here, we analyzed the expression of PTTG1 in oral SCC and evaluated the mechanism for invasion of oral SCC cell lines (YD-10B and YD-15) depends on PTTG1 expression by siRNA. In addition, the markers expressions of EMT were analyzed. The expression of PTTG1 in oral SCC tissues was mainly observed in cytoplasm. The invasiveness of oral SCC cells using Metrigel was significantly increased after PTTG1 plasmid transfection comparing to Mock ($p < 0.05$). MMP-9 expression by PTTG1 siRNA treatment was significantly decreased. Also, migration activities of oral SCC cells were significantly decreased after PTTG1 siRNA treatment ($p < 0.001$). In addition, down-regulated PTTG1 induced alterations of integrins in oral SCC cell lines through the phosphorylations of FAK1 and mTOR and decreased the expression of epithelial markers, otherwise, increased the expression of mesenchymal markers. These results suggest that alteration of PTTG1 could be regulated migration activity of human oral SCC through regulation of EMT.

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02. Bone Regeneration: Current and Future Strategies

02.01
Keynote: Bone regeneration perspectives: employ mechano-biological principles to foster endogenous regeneration

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Bone is a unique and highly regenerative tissue in vertebrates due to its capacity to restore pre-fracture properties. Understanding of scar less repair leads possibly to a path of true regeneration. From a material science perspective, native tissue formation and its mechano-senstivity are fascinating features of this regenerative process. The healing through formation of inter-mediate tissues (e.g. callus) proved to be mechano-responsive in type and amount of tissue formed: Demanding mechanical conditions lead to a prolonged inflammatory phase, a reduced and delayed angiogenesis, a prolonged cartilaginous phase, a larger callus and a delay of bridging in enchondral ossification. Even though it is widely known that cells are sensitive to physical signals, details of this interplay in tissue regeneration and its relevance at the various length scales are yet not fully understood. For example, mesenchymal stroma cells (MSCs) appear to be mechano-sensitive and by those mechanisms stimulate pro-angiogenic cascades. These effects are beneficial to regenerative cascades but the potential seems to decrease with aging. Another example are immune cells which apparently are specifically regulated in regeneration and their appearance depends upon the mechanical local conditions. A further understanding of underlying mechanism of the link between biology and mechanics seems to gain further understand of healing cascades, specifically under compromised healing conditions.

02.02
Enhanced attraction of M1, M2 macrophages and endothelial cells into mesenchymal stem cell - seeded fibrin hydrogels transplanted in a rat long bone fracture gap

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Beyond their multilineage differentiation capacity mesenchymal stem cells (MSC) provide a supportive cellular microenvironment due to their chemoattractive, trophic and immunomodulatory properties. Since only a limited number of MSC can be produced for transplantation, fast recruitment of additional cells to a defect site may be crucial for regeneration strategies. Aim of this study was to assess the effect of MSC on *in vivo* chemoattraction of host cells into a fibrin hydrogel transplanted in a rat long bone fracture gap.Female rats received an empty fibrin clot or male MSC in a fibrin carrier into a 2 mm fracture gap in the femur. After 3, 6, 14 or 28 days fracture callus was removed and persistence of transplanted MSC was detected by SRY gene specific PCR. Day 6 callus (n = 6 per group) was subjected to histology.Male MSC persisted at day 14, but not day 28. While only a few cells invaded the MSC-free fibrin clot, the MSC-transplant attracted many cells. Mean infiltrated area was 10% in the controls while 50% of the MSC-clot was filled with new cells. Endothelial cells and macrophages were the main invaders, partly organized along routes. M1 macrophages dominated at the migration front and M2 macrophages were more spread over the fibrin clot.In conclusion chemoattraction and fast recruitment of host cells into hydrogels is a dominant action and main advantage of MSC-transplantation. Long term persistence of MSC and contribution to the final callus is questionable.

02.03
A Platelet Rich Plasma (PRP) based membrane as a periosteal substitute with enhanced osteogenic and angiogenic properties for bone engineering

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The periosteum plays a pivotal role during bone development and repair by contributing to bone vascularization, together with being the source of osteoprogenitor cells. We propose a periosteal-like substitute engineered using a platelet rich plasma (PRP) membrane incorporating autologous bone marrow derived mesenchymal stem cells (PRP/BMSC gel membrane) to be used with an osteoconductive scaffold for the regeneration of compromised critical size bone defects. The PRP/BMSC gel membrane was engineered and optimized for optimal release of VEGF and PDGF-BB. Next, survival and proliferation of cells in the optimized PRP gel membrane with time were confirmed in addition to their capacity to undergo osteogenic differentiation. Furthermore, to evaluate possible effects of the released products from the PRP/BMSC gel membrane on surrounding progenitor cells in the injury area, we found that the PRP/BMSC gel membrane products could significantly induce the migration of human endothelial cells *in vitro*, and increased the expression of BMP-2 in cultured BMSC. The latter cells also secreted significantly higher amounts of the soluble pro-angiogenic factors such as PDGF-BB, VEGF, IL-8. Finally, the functionality of the PRP/BMSC gel membrane periosteal substitute for bone regeneration was tested *in vivo* both in an ectopic mouse model as well as in a rabbit segmental bone defect model providing evidence of its capacity to biomimic a periosteal response and enhance bone regeneration.

02.04
Concentration and selection of osteogenic progenitors using magnetic labeling based on hyaluronan expression for immediate transplantation into a canine femoral multidefect model

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Optimal bone regeneration may require the transplantation of osteogenic connective tissue progenitors (CTP-Os). Strategies that increase

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the concentration of CTP-Os and remove non-progenitors are expected to enhance bone regeneration. Hyaluronan (HA) is enriched on the surface of CTP-Os. This study tested the hypothesis that transplantation of HA-enriched bone marrow-derived cells will improve bone regeneration in the canine femoral multidefect model. In 10 canines, 48 ml of heparinized bone marrow (hBMA) was aspirated. Cells presenting HA on their surface were labelled and separated using a hexapole magnet. Samples before and after magnetic separation (MS) were assayed for cells and CTP-Os. Implants were placed in four cylindrical defect sites (diameter: 10 mm, depth: 15 mm) in the canine femur. Two allograft scaffolds were loaded with HA+ cells, and two were loaded with hBMA. Implants were evaluated at 4 weeks using microCT and histomorphometry. A mean of 7.8% of the cells partitioned to the HA+ fraction after MS. CTP-Os were significantly enriched in the HA^+ fraction, with a 3.3-fold higher prevalence than hBMA. The percent of CTP-Os retained in the graft was significantly higher for MS-processed HA+ cells (75.7 ± 22.2% vs 49.0 ± 20.4%). Histomorphometry showed that defects grafted with HA+ cells had statistically greater new bone formation and greater vascular sinus area than hBMA. MS selection of HA^+ cells enriches for CTP-Os and improves local bone regeneration *in vivo*.

02.05
Platelet rich plasma-mediated delivery of BMP-7 for segmental bone defect regeneration

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Introduction: Successful bone tissue engineering concepts include the combination of scaffolds, growth factors and cells with osteogenic potential and capacity for graft vascularisation. The development of appropriate biomaterials for bone regeneration has become a key research drive, but most biomaterials suffer from bioactivity and therefore must be combined with osteoinductive supplements. A frequently used technique is the local delivery of growth factors, such as bone morphogenetic proteins (BMP). Our study investigated the osteogenic potential of a platelet rich plasma (PRP) based delivery of low dose BMP-7 for bone regeneration.
Methods: A 3 cm segmental tibial bone defect was created in 18 adult merino sheep. BMP-7 was mixed with autologous PRP, transferred to the outer cortex of a tubular mPCL/TCP scaffold, and activated with thrombin. The scaffolds were implanted into the created defects. Empty scaffolds and the implantation of autologous bone grafts (ABG) served as controls. Bone healing was assessed after 12 month by radiology, micro computed tomography and biomechanical testing.
Results: Radiology, microCT and biomechanical testing showed significant more bone formation in the BMP-group compared to the empty scaffold group. The results of the BMP-group showed no significant differences compared to the ABG-group.
Conclusion: The use of PRP as a delivery vehicle leads to a low dose use of BMP-7 with good bone regeneration comparable to the use of ABG.

02.P01
Strongly enhanced levels of sclerostin during human fracture healing

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Introduction: Sclerostin is a negative regulator of bone formation. However, no data on the role of sclerostin in human fracture healing exist so far.
Patients and methods: Seventy five patients with long bone fractures were included into the study and divided in 2 groups. The first group contained 69 patients with normal healing. 6 patients with impaired fracture healing formed the second group. 34 volunteers donated blood samples as control. Serum samples were collected over a period of 1 year. Sclerostin levels were measured in fracture haematoma and serum of 16 patients with bone fractures.
Results: Fracture haematoma contained significantly higher sclerostin concentrations compared to patient's serum. Sclerostin levels in haematoma and in serum were both significantly higher than in controls. Highly elevated sclerostin serum concentrations were found in patients with physiological fracture healing. Whereas, sclerostin levels were decreased in patients with impaired healing.
Conclusion: This is the first study to provide evidence of strongly enhanced sclerostin levels in fracture haematoma and peripheral serum in patients with long bone fracture. The results indicate local and systemic involvement of sclerostin in humans during fracture healing. Significant differencesin the serum sclerostin concentrations between patients with physiological and impaired healing were not observed.

02.P02
Is the expression of TGF-Beta 1 after fracture of long bones solely influenced by the healing process?

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Introduction: Circulating TGF-β1 levels were found to be a predictor of delayed bone healing and non-union. To evaluate the reliability of TGF-β1 as a predictive bone healing marker the correlation between the expression of TGF-β1 and the different socio-demographic parameters was analyzed.
Patients and methods: Fifty one patients with long bone fractures were included into the study and divided in different groups according to their age, gender, cigarette smoking status, diabetes mellitus and regular alcohol intake. TGF-β1 levels were analyzed in patient's serum and different groups were retrospectively compared.
Results: Significantly lower TFG-β1 serum concentrations were observed in non-smokers comparing to smokers at week 8 after surgery. Significantly higher concentrations were found in male patients compared to the female at week 24. Younger patients had significantly higher concentrations at week 24 after surgery compared to older patients. Concentrations were significantly higher in patients without diabetes comparing to those with diabetes at 6 weeks after surgery. Patients with chronic alcohol abuse had significantly higher concentrations compared to those patients without chronic alcohol abuse.
Conclusion: TGF-β1 serum concentrations vary dependent upon smoking status, age gender, diabetes mellitus and chronic alcohol abuse at different time points and therefore do not seem to be a reliable predictive marker of fracture healing.

02.P03
Differentiation-dependent secretion of proangiogenic factors by mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are a promising cell population for cell-based bone repair due to their proliferative potential, ability to differentiate into bone-forming osteoblasts, and their secretion of potent trophic factors that stimulate angiogenesis and neovascularization. To promote bone healing, autogenous or allogeneic MSCs are transplanted into bone defects after differentiation to varying degrees down

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the osteogenic lineage. We hypothesized that the proangiogenic potential of MSCs was dependent upon their stage of osteogenic differentiation. After 7 days of culture, we observed the greatest osteogenic differentiation of MSCs when cells were cultured with dexamethasone (OM^+). Conversely, VEGF protein secretion and upregulation of angiogenic genes were greatest in MSCs cultured in growth medium (GM). Using conditioned media from MSCs in each culture condition, GM-conditioned media maximized proliferation and enhanced chemotactic migration and tubule formation of endothelial colony forming cells (ECFCs). ECFCs seeded on microcarrier beads and co-cultured with MSCs previously cultured in GM in a fibrin gel exhibited superior sprouting compared to MSCs previously cultured in OM^+. These results confirm that MSCs induced farther down the osteogenic lineage exhibit reduced proangiogenic potential, an important finding for bone repair.

02.P04
Osteoblast differentiation of amniotic fluid-derived stem cells irradiated with visible light

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Human cells are known to be sensitive to visible light. The effect of visible light irradiation on the expression of pluripotent genes (Oct-4, Sox2, and Nanog) in amniotic fluid-derived stem cells (AFSCs) and on the osteogenic differentiation ability of AFSCs was investigated using light emitting diodes (LEDs) at 0–2 mW/cm^2 in various wavelengths [blue (470 nm), green (525 nm), yellow (600 nm), and red (630 nm)]. Pluripotent gene expression in AFSCs was up-regulated by visible light irradiation from a LED for more than 6 h. Green light irradiation of AFSCs up-regulated the expression of pluripotent genes more significantly than irradiation with other light. The osteogenic differentiation of AFSCs was facilitated by green and blue light irradiation. Facilitated differentiation into osteogenic cells by visible light irradiation was not mediated by reactive oxygen species (ROS); alkali phosphatase activity (a marker of early osteogenic differentiation) and gene expression of osteopontin (a marker of late osteogenic differentiation) did not change significantly between AFSCs in differentiation medium with or without a ROS scavenger (vitamin C). The mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) pathway, as well as other unknown signaling pathways, may be responsible for the activation of signaling pathways that facilitate the differentiation of AFSCs into osteogenic cells upon light irradiation.

02.P05
Bilayered devices with inner bioactive nanotexture for long bone treatments

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Recent approaches for bone regeneration are commonly aimed at reproducing the native microenviroment of the natural tissue, i.e., the mineralized extracellular matrix. The manipulation of chemicals and morphological features offers the opportunity of tuning scaffold properties to stimulate 'ad hoc' cells activities and, ultimately, to trigger specific biological functionalities. Here, we propose the design of bilayered tubes for the '*in vitro*' regeneration of 3D bone segments. The bi-layer scaffold was developed by a two-step electrospinning process: the inner layer made of poly-(ϵ-caprolactone) (PCL) and Gelatin was randomly electrospun on a grounded metal plate, wrapped on a mandrel and, subsequently, coated with micrometric PCL fibers which form the outer layer of the conduit. In the *in vitro* studies on human mesenchimal stem cells, we observed that bilayered scaffolds not only are able to support cell attachment and proliferation but they can also promote osteogenic differentiation of hMSC and mineralization, thus providing to a suitable environment for *in vitro* bone formation. In this context, the covering of a nanotextured layer - with peculiar topographic and biochemical cues to improve cells activities - by microfibres, mainly ensures the mechanical stability required to guide the forming bone during the regeneration process. Hence, the proposed device can be considered an innovative scaffold to be successfully used as bone conduit for long bone treatments.

02.P06
Analysis of osteoblast viability and activity on ceramic βTCP/TiO2 biomimetic scaffolds

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Calcium-based substitutes are widely used in bone tissue replacement. In particular, hydroxyapatite (HA) and β-tricalcium phosphate (βTCP) are attractive bioactive ceramics with good osteoconductive properties but with poor mechanical strength for use in load-bearing applications. Here, we have combined the high resorbability properties of βTCP with the high mechanical strength and bioactivity of titania (TiO2). βTCP/TiO2 macroporous scaffolds were developed starting from HA and TiO2 powders, sintered at 1250 °C. Sterile scaffolds were assayed *in vitro* for osteoblast proliferation, activity, attachment and viability, at 1, 3, 7 and 14 days after seeding of human MG-63 osteoblast-like cells. Commercially available biomimetic HA bone grafts were used as controls. Scaffolds showed a high proliferation rate, high viability and high colonization rates, comparable to controls. Moreover, a significantly higher activity of the osteogenic marker alkaline phosphatase was found at 3, 7 and 14 days after seeding, with the highest activity after 3 days. These results demonstrate that the *in vitro* behaviour of βTCP-TiO2 scaffolds is similar to the commercially available HA scaffolds, with high biocompatibility and induction of cell proliferation and colonization, but moreover with better induction of osteoblast activity, indicating its higher suitability as biomimetic scaffolds for bone regeneration.

02.P07
Skeletal stem cell isolation using perivascular marker CD146, CD105 and STRO-1: A comparative investigation between single and dual labelling

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Skeletal stem cells (SSCs) provide an ideal cell source for bone tissue engineering strategies, although their homogeneous isolation from human bone marrow (HBM) remains a significant unmet challenge. Stro-1 is a robust SSC surface marker, however Stro-1$^+$ populations exhibit variable colony forming unit-fibroblastic (CFU-F) capacity and osteogenic differentiation potential. The present study investigates the use of pericyte marker CD146 and endothelial marker CD105 as SSC enrichment markers. Marker expression was quantified by flow cytometry before MACS and FACS isolation. Isolated populations were characterised *in vitro* for CFU-F capacity, alkaline phosphatase (ALP) activity and osteogenic gene expression, and functionally assessed *in vivo*. CD146$^+$ and Stro-1$^+$ populations exhibited both enriched CFU-F capacity, and new bone matrix deposition *in vivo*, compared to control and CD105$^+$ populations. Interestingly, gene expression analysis of

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select osteogenic genes showed Stro-1^+ and $CD146^+$ populations were not significantly different. Indeed, a small HBM stromal cell fraction (2.53%) exhibited positive immunolabelling for both Stro-1 and CD146. The data presents CD146 as an additional SSC enrichment marker to Stro-1, which targets a narrower cell population. However, insignificant molecular difference between Stro-1^+ and $CD146^+$ populations highlights a further requirement for novel SSC specific markers to isolate clinically homogeneous SSC populations.

02.P08
Elucidating skeletal development to inform tissue regeneration – lessons from organotypic ex vivo chick femur culture

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Enhancement and application of our understanding of skeletal developmental biology offers innovative strategies to bone tissue regeneration. We have used a 3D embryonic chick femora culture system to investigate effects of key growth factors on skeletogenesis. Microinjection of distinct skeletal cell populations into the femurs, or combination with a chick chorioallantoic membrane (CAM) system, further widen the potential of the model. Isolated chick femurs were placed in organotypic cultures with 25 μM vitamin D3, 5 ng/ml TGF-β3, or 100 ng/ml PTHrP. In addition, femurs were microinjected with chick osteoclasts to assess bone remodelling. Cultures were analysed by μCT, and histochemistry (proteoglycan / collagen production, PCNA, STRO-1, TRAP activity). To confer an angiogenic component, femurs were also incubated on the CAM of E10 chick eggs to assess vessel invasion. Stimulation of femurs with vitamin D3 or PTHrP initiated osteogenesis, enhancing bone formation and increasing collagen I and STRO-1 expression. In contrast, TGF-β3 elicited a chondrogenic effect, with significant decreases in bone tissue. Microinjection of osteoclasts modulated growth factor activity. This study demonstrates the successful use of organotypic femur cultures as a model for bone development, providing a tool for analyzing key temporal stages of skeletal development, and a paradigm for translation of bone development to improve scaffolds and skeletal stem cell treatments for regenerative medicine.

02.P09
Calcium sensing receptor (CaSR) plays a key role in bone tissue regeneration

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Introduction: Calcium phosphate glasses, such as G5, are bioactive, biodegradable and release Ca^{2+}. Previously, we proved that extracellular Ca^{2+} induces angiogenesis on rEPC and osteogenic differentiation on rMSCs. Our aims are: To evaluate the CaSR's role on rMSC's differentiation. To test the G5's effects on HUVEC-hMSC *in vitro* and CaSR's role.
Methods: rMSC were isolated from bone marrow. HUVEC-hMSC were supplied by Dr. Joëlle Amedeé. CaSR's role on ALP, Col Ia1 genetic expression and mineralization were measured on rMSC treated with 10 mM Ca^{2+} + 5 ng/mL AntiCaSR (blocked CaSR). hMSC and HUVEC were seeded on 3d PLA and PLA/G5 scaffolds by 24 h and 72 h then we performed Immunofluorescence (IF) against CaSR, VECad and CD31 expression. qPCR to measure CaSR, cbfa, VECad and CD31. ALP expression was evaluated also.
Results: Calcium's dependent ALP and Col Ia1 upregulation and chemotaxis were inhibited on rMSCs with blocked CaSR. Analogous results were observed on mineralization. Assays show the CaSR modulates osteogenic differentiation on rMSC. Whereas, IF reveals that mostly of HUVEC and hMSCs express CaSR. HUVEC on PLA and PLA/G5 express strongly CD31 and VECad. Cocultured hMSC seeded on PLA/G5 express ALP stronger than cells on PLA. Otherwise, qPCR reveals hMSC on PLA/G5 has the highest experssion of cbfa.
Conclusion: Ca^{2+} and CaSR are responsible of osteogenic effects on MSCs. Hence to control the Ca^{2+} release from G5 could be a successful strategy on bone tissue engineering.

02.P10
Modification of mesenchymal stem cells with a novel cell-surface reactive polymer for applications in bone disease

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Bone destruction is one of the most prevalent problems due to injuries on the battlefield, accidents, or cancers affecting the bone. Our laboratory has explored how to influence the propensity of circulating cells to associate with bone by covalently attaching a custom designed polymer to the cell surface. The polymer has bisphosphonate groups attached at one end, which enables partitioning to bone and a terminal NHS group at the other end that enables binding to the surface of cells. We tested the ability of the polymer to bind cells and found that the polymer bound both HL60 and mesenchymal stem cells and had no detectable effect on viability or proliferation. Further, cells that were modified with polymer containing bisphosphonate bound bone in significantly higher numbers than cells that were modified with a control polymer lacking bisphosphonate. Thus, this polymer has the attributes necessary to change the partitioning of cells from the circulation to the bone while its actual performance *in vivo* remains under investigation. Flexibility in the synthesis of the polymer allows for the attachment of different targeting molecules and/or binding moieties increasing its utility.

02.P11
Exploring the growth factor-binding properties of osteopontin

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Wound healing is a complex process where the immune system plays an important role in guiding tissue regeneration.In this context, the study of extracellular matrix proteins involved in the crosstalk between the immune system and the regenerative microenvironment could open new and promising perspectives in regenerative medicine. Osteopontin (OPN) is a extracellular matrix glycoprotein containing both heparin- and integrin-binding regions that play an important role in both inflammation and bone remodeling. Since the growth factor-binding properties of extracellular matrix proteins are fundamental in creating the appropriate regenerative microenvironment, we sought to explore the possible growth factor-binding properties of OPN. Taking advantage of an ELISA-based approach we tested growth factors from different families. Interestingly, we were able to indentify several binders, among them VEGF-165, FGF-2, PDGF-BB and BMP-2. Moreover, we showed that the binding of OPN to PDGF-BB enhances its effects on smooth muscle cell migration and sprout formation. The use of wild type or engineered OPN in combination with growth factors could open interesting perspectives for the development of novel biomaterials for bone regeneration. Indeed, the capacity of OPN to bind hydroxyapatite and growth factors, together with its ability of modulating their activi-

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ties could give rise to a novel class of bone grafts where OPN could help create an appropriate regenerative microenvironment.

02.P12: Multiscale modeling of sprouting angiogenesis and its importance for bone regeneration

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Angiogenesis strongly determines bone regeneration. The current computational models of angiogenesis represent the process of tip cell selection either at a large scale with phenomenological rules or at a small scale using detailed agent-based models. This study developed a true multiscale model of sprouting angiogenesis during bone regeneration, which consists of: (i) a tissue level describing the various key processes of bone regeneration with 11 continuous variables: mesenchymal stem cells, fibroblasts, chondrocytes, osteoblasts, fibrous matrix, cartilage, bone, oxygen and osteogenic, chondrogenic and vascular growth factors, (ii) a cellular level representing the developing vasculature with discrete endothelial cells (ECs) (iii) an intracellular level that defines the internal dynamics of every EC (Dll4-Notch1 signaling). The bone regeneration process was predicted by the model in accordance with experimental and in silico results. The multiscale model correctly recapitulated many experimentally observed aspects of tip cell selection: (i) the 'salt and pepper' pattern of cell fates, (ii) an increased tip cell density in case of Dll4 inhibition and (iii) excessive tip cell numbers in high VEGF concentrations. In conclusion, the proposed multiscale method is a useful tool to investigate possible biological mechanisms across different time and spatial scales, thereby contributing to the fundamental knowledge of angiogenesis within the context of bone regeneration.

02.P13 Analysis of bone material during osteotomy healing in rats

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Bone healing is a complex regenerative process with the unique capacity to fully recover the tissue's original material properties and structure. However, the regeneration of large defects remains a major challenge, as the fundamental processes of tissue formation are not yet fully understood. The main aim of our study was to better understand these processes during bone healing. Healing of femurs in a rat osteotomy model with a 1 mm sized defect was analyzed at 2, 3, 4 and 6 weeks after osteotomy to prove our hypothesis that bone formation during healing happens in two successive waves. This hypothesis is based on previous results from a sheep osteotomy model. Furthermore, we compared the uneventful healing situation with a critical-sized bone defect (5 mm) model in rats where the bone does not manage to bridge the osteotomy gap. We investigated the micro-mechanical and structural properties of callus and cortical regions by X-ray scattering, nanoindentation and backscattered electron imaging. By this we determined the average mineral particle thickness, their degree of alignment, the local elastic indentation modulus and the calcium content. In the uneventful healing situation, initial results showed that the early callus material in rats is rather disordered. The indentation modulus is lower in callus regions compared to cortical regions. These investigations of material properties along the course of bone healing allow a better understanding of mineral tissue formation.

02.P14 Microparticles for sustained growth factor delivery in the reconstruction of critical-sized segmental tibial bone defects

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The regeneration of large bone defects is a serious clinical problem. Spontaneous regeneration to fill large defects is limited, as such, tissue engineering has evolved to create scaffolds for bone repair.This study combined mechanically supportive, highly porous polycaprolactone scaffolds with a controlled delivery system comprising polymeric microparticles containing multiple growth factors. This delivery system afforded spatio-temporal control over released factors.Growth factor activity has been verified *in vitro* and microparticle formulations have been tailored towards desired release kinetics for the different growth factors. It is intended that early release of Vascular Endothelial Growth Factor will promote early vascularisation of the defect site. Subsequent release of Bone Morphogenetic Protein 2 from a different delivery formulation should promote osteogenic differentiation of newly migrated cells in the defect site leading to increased bone regeneration. This approach is under assessment *in vivo* in a critically-sized segmental model involving an ovine diaphyseal resection. Our x-ray data at 3 and 6 months demonstrate significantly higher bone regeneration in growth factor-loaded groups compared to scaffolds alone.Controlled release systems for delivering specific growth factors have the potential to drastically increase the applications in which growth factor treatments could be therapeutically applicable and cost effective.

02.P15 Evaluation of osteoconductive scaffolds in the canine femoral multi-defect model

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Background: In the military and civilian populations, treating large segmental bone defects in a compromised tissue bed remains an unsolved clinical challenge. This project was designed to compare osteoconductive scaffolds and enable selection of the most promising substrate on which to add cells and/or bioactive surface modifications.
Method: Scaffolds formulated with polymers such as PLGA, PLCL, tyrosine derived polycarbonate (TyrPC), poly(propylene fumarate) (PPF) were evaluated in the canine femoral multi-defect model. Mineralized cancellous allograft (MCA) bone provided a comparative reference to the current clinical standard. Percent bone volume within the defect was assessed at 4 weeks using both micro-CT and limited quantitative histology.
Results: Bone formed at the periphery of all scaffolds with varying levels of radial ingrowth. Two scaffolds, TyrPCPL/TCP and PPF4SLA/HA proved to be significantly better but were not significantly different

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from each other. No polymer scaffold achieved the level of performance of MCA (ANOVA $p < 0.05$).
Conclusions: MCA produced a rapid and advanced stage of bone formation and remodeling throughout the defect, greatly exceeded all polymer scaffold performance. Ongoing work is using MCA to evaluate advanced methods for transplantation of autogenous cell using a chronic caprine tibial defect model. Ongoing work is also evaluating the potential for modification of calcium containing polymer surface coatings and the delivery of BMP-2.

02.P16
Development of a nano-composite drug eluting bone plug enhancing fixation of screws in low quality bone

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Revision surgery due to screw-cutout is a frequent complication after the fixation of osteoporotic fractures. In our study we target the development of a new drug eluting bone plug that enhances both primary and secondary stability of screws in low quality bone. This nano-composite implant is designed to be inserted in a pre-drilled hole prior to screw insertion. The bone plugs are fabricated from a hybrid composite material consisting of a polylactide (PDLLA) and hydroxyapatite-nanoparticles (nHA) loaded with the bisphosphonate Zoledronate (Zol), a potent inhibitor of bone resorption. A solvent evaporation technique was used to form composite microspheres with a nHA/Zol-content of 5–25% and a diameter of 100–500 μm. In a second step, the microspheres were fused in order to get tube-shaped plugs with outer diameters down to 1.5 mm. Handling and pull-out tests in artificial bone have been performed to optimize the mechanical properties and the material composition of the bone plugs. These tests confirmed that the plugs can resist the shear force during insertion with fragments evenly distributed around bone trabeculae once the screw is inserted. Pull-out tests in artificial bone with a structure similar to human bone have shown that the presence of a bone plug improves the pull-out force of a screw by 16.4%. An animal study using a rat model including dynamic histomorphometry based on micro-CT scans will provide information about the drug effect *in vivo*.

02.P17
Delayed cell injection of allogenic mesenchymal progenitor cells for bone regeneration in an ovine critical-sized segmental tibial bone defect

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Introduction: Mesenchymal progenitor cells (MPCs) represent an attractive cell population for the concept of bone tissue engineering. Their special immunological characteristics suggest that MPCs can be used in an allogenic application. The results using the current cell-based concepts are discussed controversially. This might be due to the high rate of cell death caused by local hypoxia and low nutrition supply in large bone defects. Therefore, we present a novel technique of delayed cell delivery for large bone defect regeneration, to improve the outcome of cell transplantation for bone regeneration.
Methods: A 3 cm segmental tibial bone defect was created in 18 adult merino sheep. Ovine MPCs were isolated from bone marrow aspirates, expanded and cultured with osteogenic media for 2 weeks before implantation. 4 weeks after the implantation of the scaffolds, the cells were injected percutaneous into a mPCL/TCP scaffold. Bone healing was assessed after 12 month by radiology, micro computed tomography and biomechanical testing.
Results: Delayed injection of allogenic cells did not lead to a local or systemic rejection. Radiology, microCT, biomechanical testing and histology showed significant more bone formation in the cell groups compared to the empty scaffold group.
Conclusion: This novel procedure of cell delivery could overcome the current problems of cell-based tissue engineering and serve as a baseline for the translation of novel concepts into clinical application.

02.P18
Growth factor-loaded microparticles to promote the natural healing process of bone

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Critical sized defects in bone resulting from trauma and primary tumour resections have presented obstacles to the current treatment for bone repair. The identification of growth factors which play key roles in tissue regeneration have attracted great deal of attention in the past decade, however, the outcome from several clinical trials has been largely disappointing. The results from previous studies have suggested that spatio-temporal control is crucial to achieve optimal therapeutic effects of growth factors. Therefore, scaffolds that can regulate such a release represent an attractive therapeutic path for bone tissue engineering. In the current study, the effect of different growth factors on osteogenesis was initially studied in organotypic culture of the chick femur. Different protocols were developed to produce various formulations and sizes of microparticles containing selected growth factors using single and double emulsion techniques. Assessment of *in vitro* release kinetics showed that tailored release of growth factors was achieved by the inclusion of a triblock co-polymer in the microparticle formulations. Finally, the microparticles were transplanted into the chick femur and cultured for 10 days. The results from release of growth factors from microparticles by in situ hybridisation and immunostaining demonstrate the potential for controlled spatio-temporal release of bioactive growth factors for chondrogenesis and/or osteogenesis in the chick femur.

02.P19
Influence of low level laser therapy during bone repair in rats

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Laser therapy has emerged as an efficient noninvasive treatment to stimulate osteogenesis and to accelerate bone healing. In this context, this study aimed to assess the effects of LLLT on healing in a model of tibial bone defects in rats. Animals were randomly distributed into two groups: control or laser-irradiated group. Were submitted to laser irradiation (GaAsAl, 830 nm, 120J/cm^2, 100 mW) started immediately after surgery. Animals were sacrificed on day 15 post-injury. The qualitative histological analysis revealed the control group presented inflammatory infiltrate and minor amount of granulation tissue, eventually,

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woven bone with no interconnected trabeculaes was observed. The laser irradiated animals demonstrated mild delimitation of the bordes of the injury, no inflammatory infiltrate and interconnected concentric trabeculaes. Still, the animals of this group displayed a moderate amount of newly formed bone and a better tissue organization compared to control, corresponding to a more advance stage of bone repair. Also, the laser trated animals showed a higher amount of newly formed bone was observed at 15 days post-surgery. The immunohistochemical analysis showed that laser irradiation produced a higher expression of COX-2 and a positive immunoexpression of RUNX-2 at day 15 post-surgery. Our findings indicate that laser therapy improved bone healing, by accelerating the development of newly formed bone and activating the osteogenic factors on tibial defects.

02.P20
Autologous serum improves matrix remodeling and bone formation in the sheep model

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The sheep AV-loop model was established in the last years to engineer vascularized transplantable tissues in clinically relevant size. Bone matrices have been vascularized and ectopic bone parts were engineered in this model, but previous studies failed to form a bone block which is stable enough for transplantation in a critical size bone defect. The present study now aims at engineering an axial vascularized bone block with sufficient primary stability.For that purpose the primarily stable bone substitute NanoBone® was tested subcutaneously with directly re-transplanted or expanded autologous MSC both in combination with and without BMP-2. NanoBone® was saturated in fibrin, autologous serum or cell culture medium prior to implantation. Constructs were explanted up to 12 weeks. Autologous serum led to increased remodeling and earlier bone formation compared to fibrin within NanoBone® scaffolds. RT PCR analyses proved the upregulation of osteogenic genes compared to control. Best results were reached using 6×10^6 expanded MSC in combination with 60 μg/ml BMP-2 in autologous serum. In pilot experiments NanoBone® blocks were implanted in the sheep AV-loop model and showed an increasing vascularization pattern after 12 weeks. The next step will be the transplantation of an engineered bone block in the sheep AV-loop model into a critical size tibial defect. In the future this concept could possibly replace current therapeutic concepts for treatment of bone defects.

02.P21
A top-down/bottom-up strategy to develop a bioactive periostium graft

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This work aims to develop highly bioactive membrane scaffolds based on elastin-like polymers (ELPs) and multiple signals designed to stimulate mesenchymal stem cell (MSC) proliferation, osteoblastic differentiation, mineralization, and angiogenesis during bone regeneration. Four ELPs exhibiting bioactive epitopes designed to promote either MSC adhesion (RGDS), endothelial cell adhesion (REDV), or mineralization (HAP), were synthesized using standard recombinant protein techniques. The ELP membranes were fabricated by a recently reported drop-casting/evaporation technique and exhibited, in addition to the inherent epitopes, topographical patterns designed to enhance MSC proliferation and osteoblastic differentiation. Rat MSCs were used to evaluate the effects of these signals and analyzed by scanning electron and confocal microscopy, and a custom-made MATLAB program for high-throughput image analysis. Cells on ELP membranes with either RGDS or REDV exhibited faster adhesion and enhanced spreading. Increased proliferation was observed in cells growing on membranes with RGDS alone or both RGDS and HAP. Surface topographies also induced specific effects on cell behaviors. These results demonstrate the capability to create thin molecularly designed membranes with both physical and biomolecular signals designed to orchestrate multiple biological processes. This work is part of our ongoing effort to develop a multifunctional periosteum graft to enhance bone regeneration.

02.P22
Improving bone reconstruction in the rat ulnar nonunion model via controlling human recombinant bone morphogenetic protein-2 release

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Introduction: Congenital or acquired bone defects are major problems in orthopedic surgery. In this study, we conjugated a hydrophobic polyester compound, BOX, into the PLGA/PEG thermal hydrogel for control release model. In the past few years, we had already known that BMP-2 plays an important role in bone formation. The aim of this study, we created a model of critical-sized detect and design implants with osteoinductive growth factor for bone remodeling.
Materials and Methods: 45 female Wistar rats were assigned into five groups ($n = 9$), and created a 5-mm segmental bone defect on bilateral ulnas. Through this study, we can validate the efficiency of collagen sponges and BOX hydrogel cooperating with rhBMP-2 on the critical-sized defect healing.
Results: Through the data, we demonstrated the efficiency of Collagen/BOX implants combined with BMP-2 has better osteoinductive effect and can promote fracture healing on critical-sized bone defect model. Among HE staining of bone defect sections in these two experimental groups at 4 weeks after surgery demonstrated that there were regenerated bone elements formation, such as endochondral cells, osteoblasts and osteocytes. We found that bone formation at Collagen/BOX group combined with the vascular penetration of new blood vessels into the redeveloped area.

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Conclusions: The results indicated that Collagen/BOX implants adding BMP-2 would provide the possible clinical applications in orthopedic surgery and regenerative medicine.

02.P23
2,5-dimethoxy- 2,5-dihydrofuran crosslinked chitosan for bone tissue engineering application

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Biomaterials have emerged as a powerful regulator of the cell-microenvironment interactions for modern medicine. Biomaterials provide sites for cell attachment and initiation of matrix generated cell signalling pathways. High-throughput techniques of genomics and proteomics allow screening of a large number of biomaterial scaffolds thereby improving its efficacy. Chitosan is the second most naturally abundant polymer and is non-toxic, biodegradable and biocompatible. Despite being extensively exploited in biomaterials research, chitosan fibers produced in alkaline bath through pH mediated gelation suffers from high degradation rate. Crosslinking of the fibers with various chemical agents is performed to overcome the problem of instability. In this study 2,5-dimethoxy- 2,5-dihydrofuran (DHF) at various concentrations is used to crosslink chitosan. The furan ring of DHF opens to form butenedial in acidic conditions aided by mild temperature. The dialdehyde formed is used to covalently crosslink chitosan. Though crosslinking strategy of DHF is similar to glutaraldehyde, its nontoxic nature makes it highly biocompatible. SEM results showed formation of microfibers (25–65 μm in diameter). Physicochemical properties of crosslinked and uncrosslinked fibers were compared and their capability to support the attachment, spreading, and proliferation of osteoblast cells were studied. Transcriptomic and proteomic expressions of selected osteoblast genes were assessed.

02.P24
Fabricating and Evaluating the Different ratio of TCP:HA as scaffold for bone tissue engineering

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Tricalcium phosphate (TCP) and hydroxyapatite (HA) are different in their resorbablity and strength. In view of the need for custom-made scaffolds for bone tissue engineering purposes, we fabricated a series of scaffolds with different proportions of TCP/HA and evaluated their physicochemical & biological properties. The porous β-TCP/HA blocks were fabricated via a combination of gel casting & sintering methods. X-ray diffraction profile, microstructural and biomechanical properties were evaluated. The scaffolds were then placed subcutaneously into New Zealand White rabbits for three months to access local tissue reactions and biodegradation rates. XRD analysis showed maintenance of TCP/HA ratio after sintering. Compressive strength was highest in 100%TCP (8.42 ± 0.9MPa) and decreased with reducing TCP proportion. Scanning electron micrograph revealed interconnected pores in all ratios with increased porosity and pore size with increasing HA proportion. Highest was scored by 100%HA (76.2 ± 4.8% porosity; 1241.667 ± 118.14μm mean pore diameter). All ratios were biocompatible and histologically showed no significant differences in local tissue reactions. In vivo CT-image analysis showed that TCP/HA80:20 degraded fastest, with a reduction ratio of 0.43 ± 0.36 by 3 months. This study described a reproducible method for fabricating scaffolds for tissue engineering according to the desired shape and dimensions. The profile of the different TCP/HA ratios can be used as a guide for selecting the ideal ratio for different applications.

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03. Tissue Engineering from Lab via Translation to Clinical Application (TERMIS thematic group) (in coop. Bone-Tec)

03.01
Keynote: Signals in biomaterial constructs to improve bone vascularization

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For bone vascularization the three main components of translational strategies, namely biomaterials, biosignals and cells, can be used in various combinations. These range from a minimalist approach, in which molecular self-assembly of an injectable polymeric system exposes a bioactive signal in the regenerative niche, to a maximalist strategy with autologous cells pre-seeded on to a biomaterial scaffold under the action of growth factors. This presentation will examine ways of using principles of cellular communication to better advantage in translational strategies to improve bone vascularization. In the past years our group has demonstrated the use of cellular crosstalk from heterotypic interactions to accelerate vascularization. Thus, osteoblasts can drive endothelial cells to form lumen-containing microvessel-like structures in an in vitro network which can be rapidly inosculated in vivo. Biomaterials could also be functionalized using bioactive signals, such as early embryonic genes, including sonic hedgehog. A final alternative is the promotion of vascularization via modulation of the chemical composition of the biomaterial. In vitro methodology with the use of primary cells of human origin is a well-established possibility to elucidate biological mechanisms. Nevertheless, proof of concept in a bone application in vivo is still essential as a bridge to clinical translation. (Support from the BMBF German-Chinese Cooperation in RegMed)

03.02
Keynote: Exploring and engineering the cell-material interface for regenerative medicine

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This talk will detail materials based strategies to influence cell behaviour and differentiation. Both fundamental aspects of the cell-material interface will be discussed as well as potential applications throughout the field of regenerative medicine. In particular the influence of substrate mechanics, chemistry and interfacial energy on human stem cells will be presented. Additionally a thorough materials analysis of tissue engineered bone will be presented. Indeed many different cells are used in bone regeneration applications but it is not always clear if they produce a material that mimics the structural and compositional complexity of native bone. By applying multivariate analysis techniques to micro-Raman spectra of mineralized nodules formed in vitro, we have revealed cell-source-dependent differences in interactions between multiple bone-like mineral environments. These recent findings will be discussed here. Understanding the biological mechanisms of tissue formation in vitro that contribute to cell-source-specific materials differences may facilitate the development of clinically successful engineered tissue. This work is funded from several sources including the EPSRC and the ERC.

03.03
Keynote: Calcium phosphate cement as drug delivery vehicle for bone regeneration

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Autologous bone grafts are still considered as the golden standard, albeit that several major drawbacks are related to the transplantation of autologous bone. Consequently, research is focusing on the development and evaluation of (synthetic) materials to replace autologous bone in grafting procedures. Calcium phosphate cements (CPC) represent a good candidate material to use as bone substitute. Besides, CPC can be used as a carrier for local and controlled drug release. Several aspects need to be considered regarding the incorporation of drugs to CPC. First of all, it needs to be verified that the setting reaction and hardening mechanisms and physico-chemical properties do not alter after the addition of drugs. Secondly, the drug release kinetics in vitro needs to be characterized, e.g. by in vitro release experiments with radioiodinated drugs of interest. Subsequently, a release profile is obtained by measuring in time the remaining activity in a gamma counter. This needs to be followed by an assessment of the in vivo efficacy of the drug delivery carrier, after which the clinical use must be evaluated. The efficacy needs to be analyzed because the activity of the drugs and/or bioactive molecule might be lost due to the chemical reactions during cement setting, and therefore the drug and/or bioactive molecule will have no clinical effect. The present lecture will discuss the development and possible use of CPC as a drug delivery system to support bone regeneration.

03.04
Keynote: Strategies for vascularised bone tissue engineering: combining honeycombed-like scaffolds, biaxial bioreactor, growth factors, co-culture of stem cells

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The lack of angiogenesis is the main challenge limiting factor in the clinical introduction of bone tissue engineering (BTE) approaches for the repair of large bone defects. We have successfully demonstrated bony regeneration in rodent models through the use of 3D resorbable honeycombed-like scaffolds in a novel biaxial bioreactor. Dynamic culture using the bioreactor resulted in efficient mass transport, homogenous distribution of cells and uniform exertion of shear forces along the scaffold filaments. Our scaffolds (CE mark and FDA-approved) have been implanted in more than 1500 patients, reported with high rates of success. For example, scaffolds for burrhole and cranioplasty showed bone growth within 3 months. Scaffolds infused with BMP2 showed good bone formation in the mandible. Large cranial reconstruction using the scaffolds and bone marrow aspirates showed prom-

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ising early-term results. However, there remains a lack of clinical focus with regards to the need for vascularisation and its potential effects on long term bone remodelling. Recent investigations relating to coculture systems have demonstrated the proof of concept that the addition of endothelial lineages to coculture systems enhances vascularisation within the scaffold construct. More evidence relating to the quality of blood vessels formed and bone quality needs to be demonstrated. This presentation will provide an overview of the current status of engineering vascularised bone.

03.05
Keynote: Bone engineering from in vivo to clinical application

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Regenerative medicine in the field of bone engineering uses compositions of biomaterials, cells and growth factors. Many different biomaterials exist with different properties. Some have mechanical properties similar to bone, others have a composition close to the bone extracellular matrix. However, until now no perfect scaffold exists. Nevertheless, testing of scaffolds in relevant in vivo models has shown that some induce successfully bone regeneration. To optimize the scaffolds, stem cells can be added. These can be derived from adipose tissue or bone marrow. Bone marrow can be directly applied to the scaffold without being processed. This is an advantage in light of EU legislation. Finally, growth factors like BMP-2 and BMP-7 are approved for some clinical bone applications. Strategies were tested in a non-union femur defect model. This enables investigation of different time points, concentrations and compositions. Finally, we have tested an FDA approved PCL-TCP scaffold filled with bone marrow and BMP-7 in a pseudarthrosis in a patient suffering from this condition since more than 2 years. The composite was put together in vivo. The construct was covered with a PCL membrane in order to inhibit fibroblast ingrowth from the periphery. Moreover, it could give a guide to periost cells. Within 3 months, bone formation was observed. The process is still ongoing. In conclusion, several approved components exist and clinical translation in specific cases seems feasible.

03.06
Keynote: Bone development towards artificial surfaces

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A large number of papers on bone-TE have been published concerning bone-to-bone situations, for example non-unions and critical sized defects. However, developing new bone to artificial surfaces, such as implant surfaces or non-bony biological surfaces, e.g. root surfaces of teeth, is still regarded a major challenge. Suitable scaffolds, signaling molecules and cells (e.g. MSCs, platelets) are necessary to achieve good results. Requirements for close contact of newly grown bone to artificial surfaces are:○ Rough surfaces (micro- or nano-structured), so that osteoblasts are able to adhere to.○ Optimal surgical approach with preservation of the periosteum for nutrition. Similar conditions appear in the development of vertical bone in periodontal defects without existing bony walls. Results from the following categories will be presented:○ Bone to implant surfaces using different scaffolds, cells and rhBMP-2.○ Bone to exposed implant surfaces, which means implant surfaces covered with biofilms. In these cases the complete disinfection of implant surfaces carried out by photodynamic therapy is the most important initial step. ○ Bone to exposed root surfaces. 20 patients with 48 periodontal defects have been treated and examined in a 2 years follow-up.

03.P01
Expression of osteogenic genes and surface molecule during osteogenesis on bone marrow stromal cells by bone forming peptide 3 derived from BMP-7 prodomain

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Bone forming peptide 3 (BFP 3, apply for a patent for domestic), from BMP-7 derived peptide, prepared peptide library screening methods. The aim of the present study was to investigate the effect of BFP 3 on ALP, osteocalcin, and CD44 during osteoblastic differentiation of mouse bone marrow stromal cells. D1 cells were cultured in ODM for 6 days and treated with BFP 3 for 1 days and then subjected to various tests MTT assays, alizarin red S staining, and ALP and osteocalcin gene expression, fluorescence microscopy, flow cytometric analysis and animal experiments. BFP 3 has alpha helix structure. D1 cells were found to differentiate into osteoblasts in the presence of ODM. Osteogenic differentiation was enhanced when cells were treated with BFP-3, as determined by alizarin red S staining and osteogenic genes (ALP and osteocalcin) mRNA expression, and flow cytometric analysis for CD44 surface molecules. Furthermore, X-ray images showed that BFP 3 enhanced bone formation on animal experiments. These findings suggest that BFP 3 enhances osteogenic differentiation via ALP, osteocalcin, and CD44 activation when administered to bone marrow stromal cells in osteogenic differentiation medium.

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03.P02
Interactions between bone and vascular repair in a composite tissue limb injury model

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Although severe extremity trauma often includes skeletal and vascular damage, the effect of concomitant vascular injury on segmental bone defect repair is yet to be investigated. To this end, we developed a novel composite limb injury model by combining a critically-sized segmental bone defect with surgically-induced hind limb ischemia (HLI). Based on reported clinical and experimental observations, we hypothesized that HLI would impair bone regeneration. S-D rats received unilateral 8 mm femoral defects alone (HLI-, $n = 13$) or in combination with HLI (HLI+, $n = 15$). Defects were treated with 2 μg rhBMP-2 via a hybrid nanofiber mesh/alginate hydrogel system. Interestingly, μCT analysis revealed enhanced bone regeneration in the HLI+ group as early as week 4 ($p < 0.01$) that was sustained through week 12 ($p < 0.001$). μCT angiography at week 12 revealed no differences in blood vessel volume in the thigh, suggesting a robust neovascular response to ischemia. Morphological differences persisted, however, as the HLI+ group possessed a more interconnected network of smaller diameter vessels ($p < 0.001$). Ongoing experimLents are studying vessel networks early in the healing process in order to better interpret our results. While counter to our hypothesis, our results are notable and suggest interactions in the cellular and molecular mechanisms of vascular and bone repair. A better understanding of such mechanisms

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may have important clinical implications for improving trauma patient treatment.

03.P03
A collagen-targeted RGD biomimetic peptide to enhance bone formation

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Osteogenesis is a complex, multifactorial process in which many different signals interact. The bone morphogenetic proteins (BMPs) are the most potent inducers of osteoblastic differentiation, though very high doses of BMPs in combination with collagen type I formulations have to be used for clinical applications. Although integrin-binding arginine-glycine-aspartate (RGD) peptides have shown some promising abilities to promote the attachment of cells to biomaterials and to direct their differentiation, the linking of these peptides to collagen sponges implies chemical manipulation steps. Here we describe the design and characterization of a synthetic RGD biomimetic peptide (CBD-RGD), consisting of a collagen-binding domain derived from the von Willebrand factor and the integrin-binding RGD sequence. This peptide was demonstrated to bind to absorbable collagen type I sponges (ACSs) without performing any chemical linking, and to induce the differentiation of mouse preosteoblasts and rat bone marrow-derived mesenchymal stem cells. Furthermore, in vivo experiments showed that ACSs functionalized with CBD-RGD and loaded with a suboptimal dose of BMP-2 formed ectopic bone in rats while non-functionalized sponges loaded with the same amount of BMP-2 did not, indicating that the combination of this biomimetic peptide with the currently used collagen + BMP system might be a promising approach to improve osteogenesis and to reduce the doses of BMPs needed in clinical orthopaedics.

03.P04
Comparative experimental-morphologic study of the influence of calcium-phosphate materials (Chronos, Cerosorb, Collapan, Ostim) on reparative osteogenesis activation

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Last years in traumatology-orthopedic practice are widely used calcium-phosphate materials for filling bone defects and activation of bone formation. However, there are no works devoted to the comparative study of these materials.Comparative experimental-morphologic study of the influence of calcium-phosphate materials, i.e. ß-tricalcium-phosphate ceramics ChronOs («Mathys Medical Ltd», Switzerland) and Cero-sorb («Curasan», Germany), biocomposite material Collapan, containing synthetic hydroxyapa-tite, collagen, and antibiotic («Intermed-apatit», Russia), synthetic hydroxyapatite in the form of paste Ostim («Osartis», Germany) upon the activization of reparative osteogenesis was per-formed in 170 male rats. Preparations were implanted into metaepiphyseal defect of the tibia. The material was investigated histologically on days 30, 60 and 90 after operation. It was detected that all test materials promoted the formation on their surface of new bone; however the most marked activation of reparative osteogenesis was observed at implantation of Collapan and somewhat less at Ostim implantation. ChronOs and especially Cerosorb showed delayed substitution by bone growing into their pores. The obtained data were the basis for successful use of the Collapan for activation of reparative osteogenesis and prevent of the development of infection in the complex treatment of patients with comminuted fractures of long bones of the extremities.

03.P05
BMP-2 mediated tissue regeneration is attenuated in a rat model of composite bone and muscle injury

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Composite injuries involving bone and surrounding soft tissue comprise one of the most challenging musculoskeletal conditions to return to normal function. Our objective was to develop a composite injury model by combining a segmental bone defect model with an adjacent muscle injury and characterize tissue regeneration after treatment with a BMP-2 delivery system. Rats were placed in three groups: muscle injury (8 mm diameter empty defect in the quadriceps), bone injury (8 mm critically sized defect in the femur), or composite bone and muscle injury ($n = 6$ per group). Bone defects were treated with 2 μg of BMP-2 delivered in pre-gelled alginate and a nanofiber mesh. Bone regeneration and angiogenesis were quantitatively assessed using uCT and limb function was assessed using gait analysis and muscle strength measurements. At 12 weeks regenerated bone mineral volume was attenuated in the composite injury group as compared to the bone injury group. Composite injury animals showed significantly reduced gait and muscle function as compared to the animals in the single tissue injury groups. Vascular volume increased at 3 and 7 days in the bone injury group which was not observed in the composite injury group; both groups showed a comparable significant increase at 14 days as compared to control. This composite injury model produced severe functional and structural deficits and provides a clinically relevant screening platform which can be used to test novel multi-tissue therapies.

03.P06
Bone grafts engineered from human adipose-derived stem cells in dynamic 3d-environments: a top-down versus bottom-up approach

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Introduction: Modular tissue engineering (TE) is promising to overcome the limits in traditional TE. Adipose tissue derived stem cell (ADSC)-laden microcarriers can be used as building blocks that can assemble and create large bone grafts. However, the bone forming capacity in a bottom-up approach is often not compared with classical top-down approaches. In the present study, the feasibility of ADSC on microcarriers was compared with scaffolds to form bone TE grafts.
Materials and Methods: ADSC were phenotypically analyzed by flow cytometry, followed by a 2D osteogenic differentiation assay. For 3D analysis, cells were seeded on collagen-based scaffolds/microcarriers and cultured in static/dynamic conditions. The bone-like morphology and response to hydrodynamic shear was evaluated by (immuno)histochemistry and gene expression (Runx2, col I, BSP, OCN).
Results: After 6 weeks of dynamic culturing, scaffolds were colonized until the center in contrast to static cultures, where only the edges were colonized. Cells on scaffolds showed a downregulation of Runx2 and colI, in contrary to microcarriers where an upregulation of Runx2, col I, BSP and OCN was demonstrated. Large bone grafts with sizes of 2 cm^2 can be obtained in this bottom-up approach with a cell/surface area ratio 25–40 times lower than in the traditional top-down approach.

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Conclusion: This study reveals the potential of ADSC-laden microcarriers as building blocks to create macrotissues in a bottom-up approach.

03.P07
Ectopic bone formation by human MSC on different bone replacement materials

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A promising approach to tackle the challenge of large non-healing bone defects in regenerative medicine is the combination of biological or synthetic bone replacement materials and human mesenchymal stem cells (MSC). However, synthetic bone substitutes like ß-TCP do not form bone with all donor MSC cultures. Aim of our study was to investigate whether MSC show superior bone formation on biological bone replacement materials than on β-TCP and if collagen is an important component of success.Multilineage potential of donor MSC was confirmed in vitro. We compared biological collagen-containing (Duross+, Tutobone) and collagen-free (Duross-, Bio-Oss) bone substitutes with β-TCP in vivo. Granules (0.25–1 mm) were seeded with MSC from nine donors and transplanted subcutaneously into SCID mice. Lowest donor variability was evident for Duross+ and β-TCP, but bone formation failed for MSC of one donor with all materials. Histomorphometry revealed that Duross+ was most successful and mediated significantly more neo-bone formation than β-TCP ($p = 0.003$), Duross- ($p < 0.001$) and Bio-Oss ($p < 0.001$). Tutobone showed a higher bone formation compared to Bio-Oss ($p = 0.027$). Newly formed bone was of human origin in all groups. While β-TCP performed similar to several collagen-free biological bone substitutes, the collagen component in biological bone substitutes seems favourable for neobone formation by MSC. Collagen combined with β-TCP might represent a potent approach for quality improvement.

03.P08
Discontinuous release of Bone Morphogenetic Protein-2 loaded within interconnected pores of honeycomb-like polycaprolactone scaffold promotes bone healing in porcine tibial and rabbit ulnar defect model

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We have selected a threedimensional polycaprolactone (PCL) scaffold with an interconnected honeycomb-like porous structure to provide a conduit for vasculature ingrowth as well as an osteoconductive pathway to guide recruited cells responding to a unique triphasic release of osteoinductive bone morphogenetic proteins (BMP) from these PCL scaffolds. We hypothesize that the use of recombinant human bone morphogenetic protein 2 (rhBMP2)-PCLconstructs promotes rapid union and bone regeneration of a large defect. We conducted three groups of application of PCL scaffold with or without rhBMP-2 (Group I: 5 15 mm sized porcine tibial defect treated with PCL scaffold, Group II: 5 15 mm sized porcine tibial defect treated with PCL scaffold coated with rhBMP-2, Group III: 10 15 mm sized rabbit ulnar defect treated with PCL scaffold coated with rhBMP-2). Results of Group II and Group III demonstrated enhanced bone healing with greater amount of bone formation and bridging under plain radiography and microcomputed tomography imaging when compared with an empty PCL and untreated group after 8 weeks postimplantation. Quantitative measurements showed significantly higher bone volume fraction and trabecular thickness, with lower trabecular separation in the rhBMP2-treated groups. This study serves as preclinical evidence demonstrating the potential of combining osteoinductive rhBMP2 with our PCL constructs for the repair of large defects in a large animal model.

03.P09
Accelerated calcium phosphate cement degradation due to incorporation of glucono delta-lactone as porogen

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Injectable calcium phosphate cements are frequently used for filling of bone defects due to their excellent osteocompatibility, but their poor degradability limits complete regeneration of bone defects. Porogens that produce acid by-products are particularly attractive to create macroporosity in situ since calcium phosphate cements (CPC) degrade by acid dissolution. The aim of the current study was to investigate if glucono delta-lactone (GDL) can be used as acid-producing porogen for incorporation into CPC without compromising the osteocompatibility. In vitro studies (microCT, RP-HPLC, XRD and SEM) were performed in order to optimize the composition and handling properties of CPC-GDL composites, while an in vivo study (orthotopic implantation in femoral condyles of rabbits) was carried out for CPCs containing either low (10 wt%) or high (30 wt%) amounts of GDL. CPCs containing poly-lactic-co-glycolic (PLGA) or gelatin microspheres were used as control. In vitro studies confirmed that CPCs containing GDL were injectable and self-setting, while a considerable amount of porosity was formed already within 1 day of incubation in PBS. Histomorphometrical evaluation after 6 weeks of implantation revealed that CPCs containing 10% of GDL degraded considerably faster and were replaced by more bone tissue than the other experimental groups. In summary, it can be concluded that addition of 10% of GDL to injectable CPCs results into considerably improved degradation characteristics.

03.P10
Bone-like tissue induced by rhBMP-2, rhBMP-4 and rhBMP-7 in vitro has ossification potential after implantation

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Introduction: Recent experiments have attempted to induce bone from immature muscular tissue (IMT) in vitro using crude BMP and e-PTFE as a scaffold. Meanwhile, recombinant human BMP (rhBMP)-2, -4 and -7 has osteogenic potential both in vitro and in vivo. The aim of this study was to induce bone-like tissue from IMT in vitro using rhBMP-2, rhBMP-4 and rhBMP-7, and to confirm this tissue has ossification potential in vivo.
Materials and Methods: Commercially available rhBMP−2, −4 and −7 were used in this study. IMTs were extracted from the 20-day Sprague-Dawley (SD) embryonic rats and placed on e-PTFE with 10 ng/μl each of rhBMP−2, −4, and −7, and cultured for 2 weeks. The cultured tissues were analyzed by histological observation and EPMA. RT-PCR was performed to examine the expression of osteoblastic genes on days 7 and 14. Furthermore, the specimens were implanted subcutaneously into the backs of SD rats for up to 3 weeks, and then evaluated by μCT and histological observation.
Results: Mineral deposition was observed by Von Kossa staining. The expression of all osteoblastic genes was confirmed on both days 7 and 14. Ca, P and O were detected in the extracellular matrix by EPMA. In addition, the strong radiopacity and ossification was confirmed at 1 week after implantation and that was stronger after 2 weeks.

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Conclusion: The rhBMPs induced IMT differentiation into bone-like tissue in vitro, and this induced bone-like tissue has ossification potential after implantation.

03.P11
Interactions between tissue growth, pore geometry and cell mechanics

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Pore architecture has a dramatic effect on tissue formation in porous biomaterials used in tissue engineering. However, the wide variety of 3D structures used indicates there is a clear need for the optimal design of pore architecture to maximize tissue formation and ingrowth. Furthermore, how precisely cells form tissue in pores of different geometry in vitro remains unclear. Thus, the first aim of this study was to characterize tissue growth solely as a function of pore geometry. Secondly, to observe how actin organization and thus the potential role of cell generated forces are implicated in tissue formation. An in vitro system with well defined open pore slots of varying width was used, providing a 3D environment for tissue formation while minimizing nutrient limitations. Tissue formation was monitored for 18 days. In addition, 48 h time-lapse video was applied to investigate tissue formation dynamics within the constructs. Results demonstrated that tissue formation was strongly affected by pore geometry. Tissue formation rate increased as pores became narrower. The level of curvature of the tissue front changed depending on the pore geometry. This was associated with distinct patterns of actin organisation depending on pore width, indicative of the role of active cell generated forces. Time-lapse recording revealed the highly dynamic nature of tissue formation and showed the effect of pulling forces exerted by the leading cells which could potentially drive tissue growth.

03.P12
Evaluation of biologic scaffolds composed of extracellular matrix for functional restoration of muscluotendinous tissue defects: Update from clinical trials

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Skeletal muscle has a remarkable capacity for regeneration following acute injury. However, there are limited therapeutic options for massive loss of skeletal muscle tissue subsequent to trauma, tumor resection, or congenital disorders. Biologic scaffolds composed of extracellular matrix (ECM) have been successfully used in numerous preclinical animal models to promote constructive remodeling of a variety of damaged tissues, including skeletal muscle. Furthermore, a recent clinical report demonstrated the ability of ECM scaffolds to promote regeneration of functional site-appropriate tissue in a patient with volumetric skeletal muscle loss. Currently, our laboratory is involved in two clinical trials aimed at assessing the use of ECM scaffolds for the restoration of structure and function in patients with extensive muscluotendinous tissue defects of the upper or lower extremities. Evaluation of tissue biopsies taken after ECM scaffold implantation show the presence of regenerating skeletal muscle tissue as well as neovascularization within the ECM device. Immunoflourescent labeling reveals multipotent perivascular stem cells (NG2+, CD146+ cells) in addition to desmin positive cells widely distributed throughout the remodeling ECM. These results are encouraging in that they demonstrate the ability of ECM scaffolds to promote the formation of site-appropriate skeletal muscle tissue, likely through the recruitment and differentiation of multipotent perivascular stem cells.

03.P13
Effect of a calcium magnesium silicate bioceramic (merwinite) for bone regeneration: in vivo

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This study investigated the effect of a calcium magnesium silicate bioceramic (merwinite) for bone regeneration in vivo, with hydroxyapatite (HA) as a control. A histomorphology analysis and histomorphometry of the bioceramics implants in rat femur defect models indicated that both in early- and late-stage implantations, merwinite promoted more osteogenesis and biodegradation than did HA; and in late-stage implantations, the rate of new bone formation was faster in merwinite than in HA. These results suggest that merwinite might be a potential and attractive bioceramic for tissue engineering.

03.P14
Apatite microbeads as a means for stem cell expansion

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A major barrier to the development of cell-based therapies is the inability to expand these cells in abundance within a single run, using the existing technique of culturing in plastic T75 flasks. In addition, existing microcarriers are made of polymers or glass-based matrices which are unsuitable for bone implant science. As such, this work aims to fabricate apatite microbeads as cell carriers. Sodium-alginate and hydroxyapatite (HA) were mixed until homogenisation. The suspension was then extruded dropwise into a calcium chloride solution whereby mircocarriers were formed. The physicochemical properties were then analysed by scanning electron microscopy (SEM), X-ray diffractometery (XRD), and fourier transform infrared (FTIR) spectrophotometry. Cell viability on these carriers was evaluated using human fetal mesenchymal stem cells (hfMSCs). SEM revealed that apatite microcarriers were of uniform size ~900 μm, and displayed good spherical morphology. At high magnification, these carriers were observed to be interconnected. XRD analysis showed that the peaks were distinct, all belonging to HA, suggesting that the carriers have a good crystallinity level. Secondary phases were not detected, confirming that HA remained stable during sintering. FTIR analysis revealed the presence of several phosphate bands and an hydroxyl band which are characteristic of HA. In-vitro work showed that hfMSCs grew well significantly on these carriers with culturing time.NMRC, SGP (NIG10nov032)

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03.P15
Articular cartilage repair by tissue engineered hyaline cartilage reconstructed from costal cartilage

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Modern Cell &Tissue Technology Inc, Korea: Several attempts have been made to explore alternative donor cells for the articular cartilage repair. In this talk, I would like to introduce two possible source for the articular cartilage repair. One would be costal cartilage which retains many proliferating cells pool even in the old age. The costal cartilage gave three-fold higher cell yield and expanded faster than the chondrocytes derived articular cartilage of the same rabbit. Upon the cell expansion in the presence of bFGF, the chondrocytes derived costal cartilage regain MSC-like phenotype and retarded senescence, which could be successfully redifferentiated to the hyaline cartilage by pellet culture or by seeding to chitosan /hyaline scaffold. Implantation of those tissue engineered hyaline cartilage facilitated to heal full thickness pore made at patella of rabbit articular cartilage without discontinuity between neighboring cartilage and subchondral bone. Human costal chondrocytes were also expanded in the presence of bFGF and redifferentiated into chondrocytes spheres even after 1 000 000-fold cell expansion, whose cell processing, safety issue, and future clinical application will be discussed.

03.P16
In vivo electromagnetic stimulation supports muscle regeneration after stem cell injection by boosting muscular metabolism and stimulating nerve ingrowth

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Introduction and Objectives: Skeletal muscle engineering should lead to muscle tissue with voluntary contractions. Therefore, the development of the desired tissue specific oxidative muscle fiber type, nerve ingrowth and formation of new neuromuscular junctions (NMJ) are mandatory. In this study, we evaluated whether noninvasive electromagnetic stimulation (NMS) improves tissue engineered muscle regeneration after muscle cell implantation.
Materials and Methods: We implanted muscle cells (MPCs) into a damaged muscle and treated the animals for 5 days with NMS (BioCon2000, 20 min, 50 Hz) and investigated the presence of synapses, clustering of acetylcholine receptors (AChRs), muscular metabolic adaptations by immunohistochemistry, RTPCR and WB.
Results: NMS caused no local or systemic damage and increased stem cell differentiation in vitro and in vivo ($p < 0.001$). Synapses density ($p = 0.01$) and clustering of acetylcholine receptors (AChR) doubled under NMS treatment ($p = 0.003$). Muscle metabolism and reconstruction were enhanced after NMS therapy ($p < 0.001$). Nerve ingrowth and branching was stimulated ($p < 0.05$) and Agrin production was boosted ($p < 0.05$). NMS significantly improved cell integration and distribution into the regenerating muscle ($p < 0.001$) and increased the number of fibres formed by implanted MPCs.
Conclusions: NMS improves functional muscle formation by implanted MPCs by boosting nerve ingrown, improving synapses formation and AChR clustering.

03.P17
Biologically modified implant surfaces for improved outcomes of osseointegration

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Osteoarthritis and osteoporosis constitute a considerable economic and social health problem. In the absence of a definitive cure, current treatment strategies rely heavily on total joint replacement (TJR). However, in skeletally diseased patients adequate and long-term device stability is compromised. Reduced peri-implant osteoprogenitor recruitment and retention are suggested to contribute to this poor outcome. Here CoBlast technology is used to produce hydroxyapatite (HA) modified titanium-4%aluminium-6%vanadium surfaces. This technology incorporates HA within the oxide layer, thereby negating issues of delamination associated with conventional coatings. The surfaces are then biofunctionalised with collagen (COL) or fibronectin (FN), to improve cell retention and attachment at the implant surface. Biofunctionalisation did not alter surface morphology (SEM) or microroughness (White light interferometery) but produced a chemically stable modification (XPS, contact angle) for a minimum of 3 months. Protein adsorption was not affected by functionalisation. Cell attachment after 4 and 24 h as well as cell viability and proliferation over 10 days was increased on functionalised surfaces. This preliminary work suggests that biofunctionalisation of clinically used implants may provide an alternative strategy for improving TJR outcomes in diseased patients. Ongoing studies are currently evaluating the osteogenicity of the modified surfaces.

03.P18
Development of a clinically feasible off-the-shelf bone tissue engineering strategy with the integrated use of human fetal mesenchymal stem cells, polycaprolactone scaffolds and a bi-axial rotating bioreactor

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Large bone defect treatment still remains a major clinical challenge, requiring effective bone grafts to achieve healing. Bone tissue engineering (BTE) strategy provides a promising approach to generate off-the-shelf tissue engineered bone grafts (TEBG) to address this ever-pressing clinical need. The clinical success of BTE strategy lies on the synergetic research efforts from 3D scaffold fabrication, stem cell research, bioreactor development and scalable bioprocessing technology. Our multidisciplinary team has successfully developed a clinically feasible off-the-shelf BTE strategy. Specifically, we fabricated 3D polycaprolactone (PCL) scaffolds using rapid prototyping technique, identified human fetal mesenchymal stem cells (hfMSC) as an ideal off-the-shelf cell sources via a thorough comparative study, developed and optimized a unique bi-axial rotating (BXR) bioreactor for BTE application, and established a microcarrier culture technique for large scale hfMSC expansion. The proof-of-concept testing in animal models demonstrated the great defect healing efficacy of this off-the-shelf TEBG generated from the integrated use of PCL scaffolds, hfMSC and BXR bioreactor. Lastly, we developed a microsurgery technique to prevascularize the TEBG, which achieved significantly better bone regeneration and vascularization than non-prevascularized TEBG. Currently, the

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first-in-human clinical trial is underway to evaluate the efficacy of off-the-shelf TEBG for large defect treatment.

03.P19
Heparan glycosaminoglycans lower the therapeutic dose of BMP-2

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Autologous bone graft remains the gold standard for the treatment of orthopaedic trauma, however donor-site morbidity and insuffient graft material limit its use. Although bone graft substitutes containing BMP-2 are efficacious, supraphysiological doses are used clinically due to its inherent instability. An important therapeutic aim is therefore to lower the efficacious dose of BMP-2. Heparin, with >90% sulfation, has been shown to bind and sustain growth factor activity by interacting with various proteins to both agonize and antagonize them. Heparan sulfate (HS), a structural analog of heparin that has a greater specificity of sulfation may provide greater selectivity in protein interactions. The results revealed that HS3 enhanced BMP-2-induced osteogenic differentiation in a dose-dependent manner. Notably, BMP-2 activity was greatly augmented in the presence of HS3 through the inhibition of noggin's antagonism of BMP-2. This resulted in prolonged ALP activity in the presence of increasing concentrations of noggin. HS3 reduced noggin/BMP-2 interactions as revealed by co- immunoprecipitation. HS3 increased the bioavailability of BMP-2 and sustained pSMAD 1/5/8 signalling over a 72 h period indicative of accelerated osteogenic differentiation. In vivo testing revealed that the delivery of HS3 alone resulted in robust bone formation that was comparable to exogenous dosing with BMP-2.

03.P20
Osteointegration enhancement by human BMSCS and biofactor enriched hydrogels in trabecular titanium™ implants

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The bone-implant contact represents the weakest point during healing of prosthetic implant. Coupling Trabecular Titanium™(TT), a porous structure device, with a hydrogel enriched with progenitor cells and osteoinductive factors may represent a promising strategy to improve implant osteointegration. This study combines TT with an ammidated carboxymethylcellulose hydrogel (CMCA), strontium chloride (SrCl2) and bone marrow mesenchymal stem cells (BMSCs). To mimic prosthesis-bone implants, TT was seeded with human BMSCs differentiated in osteogenic medium, then grafted into engineered bone. These constructs were implanted subcutaneously in athymic mice as unseeded TT and TT+CMCA or cell seeded TT+BMSCs and TT+CMCA+BMSCs. After 8 and 12 weeks, osteodeposition, bone mineral density (BMD) and osteointegration were evaluated respectively by fluorescence imaging, μCT, SEM and pull-out tests. SEM showed a dense matrix filling the interface between TT and bone scaffold in seeded constructs compared to the unseeded. Fluorescent signal was detected in bone turn over regions and in seeded constructs compared to the unseeded. μCT quantified BMD in TT+CMCA+BMSCs higher compared to the unseeded constructs. Pull-out test confirmed the increasing extraction energy in TT+CMCA+BMSCs compared to others. In this study, we demonstrate that the association of TT with a SrCl2 enriched CMCA and BMSCs represents a valid approach and a novel strategy to accelerate prosthesis osteointegration.

03.P21
In vitro evaluation of bone marrow concentrate/ Bonelike® pellets for bone regeneration

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Human lifespan has been increasing in the last decades, which contributes to the rising number of bone grafting procedures. The association of bone marrow, BM (osteogenic and osteoinductive) with an inorganic scaffold (osteocoductive) may overcome the problems or limitations of autologous bone. Human bone marrow was collected during orthopedic surgical procedure, with patient informed consent. The Biomet Kit MarrowStimTM kit was used to obtain a concentrate from bone marrow aspirate. The same BM volume/ scaffold mass ratio was used in cell cultures. Bonelike®, is a modified hydroxyapatite matrix with a homogeneous dispersion of alpha and beta tricalcium phosphate phases. The pellets used present intra (24.1%) and interpores (31%). The cell viability of the nucleated cells of the BM aspirates and concentrates was similar. Bonelike® pellets colonized with BM concentrates showed a significantly higher cell viability/proliferation compared to those seeded with the BM aspirates. Regarding ALP activity, higher values were seen in the material colonized with the BM concentrates, with a significant increase between days 9 and 23. Compared to control cultures, pellets colonized with the aspirates or the concentrates exhibited earlier maximal ALP activity. These results suggest that the association of autologous BM concentrate to an inorganic scaffold appears to be a promising approach to improve the final results of fracture healing.

03.P22
Porous polymethylmethacrylate fabricated intraoperatively as a mandibular space maintainer post tumor resection: a case series

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Due to the complications of primary mandibular reconstruction, secondary reconstruction has advantages but with less satisfactory results due to loss of soft tissue and bone void space. In this case series, three patients diagnosed with ameloblastoma of the mandible underwent resection of the tumor and placement of a porous polymethylmethacrylate (PMMA) space maintainer. This space maintainer was fabricated by mixing the powder phase of bone cement (SmartSet HV, Depuy Orthopaedics or Cobalt HV, Biomet) with Surgiflo Hemostatic Matrix (Ethicon), where the weight of Surgiflo accounted for 30% of the entire implant. The monomer phase of the cement was added to this mixture and allowed to polymerize in situ or molded by hand or a silicone cast ex vivo, creating a porous PMMA material. The defects ranged in size from a 1.5×2.0 cm full thickness notch defect to two continuity

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defects 4.0 and 4.5 cm in length. All space maintainers were held in place with a titanium plate and screws. Two cases developed small lateral mucosal dehiscences; however these did not increase in size and maintained enough of the soft tissue envelope that oral surgical approaches were used to remove the space maintainer and place the bone graft. All three cases reported a metallic taste for the first 2–3 weeks of implantation. The space maintainers were explanted 8, 14 and 17 weeks postoperatively. Porous PMMA adequately maintained space and the soft tissue envelope for secondary mandibular reconstruction.

03.P23
The importance of continuous hypoxic exposure for the culture of human fetal mesenchymal stem cells in bone tissue engineering applications

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Mesenchymal stem cells (MSC) are osteogenic and clinically attractive for use in bone tissue engineering. As MSC reside in low oxygen tensions in the bone marrow (BM) niche, we hypothesised that oxygen is a critical parameter for maintaining stem cell phenotype. Human fetal MSC (hfMSC) expanded in normoxia (21%) previously were used to study effects on growth and differentiation in 2% and 21% oxygen over 14 days. Results were compared against hfMSC isolated from human fetal BM aspirates grown continuously in 2% and 21% oxygen from initial cultures. Studies using normoxia-maintained hfMSC showed 1.3X higher proliferation ($p < 0.01$) from Day 3 and 4X increase in colony-forming units-fibroblasts (CFU-F) ($p < 0.01$) in hypoxia compared to normoxia. Alkaline phosphatase levels were lower between Day 7–14 (4.7–5.3X; $p < 0.001$), with 3.1X reduction in calcium in hypoxia ($p < 0.001$; Day 14). CFU-F and proliferation were lowered when switched from hypoxia to normoxia compared to those kept in continuous hypoxia. Short-term hypoxia maintained self-renewability as seen from its increased growth kinetics, but osteogenic differentiation was impeded. hfMSC derived in hypoxia and kept in such conditions showed more robust osteogenic capacity, with 2.9X higher calcium on Day 14 ($p < 0.001$) and higher CFU-F (6.5X; $p < 0.05$). Continuous hypoxia resulted in the most potent hfMSC isolates, presenting itself as promising culture strategy for enhancing therapeutic potential of hfMSC prior to transplantations.

03.P24
BMP-2 loaded TCP/HAP porous ceramics with a polyelectrolyte multilayer film coating

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Delivering rhBMP-2 (recombinant Bone Morphogenic Protein-2) at low but therapeutically efficient dose is one of the current challenges for bone tissue repair. In this context, Polyelectrolyte Multilayer films (PEM) represent an attractive rhBMP-2 carrier due to their ability to protect proteins from denaturation and to coat a wide variety of materials with complex geometry. Herein, we coated macroporous TCP/HAP granules with a biopolymeric PEM film to deliver rhBMP-2 in a 'matrix-bound' manner. In vitro release kinetics indicated that the PEM-coated granules sequestered significant amounts of rhBMP-2. The degree of film crosslinking influenced the quantity of rhBMP-2 trapped within the films. Bare (uncoated) TCP/HAP scaffolds were also able to retain rhBMP-2. Bioactivity of rhBMP-2 in the PEM-coated granules was confirmed on two cell markers: luciferase expression on BMP-responsive-element/Luc C2C12 cells and alkaline phosphatase activity induction on C2C12 cells. Promisingly, rhBMP-2 adsorbed onto PEM-coated and on bare granules in a lesser extent, could be stored and remained bioactive over at least 3 weeks. In vivo, both uncoated and PEM-coated TCP/HAP granules loaded with rhBMP-2 exhibited both osteoconductive and osteoinductive properties. This opens perspective for coating these bioactive PEM on other types of implantable materials, including metal alloy that do not exhibit any affinity for rhBMP-2.

03.P25
Regenerative effects of a collagen-based matrix are amplified in the presence of necrotic stimuli

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Post-natal myogenesis is mainly controlled and preserved by muscle satellite cells (SCs). When activated (exercise or necrosis), SCs mature into myotubes and fuse with damaged fibers to regenerate muscle. This study explored the impact of necrotic milieu on SCs exposed to a collagen matrix designed to promote regenerative myogenesis. Hindlimb muscle from 4 week-old C57 mice was digested and the resulting slurry was cultured on collagen-coated culture dishes for 4 days to yield adherent SCs. SCs were transferred to collagen-coating or matrix substrates (type I collagen and chondroitin sulfate-C cross-linked with EDC/NHS) ± necrotic myocyte debris (NMD) from C2C12 cells. After 24 h, morphology, mRNA levels, and supernatant cytokine levels were assessed. Matrix culture (±NMD) increased myotube number by 4.1-fold andlength by 1.7-fold ($p < 0.05$); the addition of NMD generated larger myotubes, (length by 2.5-fold and width by 1.4-fold; $p < 0.05$). Matrix-NMD cultures generated myotubes that spontaneously contract after 24 h. Matrix alone increased myogenic genes (Mef2C, MyoD, myogenin) by up to 30%; and matrix-NMD culture increased transcription of Mef2C (350%), MyoD (1210%), myogenin (1180%) and Myf5 (370%). NMD alone did not induce these changes. Matrix-NMD culture enhanced the production of FGF-2, HGF and SDF-1 (2.1-, 3.3- & 1.6-fold, respectively). To conclude, the presence of necrotic myocytes augments the collagen matrix's ability to activate myotube formation from SCs.

03.P26
Improved ectopic bone formation through hyaluronan hydrogel-mediated BMP-2 delivery

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Introduction: BMP-2 is known to enhance fracture healing when delivered via a collagen sponge. However, this method requires high very doses of BMP-2. In this study we used hyaluronan hydrogels and compared them directly to collagen sponges for their ability to efficiently deliver BMP-2 doses of 0.2, 1.0 and 5.0 μg for tissue engineering.
Materials and Methods: We used thiol-modified hyaluronan (Glycosil™) to incorporate BMP-2 and form hydrogels. We evaluated the release and subsequent bioactivity of BMP-2 in vitro. The bone-forming efficacy of both carriers was also tested in a rat ectopic model and evaluated after 8 weeks with a combination of 2D radiography, 3D μ-CT and histological analyses.
Results: Collagen sponges released essentially all their loaded BMP-2 in less than 24 h, whereas Glycosil hydrogels released less than 45% of

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their BMP-2 over a period of 28 days. Glycosil hydrogels were also able to sustain the bioactivity of their BMP-2, as shown by the enhanced ALP in C2C12 cells. Analysis by μ-CT revealed the BMP-2 dose-dependency in vivo, with 1 μg being the lowest dose required by the collagen while it was 0.2 μg for Glycosil. Overall the in vivo results demonstrated that BMP-2-containing hydrogels can induce significantly greater amounts of bone formation than collagen sponge at all doses.
Conclusions: Glycosil hydrogels show particular promise as a delivery platform for BMP-2, albeit this strategy needs to be refined in clinically relevant model.

03.P27
Sutures: a novel way of cell delivery into soft tissues

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Delivery of viable cells into soft tissues is a challenging process. We hypothesized that growing implantable human stem cells on the surface of sutures may be a suitable source of cell delivery. First, we tested whether absorbable suture material can undergo the culturing process without significant changes in its physical properties. We found that 168 h of incubation significantly diminish tensile strength and reduces in vivo absorption time, while 48 h incubated sutures do not differ significantly from the original sutures. Second, we identified the optimal culture conditions for human BMSCs to proliferate on the surface of such sutures. In comparison with fibronectin, poly-L-lysine coatings, we found serum albumin to attach the highest cell number after 48 h. Furthermore, attached cells proliferate and reach a higher cell density after 168 h. Third, we tested the in vivo biocompatibility of cell-suture constructs in an animal model of muscle injury. Cell coated sutures were implanted in the triceps surae muscle of male Wistar rats. In this in vivo experiment, we showed that already after 24 h attached cells starts to leave the suture surface and migrate into the injured tissue. We conclude that through extensive optimization it is possible to use regular absorbable sutures for culturing stem cells on the surface and then using this construct as a cell delivery tool in soft tissue injury. Supported by TÉT-SIN-CELLTHER, TÁMOP-4.2.1/B09/1/KMR-2010-0001.

03.P28
Irradiated spongy bone allografts as potential carriers for cell therapy in bone regeneration

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Introduction: Spongy bone allografts are widely used for bone regeneration. Sterilization of allografts results in the loss of cells and changes in the quality of matrix. The graft functions as a scaffold and a source of growth factors. Successful regeneration does not occur if recipient's cells do not colonize or do not differentiate to osteoblasts. A different approach is adopted:allografts are seeded with recipient's cells before implantation. The aim of the study was to examine the influence on non-standard doses of sterilization-irradiation on features of bone allografts as cell carriers ex vivo.
Materials: Spongy bone allografts from four donors, cut into cubes, irradiated with one of the doses: 25, 35, 60 and 100 kGy. Adipose derived mesenchymal stem cells.
Methods: Cytotoxity and genotoxity tests (XTT, micronuclei test) were performed as high doses of irradiation may induce formation of toxic derivatives. Adipose derived stem cells were seeded onto the bone cubes, incubated for various periods. The phenotype of cells was checked after these periods using real-time PCR. Histolology sections were prepared in order to visualize the presence and the state of cells.
Results: Neither cyto- nor genotoxic effects were observed. The duration of incubation and the irradiation dose used influence cell differentiation and hence the effectiveness of the procedure.
Conclusions: Non-standard irradiation is an attractive alternative for preparation of bone allografts as cell carriers.

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04. Hypertrophic Cartilage and Bone Tissue Engineering

04.01
Keynote: Engineering hypertrophic cartilage grafts for bone repair via endochondral ossification

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Engineering large bone grafts and healing of critical sized defects in vivo is feasible only if adequate vascularisation can be induced in the tissue. Most research has focussed on engineering bone grafts through the intramembranous ossification process. An alternative approach is via endochondral ossification in which a hypertrophic cartilaginous intermediate is formed which will remodel into bone. This pathway has the advantage that cartilage is avascular with a better capacity than bone to survive the low oxygen environment of a wound site. Hypertrophic cartilage also contains vascularisation and osteogenesis-enhancing factors to promote blood vessel in-growth and subsequent bone formation. Using the endochondral bone formation pathway is now gaining popularity with most focus being on the use of mesenchymal stem cells (MSCs) as a source of potential chondrogenic cells. Most studies of MSC hypertrophy have been in using pellet or micromass cultures. This study will consider the use of are nasal chondrocytes to prepare hypertrophic cartilage grafts. This cell source, can be expanded in vitro, and seeded onto polymer scaffolds to form a significant extracellular matrix rich in collagen II and proteoglycans. The chondrocytes can undergo hypertrophy with extensive collagen X and alkaline phosphatase distribution. When implanted into a bone defect in vivo, the hypertrophic tissue will undergo mineralisation, vascular invasion and remodelling into bone.

04.02
Osteoclast-mediated activation of devitalized engineered cartilage to generate osteoinductive grafts

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Devitalized engineered tissues with the capacity to induce bone formation are of high clinical and commercial interest. This study investigated whether devitalized hyperthropic cartilage tissues (HCT) produced in vitro by adult human mesenchymal stromal cells can be activated by peripheral blood-derived osteoclast progenitors prior to implantation to induce ectopic bone tissue formation.HCT were devitalized by successive cycles of freeze/thaw and either co-cultured with freshly isolated and sorted $CD14^+$ osteoclast progenitors from human peripheral blood, or cultured alone, in the presence of osteoclastogenic factors (MCSF; RANK-ligand) for 1 day. Samples were implanted ectopically in nude mice for up to 8 weeks. Only the co-cultured constructs generated frank bone tissue through endochondral ossification, with 3.5-fold higher mineralized volume and less remnants of cartilagineous tissue as compared to cell-free devitalized HCT.The addition of osteoclastic cells on devitalized HCT likely primed the onset of the remodeling process, which is a critical trigger of the endochondral ossification process. The activation/stimulation of off-the-shelf, devitalized engineered tissues using easily available autologous cells could represent a novel paradigm in regenerative medicine.

04.03
Engineering a bone organ through endochondral ossification

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In this study, we aimed at developing an upscaled endochondral bone organ model with a Developmental Engineering approach by using adult human Mesenchymal Stromal Cells (hMSC). Expanded hMSC were seeded onto 8 mm diameter, 2 mm thick collagen sponges (UltrafoamTM), cultured for 5 weeks in vitro under chondrogenic and hypertrophic conditions and then implanted subcutaneously in nude mice. Samples were analyzed by histology, IHC, biochemistry, flow cytometry, ISH for human Alu sequences and μCT.In vitro, samples showed a mineralized, pre-bone collar (rich in Col1 and BSP) and a hyperthrophic core (rich in ColX). In vivo, extensive remodeling with vessel ingrowth ($CD31^+$) and osteoclast activity ($TRAP^+$, $MMP9^+$) took place. Formation of perichondral and endochondral bone, and, after 12 weeks, abundant bone marrow were observed. Human cells were detected mainly in the bone in the core of the samples. Murine bone marrow cells in the newly generated bone were extracted and characterized as hematopoietic progenitors (LSK^+, clonogenic in methylcellulose), comparable to those extracted from mice femurs. This work demonstrates that hMSC can generate, through endochondral ossification, a 'bone organ' in an upscaled, ceramic-free scaffold model. The findings are relevant towards (i) the development of clinically-sized endochondral grafts for bone repair and (ii) the engineering of ectopic bone marrow niches to study and possibly regulate hematopoiesis in normal and pathologic condition.

04.04
Channelled architectures accelerate in vivo mineralisation of engineered hypertrophic cartilaginous constructs

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The formation of canals inside hypertrophic cartilage facilitates vascular invasion during endochondral ossification. Recapitulating these developmental processes during endochondral bone regeneration by modifying the architecture of tissue engineered hypertrophic cartilage may enable rapid vascularisation and mineralisation. We hypothesised that the introduction of channels into cartilaginous tissues engineered using mesenchymal stem cells (MSCs) would accelerate endochondral ossification in vivo. Bone marrow derived MSCs were encapsulated in non-channelled cylindrical hydrogels or in channelled hydrogels consisting of a longitudinal array of channels with diameters of 500 μm and centre-centre spacing of 1 mm. Constructs were maintained in chondrogenic media at 5% pO2 for 5 weeks and then switched to 20% pO2 for 1 week to facilitate transition to hypertrophy. Constructs were implanted subcutaneously in nude mice and retrieved 4 and 8 weeks

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post-implantation. Transfer of constructs to 20% pO2 significantly increased alkaline phosphatase activity. All constructs stained homogenously for alcian blue pre-implantation. Post-implantation non-channelled constructs stained positively for alizarin red around the periphery only whereas channelled constructs stained homogenously for alizarin red, indicating enhanced mineral deposition. Channels were highly cellularised post-implantation. To conclude, channelled architectures show promise for use in endochondral bone tissue engineering.

04.05
Amniotic fluid MSC behaviour in bone microenviroment: Driving host reparative response

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The repair of skeletal defects represents a substantial economic and biomedical burden. Endogenous cell homing is a modern technique that actively recruits host stem cells into a predefined anatomic location for in situ tissue regeneration. Here we used human amniotic fluid derived mesenchymal stem cells (AFSC) as chemoattractant cells to study the in vivo remodeling of the bone niche. While the in vitro osteogenic differentiation of AFSC was successfully obtained both in 2D (culture in monolayer) and 3D (culture in porous ceramic disks), the early in vivo recruitment of vessels and pro-inflammatory cells (CD14, CD31, VEGF-R2 positive cells) was likely responsible for the non traceability of AFSC (luciferase transfected) at the site of implantation and for the population of the ceramic scaffold with host's tissues of mesodermal origin such as fat, muscle, fibrous tissues. On the contrary, human bone marrow derived MSC remained in the scaffold and formed compact tissue. When AFSC were added to human osteoblasts (OB), the in vivo formed bone niche appeared remodeled in terms of vessel density and total perfusion area. Our findings demonstrate that the benefits of an in vivo therapy based on uncommitted stem cell sources, such as AFSC, may be due to the perfusion of a specific niche, with the consequent incoming of progenitors and pro-trophic factors, rather than to the mere differentiation of the stem population toward an osteo-phenotype.

04.06
MicroRNA-31 – a novel therapeutic target for bone regeneration and osteoporosis

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The decline of physiological functions during aging results from accumulation of damage in cells and tissues and from decline of the counteracting repair systems. Stem and progenitor cells are such repair systems and recently their functionality has been found to depend on the systemic environment, as factors contained in the serum of elderly individuals inhibit adult stem and progenitor functions. However, knowledge about the identity and functionality of these factors is scarce.In order to identify such factors, serum of young versus elderly healthy individuals was tested for the miRNAs and miR-31 was found higher in elderly donors. As possible source we identified senescent endothelial cells that secrete miR-31 also in vitro, packaged into exosomes. Consequently we aimed at functionally characterizing miR-31 in the context of aging and we found that exosomes of senescent cells as well as exosomes of elderly donors deliver miR-31 to adipose tissue derived mesenchymal stem cells (ASCs). Both exosomes and transfection with miR-31 alone inhibit osteogenic differentiation by downregulation of FZD3 mRNA as target in ASCs. Taken together, our data suggest that miR-31 is part of the endothelial senescence-associated secretory phenotype (eSASP), and might serve as a novel biomarker of cellular senescence and aging. In addition, it might represent a diagnostic and therapeutic target whenever osteogenesis is a limiting factor, especially in age-related diseases like osteoporosis.

04.P01
The effect of composite scaffolds on the hypertrophy of nasal chondrocytes

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Hypertrophic cartilage has significant potential for use as a graft material to repair large bone defects as it can withstand the low oxygen levels at the wound site, and undergo mineralisation after implantation through the natural process of endochondral ossification. Our aim was to prepare poly(co-lactic) acid (PLLA)/calcium phosphate scaffolds and investigate their potential for tissue engineering hypertrophic cartilage. Scaffolds were prepared by precipitating calcium phosphate within electrospun PLLA meshes using an alternative soaking protocol (1). The precipitated mineral was analysed by scanning electron microscopy (SEM) and X-ray diffraction (XRD). Scaffolds were then cultured for 42 days with nasal chondrocytes and analysed for hypertrophic features. The amount of precipitated mineral seen on and between the scaffold fibres varied depending on the number of cycles of alternative soaking performed and was shown to be a mixture of brushite and hydroxyapatite. Analysis of the tissue constructs showed that the new composite scaffolds enable cell attachment, proliferation and hypertrophic differentiation, with some evidence of vertical growth plate like cell alignment. Therefore, the composite scaffold could be a useful new material for the development of hypertrophic cartilage grafts for use in bone repair.1.Taguchi et al J Biomater. Sci. Polymer Edn 1999;1

04.P02
IL-1β modulates in vitro remodeling and in vivo bone formation by endochondral primed human bone marrow mesenchymal stromal cells

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The milieu of the fracture site contains many inflammatory cytokines. We aimed at studying the influence of IL-1β on late events involved in the endochondral bone formation by human bone marrow stromal cells (hBM-MSC). Expanded hBM-MSC were cultured in collagen sponges, using a previously established protocol (3 weeks with chondrogenic medium and 2 weeks with hypertrophic medium with or without 50 pg/ml IL-1β) and then implanted ectopically in nude mice for 5 and 12 weeks. Constructs were analyzed biochemically (calcium, glycosaminoglycane (GAG)), by RT-PCR (MMP-13), histologically (Safranin-O, Alizarin red) and immunohistochemically (cryptic fragment of aggrecan (Dipen) and with quantitative μCT (total bone volume). Samples exposed to IL-1β accumulated 38% more calcium resulting in a thicker calcified bone collar, lost 12% more GAG and expressed higher levels of MMP-13 mRNA and increased accumulation of DIPEN. After 5 weeks in vivo, IL-1β treated samples contained larger bone marrow and reduced cartilaginous areas and higher amounts of MMP-13 and DIPEN. After 12 weeks in vivo, IL-1β treated samples showed a thicker outer bone collar with increased, even though not statistically significant, total bone volume. IL-1β treatment during the in vitro

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endochondral priming of hBM-MSC resulted in and advanced degree of cartilage remodeling and subsequent more mature bone in vivo. We would like to acknowledge the European Union for financial support (OPHIS#FP7-NMP-2009-SMALL-3-246373).

04.P03
Melt electrospun calcium phosphate coated scaffolds accelerate ectopic bone formation in the context of a long term cultured cellular construct

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Calcium phosphates (CaP) obtained by biomineralisation in Simulated Boby Fluid have been used for decades to assess the mineralisation capability of biomaterials. Recently, they have been envisioned as potential agents to promote bone formation. In this study, we have fabricated and coated with calcium phosphate melt electrospun scaffolds whereby macropores permit adequate cell migration and nutrient transfer. We have systematically investigated the effect of coating and osteoinduction onto the response of ovine osteoblasts and we observed that the coating up-regulated alkaline phosphatase activity regardless of the in vitro culture conditions. Micro Computed Tomography revealed that only scaffolds cultured in an osteoinductive cocktail were capable of depositing mineralised matrix, and that CaP coated scaffolds were more efficient at promoting mineralisation. Theses scaffolds were subcutaneously implanted in athymic rats and this demonstrated that the osteoinduction was a pre-requisite for bone formation in this ectopic model. It showed that although the bone formation was not significantly different after 8 weeks, the CaP coated scaffolds were superior at inducing bone formation as evidenced by higher levels of mineralisation at earlier time points. This work demonstrated that CaP coating is not sufficient to induce bone formation; however the combination of osteoinduction and CaP coating resulted in earlier bone formation in an ectopic model.

04.P04
Pleiotrophin potentiates chondrogenic differentiation of human mesenchymal stromal cells

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Bone healing is a complicated process that passes through endochondral formation, involving mesenchymal stromal cells (MSC) as key cellular component and interactions with many growth factors, tightly regulated to give rise to a suitable bone matrix. Among these, Pleiotrophin (PTN) is overexpressed in the callus during bone healing but its role remains controversial. Our goal was to test the effect of PTN on osteoblastic and chondrogenic differentiation of human MSC in vitro. No significant effect of PTN was observed on osteoblastic differentiation characterized by RT-qPCR analysis of marker expression and calcification staining. In contrast PTN strongly increased the expression of cartilage related genes such as Sox9, collagen 2 and COMP during chondrogenic differentiation. The expression of aggrecan, biglycan, decorin and versican proteoglycans were up regulated by PTN and correlated with an increase in glycosaminoglycan content. Finally hypertrophy related genes expression such as MMP-13 and collagen 10, and hypertrophic shape of chondrogenic induced cells observed after histological staining, were increased by PTN treatment. These effects are associated with an induction of PTN receptors expression. All these data suggest a new role of PTN in bone development as an inducer of chondrogenic differentiation toward a hypertrophic state. This is of major interest since PTN could be used as a new regenerative growth factor to associate with scaffold in a bone lesion model.

04.P05
Vessel derived stem cells contribute to endochondral ossification of atherosclerotic plaque

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Pericytes, although traditionally considered supporting cells, have recently been proposed to have a more active role in the repair and pathogenesis of various vascular diseases. There is growing body of research work indicating that the vessel wall contains a number of progenitor cell niches that remain as yet incompletely defined. In this study, we hypothesized that a pericyte-like stem cell population, termed vessel derived stem cells or VSCs with chondrogenic and osteogenic potential exist in the vessel wall and in presence of the inflammatory cytokines seen in atherosclerotic environment, contribute, along with the circulating mesenchymal stem cells (MSCs), to the calcification of atherosclerotic plaques via endochondral ossification. VSCs from aortae of ApoE-/- mice and background C57BL/6 mice were isolated and characterised for cell surface markers by flow cytometry and immunocytochemistry. MSCs from bone marrow of these mice were also isolated and characterized. To assess the ability of VSCs and MSCs from normal and ApoE-/- mice to form bone, cells were seeded onto collagen glycosaminoglycan scaffolds and primed chondrogenically in vitro followed by subcutaneous implantation for 8 weeks. Chondrogenically primed constructs from both cell types (VSCs and MSCs) showed the ability to form bone by endochondral ossification in vivo in both ApoE-/- and C57BL/6 mice. Assessment of quantity and quality of bone formed is currently being performed.

04.P06
Chondrogenic and hypertrophic differentiation of human multipotent stromal cells in alginate hydrogel constructs

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A crucial step in endochondral bone tissue engineering is the induction of hypertrophic chondrogenic differentiation of multipotent stromal cells (MSCs) after chondrogenesis. A promising 3D culture model comprises suspension of single MSCs in alginate beads. To improve chondrogenesis and hypertrophic differentiation, pellet versus single cell encapsulation in beads was compared. We hypothesized that encapsulated pellets differentiate more readily due to cellular condensation. Cultures of three human donors were compared after 28 days of chondrogenic or hypertrophic stimulation. Further, MSC differentiation was compared in different formulations of alginate gel. Cell viability and chondrogenic or hypertrophic differentiation were assessed by (immuno)histochemistry and quantitative alkaline phosphatase and proteoglycan (PG) detection. Safranin-O staining revealed donor-dependent chondrogenesis of both groups. Quantitative analysis showed highest PG content in beads with pellets for 2 out of 3 donors. Hypertrophy, as evidenced by collagen type X deposition and mineralization, was observed in all donors

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and most extensively in beads containing pellets. No difference in viability or differentiation was observed for different alginate formulations.This study reveals that alginate encapsulation of pellets is a suitable model for hypertrophic differentiation of MSCs in vitro. This design could easily be translated into an in vivo model of endochondral bone tissue engineering.

04.P07
The influence of 3D scaffolds in endochondral bone formation

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Bone is second on the list of transplanted materials. However, the currently used natural and synthetic grafting materials show several drawbacks. Tissue engineering techniques in which stem cells are combined with a scaffold material that provides a 3d structure are of great interest. But one of the main challenges remains overcoming necrosis of the implanted cells, which is mainly caused by limited vascularisation. Therefore we use the endochondral approach, mimicking the natural pathway, by implanting cartilage which is replaced by bone. Cartilage has the ability to survive in a defect area without direct vascularisation, as it produces the growth factor VEGF. But for clinical application the scaffold material also plays a critical role since it provides anchorage, stability and strength. Our aim is to evaluate in vitro and in vivo several scaffolds as potential candidates. We seeded rat mesenchymal stem cells onto six materials; HA/TCP, PU(polyurethane), PU/HA, PCL/PLGA, PCL/PLGA/HA and Collagen I. The cells were differentiated into the chondrogenic lineage and we measured the GAG content, performed an histological evaluation and did a PCR for chondrogenic markers. On all scaffolds cartilage matrix was formed but the GAG content and the expression of (hyperthropic) cartilage markers was highest for the PCL/PLGA scaffold. Next we implanted all scaffolds subcutaneous in rats for a period of 8 weeks. Currently we are evaluation the amount of bone formed on all scaffolds.

04.P08
Silver nanoparticles stimulate proliferation and migration of chondroprogenitor cells

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Bone bridge is present in 1–10% of physeal fracture. The bony structure lead to deformity and even cease growth. Current therapy could not resume its growth potential thus there is a need for alternative solutions. One remedy is to promote chondrogenesis at the physeal defect. Silver nanoparticles (AgNPs) is a powerful medicine for skin wound for its ability to accelerate healing with excellent regeneration. The objective of this study is to scrutinise the effect of AgNPs on ATDC5 cells, a murine chondroprogenitor cell line, in terms of cytotoxicity, proliferation and migration. In vitro study showed that cytotoxicity of AgNPs is dose dependent. Concentrations below 1 μM did not show cytotoxicity in MTT assay. BrdU incorporation assay exhibited a significant increase in proliferation between 0.1–0.01 μM of AgNPs. This could be explained by the upregulation of mRNA expression of wnt-5a,-5b,-10 and 11 in real-time PCR. In addition, in vitro scratch assay showed elevated migration rate at 1 μM in 24 h. All assays were analysed using the Student's paired t test with p value lower than 0.05 considered as significant. The finding shows that AgNPs enhance the proliferation and migration of chondroprogenitor cells at appropriate concentrations with minimal toxicity. This discovery suggests that AgNPs may help in the repair of the growth plate in vivo by promoting infiltration of the chondroprogenitor cells into the injury site and improve chance of cartilaginous repair.

04.P09
Functionalization of HA/βTCP scaffolds with human platelet lysate improves bone tissue engineering

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Combination of bone substitute materials with human mesenchymal stem cells (hMSC) represents a promising clinical alternative to autologous bone graft for the treatment of bone defects. This approach has shown its efficiency, however, most of the studies lead to disappointing results, particularly regarding the degree of cell colonization and vascularization after transplantation.The human platelet lysate (hPL) is composed of many growth factors known for their osteoinductive, growth and chemoattraction effects on hMSC and endothelial progenitor cells (EPC). Our approach consisted in use of hPL coated on HA/βTCP scaffolds to functionalize biomaterials in order to induce the rapid development of a vascular network and improve the process of bone repair. This study demonstrated that coating of HA/βTCP scaffolds with hPL increases chemoattraction, adhesion, colonization and distribution of hMSC on the biomaterial. In contrast to soluble hPL, we observed no induction of osteoblastic genes. However, the coating with hPL induces the secretion of cytokines by hMSC, including vascular endothelial growth factor (VEGF), which will allow a specific chemoattraction for endothelial cells and improve in vivo bone formation. The hPL seems a promising candidate for potentiating biomaterials properties, inducing a better migration and colonization of stem cells on scaffold, whether the implanted cells and/or autologous cells recruited in situ, in order to increase in vivo bone formation.

04.P10
Platelet rich plasma enhances osteoconductive properties of a hydroxyapatite-β-tricalcium phosphate scaffold (Skelite) in critical size rabbit calvarial defects

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The regeneration of large bone defects remains a consistent challenge. Although platelet rich plasma PRP has been used for many years its use in bone repair remains highly controversial. In the present work, we evaluated in a rabbit calvarial defect, the effect of lyophilized PRP on bone regeneration when associated with a silicon stabilized hydroxyapatite tricalcium phosphate scaffold (Skelite^{TM}). Critical defects were created in the calvaria of 24 rabbits. The periosteum was removed and the defects were either left empty or with allogeneic PRP gel; Skelite particles; Skelite and PRP gel. Four animals were euthanized after 4 weeks, 10 animals after 8 and 10 after 16 weeks. Specimens were processed for X-ray microtomography (μCT) and for resin

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embedded histology. μCT analysis revealed significant osteoid-like matrix and new bone deposition in PRP+Skelite group at both 8 and 16 weeks in respect to Skelite alone. Histologically, PRP+Skelite defects were well-vascularized, highly cellular with more abundant osteoid deposition and more regular collagen fibers. Moreover, in vitro migration assays confirmed the chemotactic effect of PRP to endothelial and osteoprogenitor cells. We conclude that the addition of PRP influenced the local tissue microenvironment by providing key cryptic factors for regeneration, thereby enhancing progenitor cell recruitment, collagen and bone matrix deposition, and by creating a bridging interface between the scaffold and bone.

04.P13
Cell therapy for bone repair: an in vivo study in a Lewis rat femoral defect model

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Bone loss may be a consequence of tumours, traumas or various diseases and is still a great challenge to reconstructive surgery. MSC are multipotent cells that may offer a valuable therapeutic approach to bone loss repair. Here, we investigated MSC repair efficiency on experimental bone defects in rats, when associated with a new hydrogel scaffold (pullulan and dextran) successfully developed for cardiac tissue engineering. We compared it to known calcium-phosphate ceramics (Calciresorb 35®, HA/TCP = 65/35). Biomaterials were seeded with rat MSC and surgically implanted for 7, 30 or 90 days in femoral defects (n = 192). Quantum dot-labelled MSC were evenly distributed in the defect independently of the treatment up to 30 days. Using μ-CT imaging, mineralization labelling and histochemical analysis, we showed that MSC seeded-hydrogels induced significant bone regeneration in comparison with MSC-treated rats or empty defect (Bone volume ×1.43 and ×2.20 respectively after 30 days). However, a different bone mineralization pattern was observed depending on the considered scaffold. Using hydrogels, new bone deposition was mainly detected at the defect periphery while it was evidenced around and inside ceramics. These results indicate that MSC associated with these scaffolds positively enhance bone neosynthesis with different repair patterns. Cell therapy associated with this new hydrogel could be a promising approach towards bone loss treatments.

04.P14
The periosteum osteogenic potential for cranial bone defects in a model in vivo

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Introduction: Craniofacial defect reconstruction represents a great surgical challenge. The use of periosteum combined with bioresorbable materials is a promising approach for tissue engineering. The aim of this study was to determine the osteogenic potential of the osteogenic unit (acellular human dermis and de-mineralized bone) in nude mice with cranial bone defects.
Methods: We used samples from pediatric patients with primary cleft palate. The biopsy was digested and cell cultures were maintained in monolayer during 2 weeks, until confluence was reached. Cultures were harvested with trypsin (0.25%) and cells seeded onto the osteogenic unit. We placed an osteogenic unit in a critical-size defect of skulls of athymic mice. At 3 months mice were sacrificed, H&E and von Kossa histological analyses were performed, and immunohistochemistry for RUNX-2, Col I, alkaline phosphatase and osteopontin.
Results: We found osteoblasts, and weak positive staining with the von Kossa method. RUNX-2, Col I, and alkaline phosphatase were positive and osteopontin weakly stained.
Discussion: These results suggest that the osteogenic unit contains osteoblasts and the formed extracellular matrix is similar to immature bone.

04.P15
Nano-Hydroxyapatite/Cellulose composite scaffold for bone tissue engineering

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Bone engineering via synthetic biomaterials implies the interplay of various agents including a synthetic scaffold, bone progenitor cells and growth factors. To perform successfully the ideal scaffold should be a 3D interconnected porous structure promoting cell adhesion, proliferation and vascularisation and allowing bioactive substances to be supplied to incorporated or ingrown cells. The present work explores the preparation and characterization of cellulose/hydroxyapatite composite structures for bone tissue regeneration in maxillofacial region and their osteoblastic cytocompatibility. Cellulose/hydroxyapatite porous scaffolds were produced by freeze drying suspensions of cellulose and chemically synthesized hydroxyapatite nanoparticles. Experimental parameters including cellulose concentration, pre-lyophilisation conditions, and hydroxyapatite nanoparticle morphology were used to manipulate scaffold porosity, micron- and nano-scale topography. The biological characterization of cellulose/hydroxyapatite composites was conducted with MG-63 human osteoblastic-cells. The obtained freeze-dried bodies are highly porous, with pore size ranging from micron to nanometric scale. Furthermore, assayed composites revealed improved biological profile, by enhancing the adhesion, proliferation and functional activity of seeded osteoblastic-like cells. Attained results substantiate the suitable application of cellulose/hydroxyapatite scaffolds in bone tissue engineering applications.

04.P16
Healing rat calvarial critical-sized bone defects with PCL–TCP/fibrin scaffolds loaded with bone morphogenetic protein2-binding heparan sulfate

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The biological activity of bone morphogenetic protein-2 (BMP-2) is enhanced by glycosaminoglycans. We developed a simple purification method to isolate an HS variant (HS3) that binds BMP-2 and sustains its activity. In order to determine its efficacy in vivo, bilateral, critical-sized bone defects in the calvaria of rats were created and treated with HS3 delivered on a polycaprolactone-tricalcium phosphate scaffold (PCL-TCP). Experimental groups (n = 6/group) consisted of; (i) Empty defect, (ii) Scaffold alone, (iii) BMP-2 (10 μg), (iv) HS3 (5 μg), or (v) HS3 (30 μg). Our intent was to evaluate the efficacy of HS3 in a clinically-relevant bone defect model. We hypothesized that providing exogenous HS3 into the wound milieu would further activate endogenous BMP-2, thereby prolonging its bone inducing activity. At week 12, X-ray and μ-CT imaging showed the presence of bone infiltrating the defects, albeit to varying extents. Notably, treatment with BMP-2 and HS3 (30 μg) induced similar amounts of new bone (BV: 8 ± 2 and 7 ± 1 mm^3 respectively), that was significantly higher than scaffolds alone (4 ± 1 mm^3). All other groups failed to induce appreciable bone formation ($p > 0.05$). Histological evaluation by Hematoxylin/eosin and von Kossa/McNeals stained sections of the BMP-2 and HS3 (30 μg)-treated defects verified the presence of bone filling the defects. In conclusion, HS3 proved to be just as effective as BMP-2 in the treatment of rat calvarial critical-sized bone defects.

04.P17
In vitro and in vivo assessment of pro-osteogenic potential of three ceramics for bone tissue engineering

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For bone tissue engineering, CaP-ceramics and bioactive glass are suitable implant materials due to their osteoconductive properties. However, to the scaffold material, osteocompetent cells such as mesenchymal stromal cells(MSC) are usually combined to enhance bone tissue formation. The aim of this study was to assess and to compare both in vitro and in vivo the pro-osteogenic potential of three popular osteoconductive ceramic scaffolds(HAP/betaTCP, natural Porites coral and 45S5 bioactive glass) when combined with MSC. Scaffolds were seeded with human MSC and embedded within fibrin gel. In vitro, the hMSC proliferation and the expression of bone matrix proteins were higher into 3D bioactive glass-derived cell constructs compared to the coral- or HAP/betaTCP-derived cell constructs. These three kinds of constructs were then subcutaneously implanted in Nude mice for 2 months to assess their in vivo bone forming potential. Histomorphometry analysis provided evidence of equal bone formation into HAP/betaTCP- and coral-derived cell constructs, whereas no bone was observed onto bioactive glass-derived cell constructs. These results showed that, when combined with hMSC, HAP/betaTCP and coral ceramics are both suitable scaffolds for bone tissue engineering applications. In contrast, despite its in vitro pro-osteogenic potential, bioactive glass-ceramic needs further physico-chemical improvements before considering its use with osteocompetent cells for tissue engineering uses.

04.P18
Fabrication of polymer-ceramic composite bone scaffolds with controlled multi-scale porosity

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Introduction: Traditional scaffold fabrication methods have largely been unsuccessful in controlling the internal architecture to a high degree of accuracy or homogeneity. Creation of such scaffolds with controlled macro-micro pores is critical for mass transfer and mechanobiology investigations. Here a reproducible manufacturing technique based on Indirect Solid Free Form Fabrication (ISFF) for producing ceramic-polymer bone scaffolds with controlled macro-micro pore size is presented.
Methods: Cylinders with 8 mm diameter and 8 mm height with interconnected 600 μm global and 80–200 μm controlled local micro-pores are designed in Solidworks® and fabricated with two RP systems (SLA Viper and Projet HD3000). HA with appropriate binders is cast into designed mold and burned out to remove the polymer based mold. The HA mold was sintered at elevated temperatures to get rid of organic binders and then immersed into molten biodegradable composites of PGA, PCL, and PEG (Sigma-Aldrich).
Results: SEM images and Micro-CT analyses (Skyscan 1172) show that composite scaffolds were successfully fabricated with interconnected desired pores. Stiffness and yield stress of resulting scaffolds are measured according to ASTM standards confirming their predicted values.
Conclusions: Scaffolds with pore sizes of 80–600 μm were attainable via ISFF method motivating its use for scaffolds with optimal permeability and connectivity providing tissue ingrowth and mechanical strength.

04.P19
The potential of highly orientated scaffold pore architecture for bone tissue regeneration

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The utilization of biomaterials is one of the most promising strategies for the regeneration of large tissue defects. Their success, however, is depending on the ability to guide cell behavior towards functional tissue formation. In this study we investigate the potential of a macroporous collagen-I guiding structure (porosity 98.5%, pore size 80 μm) to direct cell migration and align newly formed extracellular matrix (ECM) for improved bone healing. Migration of human and rat mesenchymal stromal cells (MSCs) into the highly oriented scaffold and orientation of ECM deposited by human and rat primary fibroblasts were analyzed in vitro. For in vivo evaluation, the scaffold guiding structure was implanted in a 5 mm defect of a rat osteotomy model.MSC migration along the scaffold pores at day 3 was 2.6(±0.4) and 3.1(±1.0) fold higher compared to migration perpendicular to pore orientation for human and rat MSCs respectively. Multiphoton microscopy revealed a long range alignment of ECM fibers within the scaffold pores. In vivo studies proved soft and mineralized tissue structure following the scaffold pore orientation. Advanced mineralized tissue formation was found in animals with scaffold pore orientation along the bone axis compared to perpendicular orientation. The study highlights the potential of scaffold based guiding structures to direct cell migration and align ECM formation. This opens new possibilities for the regeneration of oriented tissue structures like bone.

04.P20
Enhancing the cellular response to PEEK substrates for bone contacting applications

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The ability to control cell-material interactions effectively is the key to a broad range of applications in tissue engineering and regenerative medicine. Polyetheretherketone (PEEK) has been widely used as a biomaterial due to its mechanical properties, biocompatibility and chemical stability. However, the surface properties of PEEK have limited its

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use in bone-contacting applications. Here we present the development of a tailored polymer coating providing significantly improved bone cell responses that can be applied to both solid and porous PEEK substrates. Coatings were deposited on solid and porous (73% porosity, 600 μm pore diameter) PEEK samples by RAFT-based graft polymerisation of homo- and copolymers representing different ratios of selected monomers. Coatings were characterised using XPS while cell culture experiments were carried out using human Saos-2 bone-derived cells. Cell attachment and proliferation were evaluated over 7 days while mineralisation experiments were carried out for 21 days. Screening and optimisation experiments yielded a copolymer containing 33 mol% phosphate methacrylate and 67% butyl methacrylate which had the largest effect on cell attachment and growth while also resulting in an approx. 4-fold increase in mineralisation. Importantly, an even distribution of the coating throughout the pores of porous PEEK samples was demonstrated. This tailored coating is currently undergoing in vivo testing in an ovine animal model.

04.P21
Production and characterization of chitosan/calcium phosphate composite microspheres for bone tissue engineering therapeutics

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In this work chitosan/calcium phosphates (CS/CP) composite microspheres were produced. The calcium phosphates used were hydroxyapatite (HAp) and b-tricalcium phosphate (β-TCP). Acetylsalicylic acid, a model drug, was encapsulated in the microspheres in order to determine the therapeutic efficacy of the microspheres. CPs were produced by sol-gel method and microspheres were produced by microfluidics. The microspheres were characterized in terms of morphology by scanning electronic microscopy (SEM) and optical microscopy. Encapsulation efficiency and drug delivery profile were studied. CS microspheres with an average diameter of 337 μm and a smooth surface were obtained. The composite microspheres with different amounts of HAp and β-TCP presented the same morphology but a rough surface due to the presence of the ceramics. The microspheres released about 20% of the drug on the first day and about 50% of the drug in 16 days. These results seem to reveal that a surface erosion mechanism is responsible for the drug release. Results will be interpreted on the basis of data available in literature.

04.P22
Strontium and calcium ions as liquid supplementation in cell culture media do not exert the same inhibitory effect on osteoclastic resorption capacity as the same ions released from strontium containing calcium-phosphate/carbonate powder

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The literature suggests that strontium (Sr) inhibits the osteoclastic resorption of bone. Previous studies have shown that Sr ions released from a Sr containing calcium-phosphate/carbonate (Ca/P/C) powder indeed inhibit the osteoclastic resorption capacity. This study investigated if this inhibitory effect can be mimicked upon supplementation of cell culture media with Ca/SrCl2 in the same divalent ionic strength that have previously been found in conditioned media. Precipitated Sr containing Ca/P/C material was dried, milled, sterilized and incubated with cell culture medium on a shaker. After the conditioning process the remaining powder was eliminated by sterile filtration. After elemental analysis (ICP-OES) of this 'positive control' an equal amount of divalent ions was added as CaCl2 and SrCl2 to cell culture medium. These media were used to incubate a rabbit bone marrow derived cell suspension containing mature osteoclasts on bovine bone slices. After 48h the resorption was determined using a pit assay that quantified the resorbed area as percent of the total bone slice area. Supplemented Sr and Ca ions as well as both ion species alone inhibited significantly, but the conditioned media inhibited significantly stronger the resorption capacity of osteoclasts. Our results suggest additional factors other than the $Sr/Ca2^{+}$ content influence OC activity; Cl- anions may interfere with the inhibitory effect of Sr and Ca ions.

04.P23
Coralline scaffold combined with autologous stem cells for de novo reconstruction of bone defect in sheep

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Introduction: Tissue construct engineered from osteoconductive scaffolds and mesenchymal stem cells (MSC) have been extensively studied for repairing large bone defects, but bone union did not consistently occur. Several authors have emphasized that balanced scaffold resorption and bone apposition must be obtained for bone healing. To address this question, tissue constructs made of Acropora coral, a natural fully resorbable scaffold, were implanted with or without MSC in a sheep.
Materials and Methods: Metatarsal segmental critical size defect was created and filled with a poly-methyl-methacrylate spacer. The spacer was removed 6 weeks later and the defect was filled either with Acropora scaffold (Group1, $n = 4$) or with Acropora scaffold loaded with autologous MSC isolated from bone marrow (Group2, $n = 5$) (105 MSC seeded per cube of Acropora -3 × 3 × 3 mm- on week before).
Results: Six months after implantation, animals were sacrified and each defect was assessed by micro-computed tomography and histology. Acropora resorption was almost complete in Group1 and in Group2 (99.2 ± 0.8% vs. 95.8 ± 4.7%). The amounts of newly formed bone in Group2 were significantly higher than in Group1 (1357 ± 471 vs. mm3677 ± 227) allowing a full bone regeneration in one animal.
Conclusion: This study established Acropora/MSC could be successfully used to obtain bone regeneration in sheep since a bone repair associated with an almost fully resorption of the scaffold was observed.

04.P24
Biological applications of alternating current electric fields via various electrodes for bone tissue regeneration

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Various electric fields could be integrated with tissue regenerations in aspect of cellular homeostasis and healing. Electric stimulation has been known to initiate signaling pathways and provides a technique to enhance osteogenic differentiation. The application of electric fields can be used as substantial therapeutic tools, while the mechanisms between scaffolds and cells under the electric simulation are still

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researched. The purpose of this study is to track the differentiation profile of osteoblast-like cells (MG-63) undergoing osteogenic differentiation during exposure to electric fields. To achieve this goal, we designed several electrodes, parallel and interdigitated electrodes to apply the fields to the cells. From our biological results, the electrical stimulated groups promote osteosarcoma differentiation by inducing alkaline phosphatase (ALP) activity, cytoplasmic calcium (bone mineralization), and total protein and collagen synthesis in osteoblast-like cells (MG-63), respectively. From our study, the electrical stimulation of the scaffold is a useful technique to improve osteogenic differentiation of the osteoblast-like cells (MG-63).

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04.P25
Electrospun poly(lactide-co-glycolide): hydoxyapatite composite membranes intended for bone tissue regeneration

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Membranes are used in dental implant and periodontal surgery to exclude soft tissues from the surgical site where bone tissue healing is required. However, commercially available synthetic membranes contribute little towards bone tissue regeneration beyond this barrier function. Electrospinning represents a potential method to fabricate resorbable polymer membranes or meshes with added hydroxyapatite (HA), and the aim of this research was therefore to evaluate the suitability of electrospinning for the preparation of these composites. HA is an osteoconductive and highly biocompatible material, and was obtained from commercial suppliers and synthesised via wet chemical precipitation. The HA was characterised using routine methodology including x-ray diffraction (XRD). For electrospinning, poly(lactide-co-glycolide) (PLGA) was dissolved in dichloromethane at 20 weight % and spun between 15–18 kV. HA was incorporated into the membrane by adding it to the polymer-solvent dope between 5 and 20 weight %. Addition of HA to the polymer-solvent dope was shown to be an effective method for incorporating ceramic into the fibres.

04.P26
Nanocrystalline spherical hydroxyapatite granules for bone repair - in vitro evaluation with osteoblasts and osteoclasts

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The majority of hydroxyapatite (HA) bioceramics for bone repair is obtained by sintering at temperatures above 1100 °C leading to dense materials with low porosity and poor resorbability due to the loss of nanocrystallinity. A nanocrystalline microstructure with high porosity and surface area can be achieved by ionotropic gelation of an alginate/HA slurry followed by removal of the polymer at 650 °C (1). We applied this method for the generation of spherical ceramic granules manufactured by droplet extrusion of alginate sol containing nanocrystalline HA powder in the presence of calcium ions and subsequent thermal elimination of alginate at 650 °C. In some cases the resulting microspheres were coated with an additional SiO2 phase. Non-sintered granular HA materials were seeded with osteoblast-like cells and cultivated for up to 4 weeks. Cells attached, proliferated and differentiated on the nano-crystalline HA granules. Proliferation rates and specific ALP activity was comparable to those achieved on the already established granular bioceramic Cerasorb M® (Ossacur, Germany). In order to analyse the potential for active resorption osteoclasts derived from human monocytes were cultivated on the surface of the spherical granules. Scanning electron microscopy demonstrated the formation of osteoclast-like cells on the surface of nanocrystalline HA granules.

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04.P27
Strontium-modified chitosan/montmorillonite composite scaffolds for bone tissue engineering

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Addition of montmorillonite (MMT) to chitosan (C) scaffolds (C/MMT) has generated interest to improve mechanical properties for bone tissue engineering applications.1 Due to the intrinsic ability of MMT to incorporate cations, we developed scaffolds combining C and strontium-modified MMT (C/Sr-MMT) to further utilise the osteoconductive properties of strontium. Scaffolds were fabricated via lyophilisation and characterised using XRD, SEM and ICP-OES. Primary human osteoblasts (hOBs) were seeded upon C/Sr-MMT and C/MMT and cultured for up to 28 days. Cell attachment, proliferation, metabolic activity and morphology were evaluated using LIVE/DEAD® staining, DNA content, MTT assay and SEM, respectively. The results showed that C was successfully incorporated between the MMT layers, resulting in highly porous composite scaffolds.When incubated in cell culture medium, $Sr2^{+}$ was released from C/Sr-MMT, along with higher levels of $Ca2^{+}$ and $Mg2^{+}$ than C/MMT. hOBs attached, spread and infiltrated the scaffolds over the culture period. C/Sr-MMT displayed higher cell proliferation than C/MMT, an effect which may be due to the ion release. Taken together our results indicate that C/Sr-MMT scaffolds are a promising biomaterial for bone tissue engineering.Acknowledgement: Financial support for these studies has been provided by TUBITAK(Code 2219-Research Project)

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04.P28
Evaluation of polymer coated hydroxyapatite ceramic scaffolds

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The main limitation for use of the porous calcium phosphate ceramic scaffolds, in the cases where high load resistance is necessary, lies in the poor mechanical resistance associated with their porosity. The balance between porous structure and initial mechanical strength is significant to provide the necessary parameters of porous bioceramic scaffold for bone ingrowth under physiological stress in vivo. A possible approach to achieve better mechanical performance of the implant would be to develop a polymer-ceramic composite material. The addition of a polymer phase to a ceramic scaffold has been shown to enhance toughness and strength. In current research incorporation of a biodegradable polymer into the ceramic scaffold to increase the

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mechanical properties was studied. Poly L-lactide (PLLA) and Polycaprolactone (PCL) was chosen as biocompatible polymer materials. Results showed that the porous polymer-ceramic composites shows significantly higher mechanical properties compare to pure porous ceramic scaffold. The in vivo investigations ceramic scaffolds coated with polymers and non coated ceramic scaffolds were implanted subperiostally in angular region of lower jaw in nine rabbits. The Biotin and streptavidin immunohistochemical method was used to detect the expression of: defensin beta 2, tumor necrosis factor alpha, bone morphogenic protein BMP 2.

04.P29
Investigating the factors directing the process of in vitro mineralisation

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Bone cells, including bone derived stem cells and osteoblasts, interact with each other as well as extracellular matrix (ECM) through biochemical and mechanical cues, maintaining their specificity and homeostasis of the tissue. From the bone tissue engineering perspective, bone marrow stromal cells (MSC) pose as a great tool as they can differentiate within the injured areas. Their role in bone is much wider and exploring it in detail would contribute much to the cell based bone regenerative medicine. In this study, we focused on cellular communication between MSC and osteoblast cells (MG63) and its effect on the in vitro mineralisation. For this, a novel culture system based on type I collagen-hydroxyapatite (CHAP) gel was utilised. This gel was stable in long-term culture and both cell types remained viable during that time. For co-culture experiments, 50 000 cells of one cell type were plated in monolayer on 13 mm diameter Thermanox discs and overlaid with CHAP gel containing 250 000 cells of the other type or the same as control. Results showed that maintaining cells in separated 3D co-culture, or MSC in co-culture with MG63 embedded in gel, was not sufficient to induce mineralisation of these cell types. Only when MG63 cells were cultured on discs with MSC cells in the gel was mineralisation induced. These data suggest that paracrine cell-cell communication, together with surface elasticity, is required for ECM mineralisation in the bone cells.

04.P30
The effect of control-released basic fibroblast growth factor incorporated in β-tricalcium phosphate for cranial regeneration in a murine model

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Introduction: In cranioplasty, we usually use the unabsorbable material or autologous bone. But there is a risk of infection or donor site mobility. To overcome this, beta-tricalciumphosphate(beta-TCP) has been developed, but downside of this is the time for osteoinduction. We have previously developed basic fibroblast growth factors (bFGF) impregnated gelatin, which sustains growth factors and gradually releasing it from gelatin. The purpose of this study is to evaluate the efficacy of our new material for bone regeneration in a murine cranial defect model.
Materials and Methods: Bilateral cranial defects of 4 mm in diameter were made in 10-weeks-old male Wistar rats (n = 24). Each flap received the following bone graft; Group1: 20 μl saline as control, Group2: beta-TCP disk in 50 μg bFGF, Group3: beta-TCP disk in 50 μg bFGF-incorporated gelatin hydrogel. Bone regeneration and beta-TCP resorption were assessed by Computed Tomography (CT) and histological analysis at 1, 2 and 4 weeks postoperatively.
Results: In CT scan, there was an increase in radiolucency of beta-TCP in group3. In histological analysis, at 2 weeks, there were many capillary formation within the pore in group3. At 4 weeks, average rate of bone formation was higher in group 3 compared to group1 and 2.
Conclusions: Our new material can accelerate bone regeneration compared to plain beta-TCP in murine cranial defect model and we believe that it is clinically applicable as an alternative material for bone graft.

04.P31
Bone regeneration using nano/microfibrous composite scaffolds

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Electrospinning is a widely used simple technique to fabricate micro- to nanometer-sized fibrous scaffolds from various polymer solutions and melts. Generally, cell is difficult to infiltrate in the nanofibrous scaffold because it has a very small pore size in spite of its high porosity. So, we designed nano/microfibrous composite scaffold to improve cell infiltration ability. Poly(ϵ-caprolactone)(PCL), FDA-approached polymer, was used main component in microfiber. Silk fibroin (SF), a biocompatible natural polymer, was used base material for nanofiber. In this study, we were fabricated SF/PCL nano/microfibrous scaffolds with various ratios to improve cell infiltration and cell viability than scaffold consists of nanofibers or microfibers. And then SF/PCL nano/microfibrous composite scaffolds were analyzed with various conditions. We found that pore size of the composite scaffolds was decreased with increasing of SF content by the SEM photographs. It means that we can easily control pore properties of the composite scaffolds. And in vitro and in vivo cytocompatibility studies of SF/PCL nano/microfibrous scaffolds showed that cell viability of composite scaffolds significantly was influenced by content of SF nanofiber in composite scaffolds. As a result of quantitative real-time PCR analysis for marker sush as ALP, OC and BSP showed a significantly increase in hMSCs culture on composite scaffolds. Therefore, SF/PCL composite scaffolds have potential for bone regeneration.

04.P32
In vivo response of scaffolds of a highly bioactive glass-ceramic (Biosilicate®)

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Biosilicate® is a novel fully-crystallized bioactive glass-ceramic of the quaternary P2O5-Na2O-CaO-SiO2 system. The stimulatory effects of this material on bone cells and on fracture consolidation was demonstrated in in vitro and in vivo studies. A previous in vivo study showed a positive response of particulated Biosilicate® in the amount of newly formed bone and biomechanical properties in tibial defects in rats. However, the effects of a three-dimensional Biosilicate® scaffold have not been studied yet. Thus, this study aimed to investigate the in vivo bone response of the implantation of Biosilicate® scaffolds using a tibial bone defect model in rats. Two groups were created (n = 30 each): control group and Biosilicate® treated group. Animals were sacrificed 30 and 45 days post-injury. Histology, immunohistochemistry and biomechanical analysis were used. Histological analysis revealed that the Biosilicate® scaffold group showed a higher amount of newly formed bone at 30 and 45 days post-surgery compared to the control group. A positive imunnoexpression of COX-2 and RUNX-2 were observed in this group, at both periods evaluated. No significantly difference was found in the biomechanical analysis.Our findings indicate that the Biosilicate® scaffolds were able to increase the amount of newly formed bone and to stimulate the immunoexpression of factors related to bone formation. However, the biomechanical properties of tibial bones were not improved.

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04.P33
Fabrication gelatinnano bio-glass bone scaffolds: in vitro and in vivo studies

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Bioactive glass has played in the bone regeneration field, due to its bioactivity, osteoconductivity, osteoinductivity. Porous Bioglass/Gelatin scaffold might be as a suitable candidate during bone formation. Nanopowders of bio-glass (BG) were synthesized and Bioglass/Gelatin nano Bio-glass scaffold was made through Sol Gel. Biocompatibility of BG scaffolds examined by cell culture. Fabricated scaffolds were implanted to rabbit's ulna. A 10 mm of the ulna was exchanged with BG scaffold into the defect site.The increase in cell aggregation on the scaffolds indicated the ability of the scaffolds to support cell growth. Scaffold implantation on ulna indicated a good healing of the bone and demonstrated that the scaffolds could be helpful for new bone formation. Based on the results, Nano Bio-glass scaffold made a significant contribution to growth and healing of the bone, as it is hoped to use it as an efficient alternative for the bonein the future.

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05. Intradiscal Technologies and Spine Surgery (in coop. GRIBOI)

05.01
Keynote: MSC for disc regeneration: when and which way?

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There is evidence that implantation of bone marrow derived mesenchymal stem cells (MSCs) into damaged discs may regenerate the tissue. MSCs have increasingly been recognized as a promising source of stem cells for tissue repair and regeneration therapies. Indeed, recent studies have shown that human MSCs have the capability to survive within the disc. Injection of human MSCs into injured porcine spinal discs, rat disc degeneration models, and bovine caudal discs in vitro demonstrated MSC survival and differentiation towards a disc-like phenotype. It is, however, not yet clear whether MSCs could also release biological factors, which will be able to stimulate the resident disc cells or activate the potential progenitor cells present within the disc. Anyhow, the present delivery approach of MSCs within the IVD has to be through injection, via the annular or end-plate route. In both cases, it would require to injure intact tissues, which would subsequently lead to further degeneration, as recently shown by (Carragee et al., Spine 2009). We have now shown that MSCs have the ability to migrate (homing) towards sites of injury and aid wound healing and tissue repair, using our whole organ culture system. This alternative finding is opening new potential strategies for the systemic delivery of MSCs within the IVD without damaging neither the annulus fibrosus nor the end-plate tissues.

05.02
Design of self-assembling peptide/ glycosaminoglycan hydrogels for spinal therapies

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This project focuses for the first time on using new peptide gels in the treatment of degenerative disc disease by providing an injectable nucleus replacement that can mimic the mechanical function of the natural tissue and restore the swelling pressure of the disc. Here the behaviour of a range of designed peptide blocks with systematic variations in their structure was studied and the design criteria for a suitable peptide hydrogel were established. The peptides were analysed using a series of complementary analytical techniques (NMR, FTIR, CDUV & TEM) to determine their behaviour at the molecular and nanoscale levels. Mechanical tests were also carried out on the peptide gels to establish their behaviour both inside and outside the disc. The results have shown that we can control the mechanical properties of the gels by allowing up to a 10 000-fold variation in the stiffness. The peptide materials were further optimised by mixing with glycosaminoglycans that are naturally found within the disc. A range of systematically varying peptide solutions were injected in discs in vitro and their behaviour evaluated under compressive loading to discover which peptide formulations restore the biomechanical function of the disc most effectively. These results demonstrate that the peptides have very real potential for the treatment of the degenerated disc and could find wider uses in other fields of regenerative medicine such as substrates for cell growth or cartilage engineering.

05.03
Inter-vertebral disc tissue engineering through design of injectable scaffolds and physiologically informed bioreactors

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Injectable scaffold loaded with human mesenchymal stem cells (hMSC) were investigated to demonstrate the feasibility of applying tissue engineering technology for the treatment of inter-vertebral disc degeneration (IVD). The biomimetic and biocompatible polyamidoamine hydrogels were explored to identify a suitable formulation of polymer composites with mechanical property representative NP tissue that can be used as an injectable scaffold for cell delivery to treat IVD degeneration. In order to understand the behaviour of hMSC in a physiologically relevant environment, hMSC loaded constructs were cultured in an environment with controlled oxygen concentration and mechanical stress. A platform evaluating the mechanical compliance of the cell-loaded constructs were also used to predicate the performance of the injectable scaffold in vivo. The bioreactor were designed with IVD products in mind, capable of applying varying fully three dimensional stress systems to deliver dynamic mechanical stimulation conditions. Such a platform with improved design could also be applied as a testing platform to predicate the behaviour of or even as a manufacturing facility for tissue engineered disc production. Engineering approaches guided and verified by biological requirements will improve the design, construction and implementation of such complex system emulating physiological conditions and facilitate the translation of technology.

05.04
Optimising microgel niches to influence adipose derived stem cells differentiation

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Cell therapy of the intervertebral disc (IVD) is limited by the lack of appropriate cell sources and the lack of knowledge on the transdifferentiation of progenitor cells to a nucleus pulposus (NP) phenotype. In this study, it is hypothesized that spherical structures composed of type II collagen (coll II) - hyaluronan (HA) will mimic the NP microenvironment and thus promote transdifferentiation of adipose derived stem cells (ADSCs) towards a NP cells-like phenotype. To achieve this aim, an optimal microenvironment was designed by varying coll II/HA concentration, cell density and amount of crosslinker. Microgels were fabricated by mixing coll II, high molecular weight HA, a PEG-based crosslinker and ADSCs. The resulting hydrogel was then self-assembled on a hydrophobic surface to generate a spherical shape and incubated for 1 h at 37 °C. The spherical hydrogels were maintained in culture for 14 days before assessing cell viability, cell morphology and gene expression. Over 80% of cell viability was maintained after 14 days of culture for each condition. Coll II concentration showed to have a significant impact on cell morphology and gene expression. When embedded in high coll II concentration ADSC were rounded in shape and had a higher coll II and aggrecan expression compared to cells embedded in lower concentrations of coll II. The resulting cellular phenotype was

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seen closer to that seen in nucleus pulposus.DISC REGENERATION project (NMP3-LA-2008-213904).

05.05
Covalent incorporation of bone morphogenetic protein-2 into fibrin-based hydrogels for intervertebral disc regeneration

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Degenerative disc disease is characterized by the breakdown of proteoglycans and matrix proteins, especially in the inner part of the intervertebral disc (IVD), the nucleus pulposus (NP), preventing the NP from performing its stress transfer functions. Several growth factors, including bone morphogenetic protein-2 (BMP-2), have been shown to increase proteoglycan and collagen content in disc cells. Studies indicated that a proper extracellular matrix environment and growth factor administration can induce reversal of disc degeneration. Since growth factors have extremely short in vivo circulation times, we have explored enzymatic, covalent incorporation of BMP-2 into fibrin, which has been shown to support NP cells. The tethered BMP-2 can be retained in the matrix until released by cells through an included plasmin-cleavable site. Recombinant human BMP-2, including amino acids for Factor XIII crosslinking and plasmin cleavage, was cloned, expressed, purified, and refolded in our lab. BMP-2 was integrated into fibrin gels formed with fibrinogen, thrombin, and preactivated Factor XIII. Extracts from hydrogels digested with trypsin were assayed on Western blots and a decrease in mobility in SDS gels of BMP-2 indicated that BMP-2 was covalently incorporated. Additionally, released BMP-2 from trypsin-digested gels was functional, as measured with an alkaline phosphatase assay. These findings indicate the potential of this growth factor delivery system for IVD regeneration.

05.06
Assessment of an intradiscal injectable hydrogel stick

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Introduction: Degenerative disc disease associated with diminished water-binding capabilities of the nucleus, leads to disc dehydration, volume reduction, altered cellular activity, and pain. When non-surgical treatment fails, alternatives to highly invasive intervention are few. The GelStix™ nucleus augmentation device is a hydrogel that exhibits similar properties to native nucleus in terms of water binding, absorbs up to 90% w/w of fluid, pressure-hydration response (similar response to published data on human cadaveric tissue)and pH. The hydrogel is a synthetic block co-polymer composed of hard and soft segments. The hydrophobic C-C backbone is highly resistant to degradation and the hydrophilic block is composed of carboxylic acid, amidine and amide pendant groups. The GelStix hydrogel implant has similar water binding capabilities to nucleus, similar modulus and functions as a buffer restoring pH. Extensive animal studies demonstrate the implant is highly biocompatible. In a 10 patient safety study, two or three hydrogel implants were delivered into each disc level of patients with discogenic bain pain and were followed up to 1 year.
Results: Patients showed a significant reduction in back pain. No complications or adverse events were observed.
Conclusions: GelStix nucleus augmentation device exhibits similar chemical and physical properties to native nucleus pulposus and holds promise as a non-surgical, minimally invasive treatment for degenerative disc disease.

05.P01
Detection of cells in human degenerated intervertebral discs that express stem cell- and progenitor markers including OCT3/4

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Introduction/aim: Low back pain is common and degenerated discs(DD) are believed to be a major cause. In non-degenerated discs presence of stem-/progenitor cells was recently reported in different mammals. Understanding processes of discdegeneration and regenerative mechanisms within DD is important. Aim of the study was to investigate presence of stem-/progenitor cells within human DD.
Material and Methods: Human degenerated disc tissues (2–3 on Thompson scale) were collected, patients (n = 12) undergoing spinal-fusion surgery. Isolation of RNA and Quantitative Real-time-PCR was performed using standard protocols. Real-time-PCR was performed with assays for: OCT3/4,CD105,CD90, STRO-1,NOTCH1. Protein expression was analyzed by IHC using antibodies: antiOCT3/4, antiCD105, antiSTRO-1, antiCD90, antiNotch1.
Results: Gene expression of OCT3/4, CD105, CD90, STRO-1 and Notch1 were found in 11/12 samples. Results were supported by findings of cells expressing the same markers on protein level which were detected sparsely in the tissue as solitary cells or cell-foci.
Discussion and Conclusion: Expression of OCT3/4, multipotency marker and NOTCH1, regulator of cell fate, mesenchymal stem cell markers CD105, CD90 and STRO-1 indicate that primitive cell populations are present within DD. OCT3/4+ cells have not previously been reported to be present in intervertebral discs. Possibly, these cellpopulations participate in repair attempts of DD and may be possible to stimulate.

05.P02
The use of adipose derived stem cells and cell-sheets for inner and outer annulus fibrosus regeneration: a comparative study with native IVD cells using two chondrogenic induction cocktails

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Intervertebral disc (IVD) regeneration hinges on the successful tissue engineering (TE) of the nucleus pulposus (NP) and annulus fibrosus (AF). The AF is a complex structure with distinct inner and outer regions. Using IVD cells for TE is difficult because extraction of IVD cells inflicts injury on healthy discs. Adipose derived stem cells (ASCs) is a potential cell source to use for AF regeneration. The objective of this study is to assess the suitability of ASCs and ASCs cell-sheets (ASC-CSs) for outer and inner AF regeneration by: (i) comparing the gene expression of chondrogenically differentiated ASCs and ASC-CSs with inner and outer AF cells, and (ii) assessing the phenotype of chondrogenically differentiated ASCs and ASC-CSs. Isolated ASCs are cultured in ascorbic acid supplemented medium to form 1 week old cell-sheets. ASCs and ASC-CSs were then chondrogenically differentiated using two chondrogenic induction cocktails. Cocktail A consists of pyruvate supplemented HG-DMEM with BMP-6 and TGF-β3, while cocktail B consists of pyruvate absent HG-DMEM with TGF-β1. Immunohistochemistry results showed that cocktail A gave a stronger phenotype expression of Type II collagen (Col) in ASCs than ASC-CSs, while cocktail B showed a distinctly stronger Type I Col phenotype expression in ASC-CSs than ASCs. Under suitable chemical cues, ASC-CSs appear to

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be more suitable for the regeneration of the fibrous outer AF, while ASCs appear to be more suitable for inner AF regeneration.

05.P03
A constrained disc organ culture model for in vitro simulation of cell therapy approaches

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Our purpose was the establishment and characterization of a disc organ culture model that allows the evaluation of the fate of injected cells under the simulation of a degenerative environment. Bovine disc punches were injected by a fluorescent GFP-cell-line, PKH67- or PKH26- labelled disc cells in each 50 μl albumin hyaluronan hydrogel, and cultured at constrained conditions. The analyses at day 1, 7, and 14 were performed by histomorphology, immunohistochemistry and DMMB assay. Constraining prevented disc swelling with maintenance of the original height. A decreased GAG content was shown by reduced Alcian blue staining and increased GAG-concentration in the conditioned media. Fluorescent cells could be detected at each sampling time point. Close to the injection site, sporadic cell clusters were found. Disc matrix around injected cells showed a more intense Alcian blue staining suggesting a locally increased GAG deposition. The detection of fluorescent cells at different time-points after injection allows ongoing characterization of cell reactions in this organ culture environment. Our finding of a locally increased GAG deposition suggests that the injected cells are metabolic active. As the organ cultures loose matrix proteoglycans with time in culture and as this environment can also be varied with regard to nutrient supply, our approach is promising to observe the fate of injected cells with simulation of a degenerative disc environment. Supported by HEALTH-F2-2008-20162

05.P04
Differentiation of nucleus pulposus cells alters their responsivity to glucose reduction and mechanical stimulation

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Department of Trauma, Hand, Plastic and Reconstructive Surgery, Ulm, Germany; Istituto Ortopedico Galeazzi, Italy: With proceeding disc degeneration and calcification of the endplate the main route of transport of nutrients is impaired. Purpose was to investigate the influence of glucose deprivation on mechanical sensitivity of nucleus pulposus (NP) cells after differentiation.Bovine NP cells were cultured in alginate beads in high glucose chondrogenic medium. After differentiation, part of the samples was exposed to reduced glucose concentration (5 mM) for 3 days prior to further reduction to 0, 0.5 or 5 mM for 24 h. Afterwards, half of all samples were mechanical loaded by hydrostatic pressure. Gene expression was analysed by real-time RT-PCR. Differentiation increased gene expression of matrix molecules while MMP13 expression decreased. Subsequent reduction of glucose concentration significantly decreased expression of matrix molecules but increased MMP13 expression. Mechanical stimulation led to greatest effects in cells, which were maintained in chondrogenic medium. In cells exposed to glucose reduction, the mechanically induced changes in gene expression were minimal. Thus, glucose deprivation revealed a strong down-regulating impact on gene expression of matrix molecules. Application of hydrostatic pressure could not change these results. However, in chondrogenic medium, hydrostatic pressure increased expression of matrix molecules and decreased expression of catabolic enzymes. These findings indicate that mechanical sensitivity of NP cells depends on environmental conditions.

05.P05
Mesenchymal stem cells promote bone tissue regeneration in a preclinical rat model of vertebral body defect

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In orthopedics, vertebral body fractures are one of the most common disorders. Effective repair can be achieved using an autologous graft, the amount of which is usually limited for transplantation and the harvest is associated with donor site morbidity. We evaluated the efficacy of human mesenchymal stem cells (MSC) combined with a hydroxyapatite scaffold in a rat model of vertebral body defects. MSC were isolated from the bone marrow of healthy donors, then cultivated, expanded and characterized under Good Manufacturing Practice guidelines. Vertebral body defects ($1.5 \times 5 \times 1.5$ mm) were created in 32 Wistar rats and either left empty (control) or transplanted with a hydroxyapatite scaffold (CEM-OSTETIC®), alone or loaded with 0.5 million or 5 million MSC. After 8 weeks the animals were sacrificed and the vertebrae dissected and analyzed. The cells' viability and attachment to the scaffold material in-vitro were confirmed by fluorescent microscopy. Histological analysis and histomorphometry showed significant bone formation in the group transplanted with 5 million MSC in comparison to a scaffold alone or loaded with 0.5 million MSC. There was no sign of inflammation or tumor formation in any group. Micro-CT analysis did not reveal any substantial deformation of the vertebral body. Our results show that MSC combined with a hydroxyapatite scaffold may represent a safe and effective alternative for vertebral body repair. Supported by P304/10/0320, AVOZ 503905703, GACR P304/11/0653

05.P06
Tissue Enigineered Construct (TEC) prevents disc degeneration after nucleotomy in a rat model

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Back pain, a significant source of morbidity in our society, is directly linked to the pathology of the intervertebral disc. We have developed a novel scaffold-free three-dimensional tissue-engineered construct (TEC) derived from cultured synovial mesenchymal stem cells (MSCs) as a unique approach for cartilage repair (W Ando et al. Biomaterials 2007). This study explores the use of TEC derived from synovial MSCs as a cell-based therapy for intervertebral disc regeneration. Synovial MSCs were isolated enzymatically from rat synovial membranes as previously reported. For development of the TEC, cells were cultured at the density of $4.0 \times 105/cm^2$ in the presence of 0.2 mM ascorbic acid 2-phosphate for 2 weeks. The monolayer cultured cell-matrix complex was then detached from cell-substratum interface by addition of shear

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stress to convert to suspension culture. The cell-matrix complex immediately starts active contraction to form the three dimensional TEC. Two most cranial tail discs of nine rats were denucleated and/or treated with TEC (controls were denucleation only), giving three discs per group per time point. At 2, 8 and 12 weeks after implantation, the animals were euthanized and discs were evaluated. At the 8- and 12-week time point, untreated group experienced severe disruption of the anulus fibrosus and end plate, whereas such degenerative changes were significantly alleviated with TEC implantation. This study showed that TEC could prevent postnucleotomy disc degeneration.

05.P07
A novel nano-biopolymer for intervertebral disc regeneration: In vitro study on nucleus pulposus cells

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A novel nano-biopolymer, based on Hyaluronic acid (HA)-Fibrinogen (FBG) conjugate, was developed to mimic native extracellular matrix for minimally invasive intervertebral disc regenerative treatment. This study aimed to evaluate different formulations of HA-FBG conjugates for their ability to provide an optimal 3D environment for nucleus pulposus (NP) cell growth and differentiation. Bovine NP cells were seeded into conjugates of different HA molecular weights (B, C) and FBG : HA ratios (2, 4) and combined with thrombin for gelation; constructs were cultured for 14 days. Constructs of pure FBG and non-conjugated FBG-HA mixtures served as controls. The FBG and FBG-HA mixtures degraded faster than the conjugate constructs, and glycosaminoglycan (GAG) accumulation was higher in the conjugate constructs. B2 conjugates showed the least degradation and retained the highest GAG by day 14. There was a trend for highest gene expression of collagen II and transcription factor Sox9 in B2 conjugates. A decrease in aggrecan and collagen II expression was observed during culture, while the NP markers carbonic anhydrase 12, keratin-19 and biglycan were maintained or up-regulated in all materials. To conclude, HA-FBG conjugate provides the cells with a 3D environment of HA as a natural NP matrix component and FBG, which facilitates gelation and provides cell adhesion and stability. This study indicates that HA-FBG conjugate may be a suitable injectable hydrogel for biological NP regeneration.

05.P08
In vitro and in vivo biological performance of modified gellan gum-based hydrogels for nucleus pulposus tissue engineering

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Ionic- (iGG-MA) and photo-crosslinked (phGG-MA) methacrylated gellan gum hydrogels have been proposed as biomaterials for supporting nucleus pulposus (NP) regeneration and/or repair. In this study, the mechanical stability and biocompatibility of these hydrogels have been evaluated in vitro. Human intervertebral disc cells obtained from herniated patients were cultured within both hydrogels, for 1–21 days. Dynamic mechanical analysis and biological characterization (Live/Dead assay, ATP and DNA quantification, PCR and immunocytochemistry) were performed after specific times of culturing. The in vitro study showed that both cell loading and culturing time do not affect the mechanical properties of hydrogels. In addition, the iGG-MA and phGG-MA hydrogels showed to be effective on supporting cells encapsulation and viability up to 21 days of culturing. In vivo biocompatibility screening was also performed, by subcutaneous implantation of both hydrogels in Lewis rats for the period of 10 and 18 days. Haematoxylin & eosin staining revealed that the hydrogels do not elicit necrosis, calcification or acute inflammatory reaction. The present study demonstrates that the iGG-MA and phGG-MA hydrogels support cells encapsulation and viability, and are well-tolerated, stable and non-cytotoxic in vitro and in vivo, thus possessing promising features for finding application as viable NP substitutes.

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05.P09
The effect of different defects and hydrogels for nucleus replacements on the biomechanical response of the intervertebral disc

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Hydrogels offer unique opportunities for regenerative strategies of the intervertebral disc. A nucleotomy necessary for the implantation of hydrogels, however, disrupts the annulus integrity and destroys natural interfaces in the disc. To clarify whether hydrogels can restore the mechanical competence of the disc an experimental test was used. Intradiscal pressure(IDP) in an ovine disc was measured in vivo for 24 h and adapted to an axial compressive test consisting of three cycles 15-min diurnal and 30-min night load. To study the fluid mechanics, 30 motion segments in different defect conditions were used: (i) INTACT; (ii) DEF-ANN: isolated annulus defect; (iii) DEF-NUC: re-implanted nucleus; (iv) DDAHA and (v) iGG-MA: two hydrogels. DEF-ANN showed no significant difference in disc height loss or IDP compared to INTACT, while DEF-NUC reduced the IDP by ~30%($p = 0.03$) and tended to increase the height loss($p = 0.2$). Both DDAHA and iGG-MA better reflected the height loss of INTACT, but caused an even stronger loss in IDP than DEF-NUC(~34%). Neither the hydrogels nor the re-implanted nucleus, assumed to be the ideal implant, could restore the mechanical functionality of the disc. Hydrogels designed to mimic the mechanical behavior of the native nucleus may fail in restoring IDP due to the destruction of natural interfaces and an inappropriate annulus closure. To regain a biomechanical equivalent of the natural nucleus, more attention needs to be paid to the anchoring of the substitute inside the disc.

05.P10
Cell-loaded gellan gum-based hydrogels for nucleus pulposus regeneration

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Limitations of current treatments for intervertebral disc (IVD) degeneration encourage the development of tissue engineering approaches. Injectable hydrogels loaded with cells can be used as substitute material for the inner part of the IVD, the nucleus pulposus (NP), and provide an opportunity for minimally invasive treatment of IVD degeneration. The NP is populated by chondrocyte-like cells, therefore, chondrocytes or mesenchymal stem cells (MSC), stimulated to differen-

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tiate along the chondrogenic lineage could be used to promote NP regeneration. Here we present an in vitro response of bone marrow-derived MSC and nasal chondrocytes (NC) to modified gellan gum-based hydrogels. Both ionic- (iGG-MA) and photo-crosslinked (phGG-MA) methacrylated gellan gum showed no cytotoxicity in extraction assays with MSC and NC. Furthermore, the materials did not induce pro-inflammatory responses in endothelial cells. MSC and NC attached and formed a monolayer on the hydrogel surface. Moreover, both cell types could be encapsulated into the hydrogels and remained viable for at least 2 weeks, as shown by live cell staining and histochemistry. Importantly, encapsulated MSC and NC showed an increased expression of chondrocytic markers in response to chondrogenic conditions. Altogether, the data confirm the potential of modified gellan gum-based materials in NP tissue engineering.

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05.P11
Evaluation of different formulations of gellan gum-based hydrogels for tissue engineering of intervertebral disc

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Gellan gum based-hydrogels present advantageous features for application as acellular and cellular nucleus pulposus (NP) substitutes due to the possibility of fine-tuning its physico-chemical and biological properties. In this study, ionic-crosslinked hydrogel discs were produced by means of mixing a raw and chemically modified material, i.e., high acyl gellan gum (HAGG) and methacrylated low acyl gellan gum (GG-MA), respectively. The hydrogel discs were characterized in terms of its mechanical properties and degradation/swelling ability. The biocompatibility of the different hydrogel formulations was assessed in vitro using NP rabbit cells isolated from the intervertebral disc. The biological performance of the developed gellan gum-based hydrogels formulations was evaluated by: (i) culturing of NP cells in the presence of the hydrogel leachables, and (ii) seeding or encapsulation of the NP cells within the hydrogels. The present work demonstrated that as HAGG content increases, the modulus of the hydrogels decreases. Moreover, the increase of the HAGG content induces a higher weight loss of the GG-MA/HA-GG formulation as compared to GG-MA hydrogel. The in vitro study revealed that hydrogels are non-cytotoxic and support the encapsulation of rabbit NP cells. The methacrylated gellan gum and formulations possessing high acyl gellan gum present tunable properties that may be interesting for application as NP substitutes.

05.P12
A novel injectable hydrogel for intervertebral disc regeneration

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Intervertebral disc (IVD) degeneration is a leading cause of lower back pain and major health problem worldwide. Current treatments do not enable disc repair and so there is interest in a tissue engineering therapy to generate functional fibrocartilage. Mesenchymal progenitor cells (MPCs) are prime candidates for such a therapy because they can be readily isolated, expanded in vitro and are able to differentiate into cartilage. However, use of MPCs will require an effective delivery system, as well as the correct physical and chemical cues to direct and support differentiation of these cells to generate new functional tissue. We have developed an injectable hydrogel system based on polyethylene glycol and hyaluronan and examined the effects of adding both bound and soluble pentosan polysulphate (PPS), a factor which has been shown to induce chondrogenesis in MPCs even in the absence of additional growth factors. We show that the gelation rate and mechanical strength of the resulting hydrogels can be tuned and have optimized the conditions required to produce gels with the desired properties for an IVD scaffold. We show that human immunoselected STRO-1+MPCs remain viable within the gels for long-term culture periods and show elevated expression of chondrogenic markers including Sox9 and Collagen-II, compared to monolayer MPCs, which is further enhanced in the presence of PPS. These data provide evidence that such a system may be of use for the treatment of IVD degeneration.

05.P13
Thermo-reversible hydrogel for nucleus pulposus replacement: feasibility under static loading in a mild papain-induced disc degeneration model

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Nucleus pulposus (NP) replacement by application of injectable hydrogels seems a straightforward approach for intervertebral disc (IVD) tissue engineering. We investigated a thermo-reversible hydrogel (TH-RHG), based on a modified polysaccharide with a thermo-reversible polyamide poly(N-isopropylacrylamide = pNIPAAM). The gel behaves like a liquid at room temperature and is gelling at >32 °C. Here we investigate the performance of the TH-RHG in situ in bovine IVD with preconditioning of human mesenchymal stem cells (hMSC) with GDF-5. IVDs with bony endplates were harvested from calf tails and cultured in vitro for 16 days (d) with induced 'cavity' in the NP using our papain disc degeneration model (PDDM). Primary hMSCs from bone marrow (ethically approved) were seeded at 4 M cells/ml in the TH-RHG and pre-conditioned with 100 ng/ml GDF-5 for 7 days. Then, the TH-RHG was injected into the IVD and kept under static loading of 0.1 MPa for 7 days. MRI was employed to image the cavity before and after loading at day 9 and day 16, and of hMSCs RT-PCR, cell viability, sulphated glycosaminoglycan synthesis and DNA content was measured at day 1, 8 and 15. MRI images confirmed a central positioning of the TH-RHG. A drop in volume across all groups in the NP region and consequently of disc height was observed between day 9 and day 16. hMSCs expressed a more 'discogenic' phenotype after exposure to the 3D organ culture system.

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05.P14
Viscoelastic properties of the nucleus pulposus change with mild intervertebral disc degeneration after CABC injection

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Intervertebral disc (IVD) degeneration causes increased mobility in spinal motion segments. Besides, early IVD degeneration alters nucleus pulposus (NP) signal on MRI indicating changed NP composition. Hence, we hypothesize that NP viscoelastic (VE) properties also change with degeneration. To test this we used our previously validated goat model. In eight goats, three lumbar IVDs were chemically degenerated using chondroitinase ABC (CABC) confirmed by radiography and MRI. Neutral zone stiffness (NZS) and range of motion (ROM) were determined sagitally, laterally and rotationally. NPs were tested in oscillatory shear experiments on a rheometer. Water content was quantified by freeze-drying. Disc height (-6%) and MRI signal (-22%) were significantly lower after CABC treatment, confirming our model. CABC-injected IVDs had overall lower NZS and larger ROM than controls. Elastic and viscous moduli varied widely between subjects, but within goats a significant decrease (-10%) was observed in NPs after CABC injection. Surprisingly, water content was unaffected by CABC, remaining at ~75%. VE moduli and water content were not related.In conclusion, CABC injections into the NP change VE properties within subjects while water content remains unaltered, as observed earlier by Boxberger et al. However, the relatively large variation between subjects was remarkable. Together, these findings raise the question what parameters regenerative strategies should aim at to achieve IVD regeneration.

05.P15
Lumbar intradiscal treatments: early evaluation of patient satisfaction in disc repair by nucleus augmentation (GelStix™)

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Introduction: There are many methods of intradiscal treatments for degenerative disc disease. This is an early report of patient satisfaction via a method of disc repair by nucleus augmentation (GelStix™); which reverses the degenerative process by restoring pressure, hydration and physiological pH to the nucleus. This novel concept proposes nucleus/disc preservation; rather than nucleus/disc replacement which is the current art in motion preservation in the lumbar spine.
Methods: Twenty two (22) patients with clinically significant lumbar disc disease as defined by back pain with or without leg pain, with an MRI proven disc lesions (disc bulge, 'black discs', contained herniations) were treated by nucleus augmentation via intradiscal, percutaneous insertion of GelStix™ between November 2011 and January 2012 (2 months). Mean follow up was 6 weeks. VAS and ODI were assessed pre procedure and at 4 weeks post intervention. The surgical technique of insertion of the nucleus augmentation device is via percutaneous 18 gauge needle; similar to discography, and can be used as part of a nucleus ablation or decompression procedure (eg RF or laser nucleus ablation).
Results: At 4 weeks VAS reduction was from 8.5 to 3.0 ($p < 0.0001$); and ODI was 25.1 to 10.2 ($p < 0.0001$). No procedural complications were noted in the study period.
Conclusion: This early experience with GelStix™, has produced good patient acceptance, reduced pain scores and improved daily function significantly.

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06. Tissue Engineering of Functional Tendons and Ligaments

06.01
Keynote: Translation for tendon engineering: efficacy, safety and standardization

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Tendon engineering is an important research area of center. In the past 10 years, tremendous effort has been made in basic and applied researches to prove the feasibility of engineered tendon repair in animal studies. To further strengthen the translational research, a particular focus has been given on the proof of biosafety of in vitro expanded cells, and biocompatibility and biosafety of scaffold materials. Furthermore, a special production line was set up in our center to produce tendon scaffold materials automatically by the machine in order to guarantee controllable and repeatable quality control. Additionally, pharmaceutical level GMP facility is under construction in our center to provide the safety control of expanded cells and in vitro engineered tendon graft. With the efficacy study in large animal studies and the accomplishment of safety approval, it is likely for us to move forward towards clinical trials of engineered tendon repair. This talk will provide an overview of related researches in our center.

06.02
The blood-tendon barrier: A new jigsaw piece for understanding the tendon niche?

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Cell therapy and tissue engineering approaches are tempting to accelerate tendon healing. A major challenge for these strategies is to understand the niche that regulates tendon cell differentiation and cell renewal.Along our attempts to characterize tendon cells we observed a population of perivascular cells expressing tendon- and neuronal stem cell associated markers. We also observed that tendon endothelial cells express the tight junction (TJ) associated marker Occludin. We therefore hypothesize that tendon vessels form a barrier between blood and tendon tissue, establishing a privileged microenvironment.By immunohistochemistry, Lasermicrodissection, qRT-PCR and transmission electron microscopy we examined human biceps and semitendinosus tendons and mouse achilles tendons for TJ associated markers and structures. Occludin, Claudin5 and ZO1 protein localize at the cell boundaries of tendon endothelial cells, as confirmed by confocal microscopy of isolated tendon vessels. Besides, these cells express mRNA encoding for Occludin, Claudin1, Claudin5 and Claudin11.The finding of TJ- like structures in human tendons is confirmed by TEM images published by others, not focussing on TJs.The finding of TJ- like structures in tendons sheds new light on the composition of the tendon niche. The role of this barrier in tendon de- and regeneration as well as its implications on tendon cell culture for tissue engineering purposes will have to be addressed by further studies.

06.03
Mechanostimulation of human mesenchymal stem cells in PHBHHx /collagen hybrid scaffolds for tendon tissue engineering applications

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Tendon injury is increasingly prevalent with a lack of an associated effective treatment. PHBHHx is a biopolymer with potential for use as a biomaterial. Here, we investigate how in vitro mechanical stimulation (MS) influences human mesenchymal stem cell (hMSC) behaviour in a PHBHHx/collagen scaffold designed for tendon tissue engineering.hMSCs were suspended in rat type I collagen gels, injected into the lumen of a porous PHBHHx tube, continuously exposed to either no growth factor additions (no GF media) FGFs 4, 6, and 8 (FGF) or BMPs 12 and 13 (BMP), and tensile strain applied for a maximum of 20 days using a BOSE biodynamic chamber. Mechanical testing, histological analysis and molecular characterisation were performed to explore differentiation. Static controls showed little or no evidence of collagen remodelling into fibril-like structures. The addition of GF to media resulted in remodelling at 5 days and extensive remodelling after 10 days whereas only slight remodelling was apparent after 10 days MS in non GF media. Tenomodulin expression increased with MS, with the addition of FGF and BMP increasing further. Tensile force significantly increased in day 10 and day 20 in non GF and BMP-supplemented constructs over static controls. Mechanical stimulation of PHBHHx/collagen/hMSC constructs promotes tenogenic differentiation which is enhanced by BMP supplementation. The generation of in vitro tendon-like constructs holds promise for future tendon repair strategies.

06.04
Three dimensional collagen scaffolds functionalised with glycomoieties

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Introduction: Proteoglycans, glycoproteins and glycosaminoglycans form the gel-like structure, in which collagen fibrils are embedded in. Glycomoieties maintain the structural stability of collagen fibrils; play a primary role in cell-cell and matrix-cell interactions; and determine cell phenotype and function. Despite these facts, there is limited work available studying the influence of sugar molecules on tendon repair.
Materials & Methods: Collagen fibres were produced through a serial extrusion in neutral buffers maintained at 37 °C. Following fibre production, functionalisation with sulphated and non-sulphated polysaccharides took place. Subsequently, biophysical (e.g. tensile testing, thermal analysis), biochemical (e.g. ninhydrin, FTIR) and biological (e.g. collagenase digestion, cell viability, metabolic activity and gene analysis) assays were carried out.
Results: Incorporation of sugar moieties in the collagen fibres enhanced cell attachment, viability and metabolic activity. No significant difference in thermal properties was observed. Mechanical analysis and gene expression assays are on-going to fully comprehend the influence of sugar molecules on the mechanical properties of the fibres and on cellular behaviour respectively.
Conclusions: These preliminary results indicate the suitability of sugar-functionalised scaffolds for tendon repair.

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06.05
Effects of BMP-2 eluting biphasic silk scaffold on ligament regeneration and bone tunnel healing in rabbits

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To tackle graft harvesting morbidity and graft-host integration issues, we have proposed a functionalized biphasic silk scaffold for complete ligament and bone regeneration. It is hypothesized that the hydroxyapatite nanoparticles (n-HA) and BMP-2 loaded ends of this silk scaffold system will enhance enthesis regeneration and bone tunnel healing, while the central one-third supports ligament regeneration. Two groups of biphasic scaffolds, distinguished by the different ends' composition, were fabricated: SF/n-HA (0.78 mg/end, Ctrl) and SF/n-HA/BMP-2 (0.78 mg/end and 29 μg/end respectively, Exp). In vitro and in vivo characterizations (8, 16, 24 weeks) using the rabbit model were performed on these rabbit MSC-seeded constructs. The bioactivity of BMP-2 was ascertained and shown to be eluting with an initial burst and a subsequent lowered sustained release. Osteogeneic genes were upregulated in both the end types compared with pure SF. Osteogeneic differentiation was further substantiated by the increased calcium deposition. Gross observation of the excised knee joints did not indicate osteoarthritis and the ligament portion was regenerated. Bone tunnel narrowing was observed with histological evidences indicating enthesis regeneration in Exp. Better integration was observed from the superior pull-out strength of Exp compared to Ctrl. The BMP-2 eluting biphasic silk scaffold was thus shown to be a promising TE solution for enhanced ligament regeneration and bone tunnel healing.

06.06
In vivo tendon engineering using skeletal muscle derived cells

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Cell source is one of the key issues for tendon engineering research and application. Considering that muscle and tendon have the same origin during development, we thus hypothesize that muscle derived cells (MDCs) may serve as the cell source for in vivo tendon engineering. Mouse skeletal muscles and tendons were harvested to extract muscle MDCs and tenocytes respectively. Cell characterization showed that MDCs are a mixed cell population that contains MyoD, Desmin and Vimentin positive cells, and both cell types expressed collagens I, III and VI, GDF-8, sceleraxis, and tenomodulin indicating the similarity between them. qPCR showed higher expression of GDF-8, collagens III and VI in MDCs and higher expression of sceleraxis, tenomodulin and collagen I in tenocytes. MyoD was only found in MDCs. Additionally, MDCs proliferated much faster than tenocytes ($p < 0.05$) with significantly higher percentage of S+M2 phase cells as opposed to tenocytes ($p < 0.05$). After in vivo implantation along with polyglycolic acid fibers in nude mouse, MDC engineered tendons exhibited much stronger mechanical properties with greater collagen fibril diameter when compared to the tendon engineered with tenocytes. Furthermore, MDC engineered tendon gradually decreased and lost the expression of MyoD and Desimin and gradually increased the expression of tenomodulin, suggesting that MDCs have been transformed into tenocyte type with increased implantation times.

06.P01
Tissue engineering for the rotator cuff

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Introduction: Rotator cuff tears remains a problem, with massive rotator cuff tears having a failure rate of repair of up to 90%, despite new surgical techniques. Tissue engineering techniques offer the possibility of generating pre-injury tendon tissue. We present a case-control study of where we place an interpositional graft in the infraspinatus tendon of the humerus.
Methods: A total of 24 sheep were operated on, with the infraspinatus tendon being surgically cut from its attachment on the humeral head. The sheep were divided into four groups, all repaired with either: empty control, novel collagen fibre with PRP, collagen sponge, and collagen sponge with PRP.
Results: Our findings showed that the tendon augmentation graft was well integrated into the tissue, with minimal inflammatory process. The material however had not yet begun to break down as expected, however there were indications that this was occurring. We found that the delivery of platelet rich plasma using the device does enhance the repair of the tendon.
Conclusion: Platelet rich plasma and tendon graft augmentation offers exciting new prospects in tendon healing.

06.P02
Development of a tissue engineered meniscal replacement

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The knee joint is the largest and most complex joint in the human body. Owing to its anatomical structure it is susceptible to injury and damage through wear and tear. The menisci reside within this joint and play a vital role in joint stability, shock absorption and load transmission. Hence, injury to the menisci leads to altered joint biomechanics, and coupled with an inability to heal due to avascularity, this leads to subsequent degeneration of surrounding tissues characteristic of osteoarthritis (OA). There are approximately a million procedures related to the meniscus every year in the United States alone, and current treatment options only delay the onset of OA and do not provide a cure. Therefore, a meniscal replacement is necessary in order to halt the progression of OA and restore native joint biomechanics. Many avenues have been investigated in the search for a meniscal replacement, from autogenous, allogeneic and xenogeneic tissue sources, to more recent tissue engineering strategies. The aim of this study was to develop a decellularised porcine bone-medial meniscus-bone construct for use as a meniscal replacement. Bone-medial meniscus-bone was dissected from six month old pigs before decellularisation using a method adapted from Stapleton et al. (2008). Decellularised bone-medial meniscus-bone was then characterised histologically, immunohistochemically, biochemically and biomechanically to assess level of decellularisation and to compare to native porcine tissue.

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06.P03
Tendon construct development in vitro

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Anterior Cruciate Ligament rupture is a vast problem in sports and occupation and has poor healing qualities. Tissue engineering of tendon and ligaments appear as a promising tool to overcome this problem in the future. Recently, we have introduced an in vitro tendon-like construct with adult human tendon cells that demonstrates similar properties to embryonic tendon. In this study we investigated the mechanical and structural development of these tendon constructs over time. Fixed length tendon constructs were prepared with tendon cells from five individuals and cultured under constant conditions. After 14, 21, 28 and 35 days the mechanical properties of the constructs were evaluated and the collagen fibril diameter was measured by electron microscopy. The tendon constructs increased over time in strength from 0.9 ± 0.1 MPa (mean ± SE) to 4.9 ± 0.6 MPa ($p < 0.001$) and in stiffness from 5.8 ± 0.8 MPa to 32.3 ± 4.2 MPa ($p < 0.001$). However, the rupture tensile strain remained constant at approximately 15%. Synchronously with the mechanical properties, the average collagen fibril diameter increased from 37.9 ± 1.9 nm at 14 days to 59.7 ± 3.9 nm after 35 days ($p < 0.001$). In conclusion, the human tendon constructs develop considerably in structure and strength over time. The average collagen diameter increased significantly and corresponded with that found in mature human tendon and ligament. Despite significant increases, the mechanical strength reached only 10% of that in human ligament.

06.P04
Investigating the biological influence of biophysical stimulation on primary human tendon stem progenitor cells (hTSPCs)

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Numbers of traumatic tendon injuries and tendinopathies are increasing, and the functional recovery is often problematic. The biology of tendon healing has been widely investigated but it is not completely understood. Among conservative approaches, pulsed magnetic fields (PEMFs) and extracorporeal shock wave therapy have been used with success, although the biological mechanisms of their action need to be further studied. We have investigated the effects of PEMFs on primary human tendon stem progenitor cells (hTSPCs) isolated from semitendinosus tendons. After accurate characterization by FACS analysis, passage 4 hTSPCs ($n = 10$) were exposed to PEMFs (1.5 mT, 75 Hz) for 4, 8 and 12 h and evaluated at different time points (0, 2, 7 and 10 days). 8 h of PEMF exposure influenced the cell proliferation at 0 day (+21% vs. untreated cells), while after 4 and 12 hours of treatment we observed an increase of 15% and 28% at 2 days, respectively. Tissue specific gene expression (collagen I, tenomodulin, scleraxis) was differently affected by PEMF treatment. A stronger effect of PEMFs was observed when TSPCs were treated daily for a week.Unfocused shock waves (0.17 mJ/mm^2; 1000 shots, 3 Hz) were also able to positively affect hTSPC proliferation, viability and specific gene expression for up to 14 days from treatment ($n = 10$). The cells were treated in plastic adherence conditions, differently from the common in vitro practice, to better mimic the physiological tendon environment.

06.P05
Novel compound polylactic-glycolic acids with adipose derived stem cells for tendon tissue engineering

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Polylactic-glycolic acids (PLGA) have been widely used for soft tissue construction but still faces the challenge of limited mechanical properties. This study aimed to explore the possibility of employ a novel compound PLGA with autologous adipose derived stem cells (ASCs) to effectively repair Achilles tendon defect in a rabbit model. Cells were seeded on longitudinally arranged PLGA fibers rolled by PLGA network followed by 5 weeks cultivation in a special stretching bioreactor to reduce degrading acids ($n = 12$ for each group) before implantion. Scaffold only were used as the control. After 5 weeks of dynamic cultivation, cell-scaffold constructs with tensile stress near 50MPa were generated in experimental group when PLGA fibers were partly degraded. At 12 weeks post operation, most of PLGA networks were still existed grossly complete but became much weaker (only 15% of normal tendon) probably contributed to scaffold degradation. At 21 weeks, parallel collagen alignment was observed at both ends, but not in the middle in histology, with tensile stress reached to 55% of normal tendon again. At 45 weeks, engineered tendons exhibited similar histology to natural tendon with relatively mature parallel collagens formation (fibril diameter around 100 nm) whith complete scaffold degradation and higher tensile stress (80 MPa). While in control group, neo-tissue was formed only at the peripheral area, which was histologically disorganized and mechanically weaker than cell-engineered ten.

06.P06
Interaction of different cell types and cells with collagen based scaffolds for meniscus tissue regeneration

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For meniscus regeneration the interaction of meniscus cells (MCs) with neighboring endothelial cells (ECs) and their collagen-rich ECM plays pivotal roles. To further examine we explored the interaction between different cell types in a co-culture-system. Furthermore we generated an electro-spinning scaffold (ESPS) and seeded with human mesenchymal stem cells (hMSCs) for observation of cell matrix interactions. Finally we developed a bioreactor to explore cell responses by culturing cell seeded scaffolds under mechanical stress.The co-culture model was established by seeding human MCs on a PET insert and human microvascular ECs on the bottom of a well plate. After 7 days RNA was isolated and the supernatant was analyzed with an endostatin assay. ESPS were fabricated by containing collagen I isolated from rat tail and collagen II from calf knees and seeded with hMSCs. Constructs were analyzed by alcianblue staining and immunhistochemistry. The bioreactor was made with a 3D construction software. By co-culturing hMCs with hmvECs the endostatin expression is increased compared to the mono-culture. HMSCs seeded on ESPS showed already at day seven a spontaneous chondrogenic differentiation as indicated by the synthesis of proteoglycans and Col II. Further investigations have to show whether a co-culture of the three cell types in 3D constructs has

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an influence on the differentiation of the cells. To simulate the forces exposed to native tissue dynamic culture experiment have to be done.

06.P07
Extracellular matrix based device for reconstruction of the temporomandibular joint (TMJ) meniscus

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For a percentage of TMJ patients the only available treatment is meniscectomy, which results in wear of the articulating surfaces and leads to corrective surgeries. No alternatives exist for reconstruction, and removal without replacement is the 'gold-standard' for patient care. Urinary bladder extracellular matrix (UBM) was investigated as a scaffold for meniscus reconstruction. Five dogs were subjected to unilateral resection and replacement with UBM. Ten additional dogs were subjected to bilateral resection and replacement on only one side with UBM. The device remodeled rapidly and was indistinguishable from new host tissue. Remodeling was characterized by mononuclear and smooth muscle actin (SMA)+ cells at early time points changing with time to SMA- cells resembling those of the native meniscus. The remodeling process showed deposition of predominantly type I collagen, the density and organization of which resembled that of the native meniscus. Ingrowth of skeletal muscle tissue was observed at the periphery of the implant and was similar to that at the periphery of the native meniscus. Biomechanical testing showed that the mechanical properties were similar to those of native meniscus. No adverse changes in the articulating surfaces were observed in implanted joints. Results of this study suggest that the UBM device may represent an effective, off-the-shelf interpositional material while also serving as an inductive template for reconstruction of the TMJ meniscus.

06.P08
A novel triphasic composite silk fibroin scaffold with zonal release of multiple growth factors via microspheres delivery for integrated ACL-bone regeneration

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Development of an integrated ACL-bone tissue is always a challenge in orthopedics tissue engineering due to the composition of three different regions (ligament, fibrocartilage, and bone) at the interface. A promising strategy is to build a biomimic stratified scaffold with appropriate cell source for the matrix heterogeneity development which mimics the multi-tissue organization of the native ACL-bone interface. It is well known that the mesenchymal stem cell can undergo multiple lineages of differentiation under induction of different growth factors. Based on this, this study focuses on design of a novel triphasic composite silk fibroin scaffold with three distinct yet continuous layers, each layer being embedded with different growth factor loaded gelatin microspheres and the multi-lineage differentiation of the MSC on the triphasic scaffold. The zonal protein distribution, protein release kinetics and bioactivity were studied before cell culture on the scaffold and then cell proliferation, migration and differentiation were investigated and both genotype and phenotype results demonstrate the heterogeneity of matrix deposition as well as the formation of the fibrocartilage interface, which indicates the great potential of this biomimic triphasic composite silk fibroin scaffold for integrated ACL-bone tissue formation.

06.P09
Induction of tenogenic differentiation in human adipose stem cells by manipulating culture medium supplements

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Introduction: The limited ability of tendon to self-repair and the limitation of treatment regimes have hastened the motivation to develop cell-based strategies for tendon repair. Growth factors (GFs) such as EGF, FGF, PDGF and TGF-β participate in tendon formation, ECM synthesis or healing, and may assist tenogenic differentiation. Thus, this work aims to establish culturing conditions that induce tenogenic differentiation of human adipose stem cells (hASCs) using these GFs.
Materials&Methods: hASCs were isolated and expanded in α-MEM basic medium. Both freshly isolated and cryopreserved hASCs (P3) were further cultured with different supplements namely basic medium with glutamine (2 mM) and ascorbic acid (0.2 mM) plus i) EGF (10 ng/ml), ii) FGF (10 ng/ml), iii) PDGF (10 ng/ml) or iv) TGF-β (10 ng/ml) for 7, 14, 21 or 28 days. hASCs differentiation into tenocytes was assessed by real time PCR analysis of the expression of collagen type I and type III, decorin, and scleraxis.
Results: In i) and ii) media, hASCs showed a tenocyte-like aligned distribution after 14 days, while in iii) and iv) it was only observed by 21 days. Overall, hASCs showed a higher gene expression in media i) and ii).
Conclusions: EGF and FGF evidence a higher potential to induce the expression of tendon-related markers in hASCs and the cryopreservation may influence the expression of these genes. This might be a useful approach to have high number of cells to use in cell-based tendon regeneration therapies.

06.P10
Effects of vibration on the proteome expression of anterior cruciate ligament cells

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Recent reports have suggested that vibration has beneficial effects on knee healing response; however, the biomechanism of these beneficial effects still need to be determined on the anterior cruciate ligament (ACL) cell level. In this study, we applied a 20 Hz vibration to ACL cells, which produced a 20% increase ($p < 0.001$) in cell activity and 17% increase ($p < 0.001$) in intracellular sulfated glycosaminoglycan (GAG) levels. In the 20 Hz vibration-stimulated ACL cells group, eight up-regulated (100~300%) protein spots were identified compared to the control group by proteomics analysis. Among these proteins, Annexin A2 and Prolyl 4 hydroxylase (PH4B) were shown to have a 71% and 16% higher expression, respectively, in the 20 Hz vibration-stimulated ACL cells group by western blotting ($p < 0.001$). These results indicate that vibration produces a positive cellular environment, and Annexin A2 and P4HB are expected to help ligament repair and ACL cell proliferation by controlling cell membrane and extra cellular matrix formation.

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06.P11
Molecular and cellular architecture of tendon and ligament: How diverse are anterior cruciate ligament and semitendinosus tendon?

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Tendons and ligaments are both dense connective tissues which are phenotypically similar, but differ in their functions. While the cellular constituents of tendon tissue including tendon progenitor cells have been well characterized, less information for ligament cells is available. We explore similarities and differences between tendon and ligament cells at a molecular and biochemical level. Therefore we used biopsy tissue of semitendinosus tendon (ST) and anterior cruciate ligament (ACL) since ruptured ACL often is reconstructed by autologous ST grafts. The expression of progenitor cell markers (Nestin, CD133, Scleraxis) in ACL and ST was analysed by immunhistochemistry and quantitative RT-PCR revealing differences in their expression levels and patterns. We also studied the expression of extracellular matrix components (Collagen I, III, Aggrecan), matrix remodelling proteins (MMP-2, -9, LOX) and Substance P, a neurotransmitter in pain perception. The mRNA expression of Collagen I and III, MMP-2 and LOX was significantly higher in ACL than in ST. Substance P was only detectable in ACL, Scleraxis and Nestin showed no differences. The ratio of Collagen I to III mRNA was significantly higher in ACL indicating structural and functional differences. Our results contribute to the understanding of tendon and ligament nature and origin, and might provide clues for new therapeutic ways in ligament regeneration or reconstruction. Supported: PMU-FFF E-09/09/051-BAH & ABT GmbH.

06.P12
A revised surgical concept of anterior cruciate ligament replacement in a rabbit model

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This particular project on Anterior Cruciate Ligament (ACL) replacement in rabbits was created for the preclinical phase of investigating a new biomaterial. This material was based on the resorbable aliphatic polyesters, i.e. poly-(L/DL)-lactide (PLA), which has been designated as an osteoconductive agent in reconstructive ligament surgery. The aim of this investigation was to assess the possibility of including a perforated tube which surrounds the graft as a means of PLA application in ACL surgery. The rabbits (Ethic Committee approval 668/09, July 7, 2009) were divided into four groups, experimental and control, and were euthanized 6 or 12 weeks after surgery for histological evaluation. The long digital extensor tendon of the right hind limb was detached from its lateral femoral condyle, as well as from the muscle belly. The interior of the knee joint with the ACL was exposed. The native ACL was removed from the joint. Porous PLA material, molded into a perforated tube, was inserted within the bone tunnel according to its length and diameter. The outer surface of the grafted tendon adhered to the inner part of the perforated PLA tube. All the tunnel and graft parameters were precisely noted. This innovative concept of PLA tube application in ACL surgery elevated the quality of healing between the tendon and bone, which has been confirmed in histologic scans.

Acknowledgements: This study was kindly provided by Professor Sylwester Gogolewski, D.Sc., Ph.D.

06.P13
Bioresorbable polylactide (PLA) beads promote tendon healing in the bone tunnel

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Objectives: The outcome of reconstructive ligament surgery is dependent on the quality of healing of the transplanted grafts in the bone tunnels. In this study, microporous polylactide (PLA) beads of a controlled pore size were impregnated with peripheral blood and evaluated in bone tunnels of rabbits to assess their potential to enhance tendon graft osteointegration after ligament reconstruction surgery.
Material: The beads were produced from poly(L/DL-lactide) - PURAC Biochem, The Netherlands. 24 rabbits (Ethics Committee approval 668/09, July 7, 2009) were divided into four groups of six animals each, euthanized after 6 and 12 weeks. The long digital extensor tendon of the right hind limb was detached from the lateral femoral condyle and then implanted in the bone tunnel which was drilled in the proximal tibia metaphysis. Porous PLA material was distributed around the graft.
Results: Histological scans confirmed the osteoconductive attributes of the biomaterial and their synergism with osteoinductive properties of the cells from the blood and bone marrow. Proliferation of the bone around the tendon [um^2] was statistically significant in both PLA groups after 6 weeks $p = 0.034$ and after 12 weeks $p = 0.014$ ($p < 0.05$ were considered) in *post hoc* Sheffe test.
Conclusions: The new bone was almost exclusively formed in these areas where PLA paste was in direct contact with the bone and tendon.
Acknowledgements: This study was kindly provided by Prof. S. Gogolewski.

06.P14
Patterning biochemical and structural cues in collagen-GAG scaffolds to drive mesenchymal stem cell differentiation for tendon insertion regeneration

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New tissue engineering strategies are needed to meet clinical needs to regenerate spatially-ordered, multi-tissue structures. Orthopedic interfaces such as the tendon-bone-junction (TBJ) are a prototypical class of such tissues. The TBJ contains a graded interface linking tendon and bone that is critical for mechanical competence but is also a common injury site. Our goal is to develop a spatially-patterned collagen-GAG (CG) scaffold to drive multilineage mesenchymal stem cell (MSC) differentiation in a spatially-selective manner to regenerate the TBJ. We have developed fabrication schemes to control alignment, mineralization, and mechanics of CG scaffolds as well as liquid-phase and solid-phase (immobilized) biomolecule supplementation strategies to mimic the structural and biochemical heterogeneity of the TBJ. Scaffold anisotropy, pore size, mineral, and biomolecule supplementation are critical regulators of tenocyte bioactivity and MSC differentiation towards tendinous, cartilagenous, and osseous phenotypes. Composite

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biomolecule supplementation strategies have been optimized to drive simultaneous proliferation (i.e. IGF-1) and lineage specification (i.e. GDF-5 for tenocytes). Ongoing work is investigating the influence of the degree of GAG-sulfation in the scaffold to control transient biomolecule sequestration and optimizing coincident matrix and biomolecule cues to drive regional MSC differentiation and tissue biosynthesis.

06.P15
Stimulation of ligament tissue formation on a silk scaffold with mechanical loading using a custom-made bioreactor system

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The aim of the study is to generate a tissue engineered silk scaffold with mechanical properties similar to human anterior cruciate ligament (ACL) that could possibly guarantee long-term clinical success in anterior ACL regeneration/replacement. The silk scaffold was made of white raw Bombyx mori silkworm fibers in a wire-rope design. The alterations in the mechanical properties due to the preparation process (sericin removal) pull-to-failure tests were performed. Furthermore cytotoxicity has been evaluated in vitro by MTT assay and BrdU-Elisa. In parallel a bioreactor system has been developed that enables the mechanical stimulation of our constructs. Mechanical properties (maximum load/stiffness) of our silk scaffolds (2023 ± 109N/336 ± 40 N/mm) were compareable to the human ACL (2160 ± 157 N/242 ± 28 N/mm [1]). Cell culture experiments proved the non-toxicity of our silk constructs. First bioreactor tests showed a significant production of ECM proteins on the scaffolds under biaxial mechanical stimulation. Moreover, real-time PCR analysis showed a significant enhanced production of ligament related genes.We could generate a silk scaffold matching the mechanical properties of the human ACL. Furthermore, we could build up a novel bioreactor system that enables investigation of cell and tissue growth on our scaffold under biaxial mechanical loading.

06.P16
A multiscale computational model of tendon to characterize the optimum microenvironment for tissue engineering

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Accurate characterization of tissue microenvironment is vital in developing successful tissue engineering scaffolds and strategies. We present a multi-scale computational model of tendon using the open-source ontologies developed for the Physiome Project with accurate descriptions of the hierarchical structure of tendon at the cellular, micro and macro levels. Using this framework, we can effectively model the influence of whole body loadings at the macro level, the influence of tissue organisation and architecture at the micro level and have cell level processes determine the activation of relevant signaling pathways. Cell information is then passed up the spatial scales to modify micro architecture and provide a macro spatial characterisation of tendon tissue. We evaluate the framework by simulating the integration of a number of different scaffold materials to tendon under physiological loading conditions. The presented modelling framework is a web-based open-source repository of models, which contributing scientists can easily use and disseminate their findings. This framework provides a 'virtual tendon scaffold simulator' for evaluating performances of different scaffold materials and designs for developing an optimal tendon tissue engineering constructs.

06.P17
Demineralized bone matrix augmented tendon-bone healing

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Background/Aim: Although rotator cuff repair is among the most common procedures inshoulder surgery, retear rates are still high and enhancements in tendon to bone fixations stillrepresent challenging problems. Administering demineralized bone matrix (DBM) is one of the attempts to deliver osteoinductive agents improving tendon to bone healing. The aim of the study was to compare histomorphology between a reattached infraspinatus tendon with DBM administered to the bone bed (DBM group) and without DBM (control group) in anovine model.
Method: From a total of eight sheep the left infraspinatus tendon was detached from its insertion and reinserted into a prepared bone bed in a transosseous equivalent fashion using suture anchors. For the reattachment of six sheep DBM was administered to the bone bed. The DBM was prepared beforehand from ovine bone according to a modified Urist protocol. All animals were sacrificed after 4 weeks and histomorphologic analysis was performed.
Results: DBM augmentation resulted in increased amounts of fibrocartilage and mineralized fibrocartilage compared to the control group without DBM. Fibrocartilage was organized and chondrocytes were orientated in the direction of the insertional collagen fibres.
Conclusion: This study showed that DBM augmentation of a tendon to bone repair leads to more organized tendon to bone interface with less scar formations, thus to a morphology closer to a normal enthesis which may reduce repair failure rates.

06.P18
Cell sources for anterior cruciate ligament reconstruction

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Mesenchymal stromal cells (MSC), multipotent cells usually isolated from bone marrow aspirates, hold great potential for regenerative medicine applications. However, because large number of cells are needed to regenerate worn-out tissues, extensive in vitro expansion is needed prior to in vivo use. Tendons and ligaments (T/L) are often injured not only in young active people but also in old people suffering from osteoarthritis, for example. Currently, surgeons use autografts or allografts to replace the damaged T/L. Upon replacement, the new T/L will be remodeled and integrate with the bone. However, due to poor vascularization during the initial stage, loss of ECM and cellularity occur in the new graft. In order or accelerate the remodeling stage and the integration with the host tissue - therefore decreasing the recovery time of

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patients - MSCs can be co-delivered during the surgical procedure. Here we show that ligament-derived cells are multipotent cells with phenotypical resemblance to MSCs. Interestingly, and in contrast with MSCs, they can be expanded in vitro, without loss of pluripotentcy, for much longer than MSCs (at passage five MSCs loose multipotentcy whereas ligament-derived cells maintain pluripotentcy up to passage 10. We believe these cells can play a key role in cell-based therapy for T/L regeneration. By gaining more knowledge on the pathways that control their differentiation, we can understand how the ligament is regenerated.

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07. Cartilage and Joints (in coop. ICRS)

07.01
Keynote: Biomaterials for autologous matrix-associated chondrocyte transplantation

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Scaffold composition and architecture are considered to be important parameters for cell differentiation and gene expression in matrix-associated chondrocyte transplantation (MACT). In preclinical experiments four different biomaterials were compared using morphological and molecular biological techniques. Major differences in cell distribution related to scaffold density, pore size and architecture were observed. Material composition influenced the quantity of autogenous matrix used for cellular adhesion. Cell bonding was further influenced by the geometry of the scaffold subunits. On scaffolds with widely spaced fibers and a thickness less than the cell diameter, chondrocytes surrounded the scaffold fibers with cell extensions. On those fibers, chondrocytes were spherical, suggesting a differentiated phenotype. Fiber sizes smaller than chondrocyte size, and widely spaced, are therefore beneficial in terms of improved adhesion by cell shape adaptation. Scaffold characteristics as well as culture conditions also highly influenced gene expression in cartilage transplants and these parameters may have profound impact on the tissue regeneration after MACT. All four biomaterials have been used in the clinical treatment of full-thickness cartilage defects of the femoral condyle in the knee. The outcome after 5 years is described with clinical parameters and magnetic resonance imaging using the MOCART score.

07.02
Tissue engineered nasal cartilage for the regeneration of articular cartilage in goats

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Compared to articular chondrocytes (AC), nasal septum chondrocytes (NC) have a higher and more reproducible chondrogenic differentiation capacity and their use would reduce donor site morbidity. Our study aimed to demonstrate safety and feasibility of tissue engineered (TE) cartilage grafts based on autologous NC for the repair of articular defects in goats.Expanded and GFP-labelled NC and AC from six goats were seeded on Chondro-Gide® (Geistlich). After 2 weeks of chondrogenic culture, 2 NC- and 2 AC-based grafts were implanted into chondral defects of the same posterior stifle joint and the repair tissue evaluated after 3 and 6 months. Furthermore, isolated cells from surrounding fat pad, ligament, synovium, tendon and patellar cartilage were tested for GFP-positivity. No complications or signs of inflammation occurred in any of the animals. GFP-positive cells in the repair tissue indicate the contribution of the implanted cells to the newly formed cartilage. O'Driscoll scores of 8.6 and 7.6 after 3 months increased to 14.1 and 12.4 after 6 months for NC- and AC- grafts, respectively. Surrounding tissues showed no or very low (fat pad ≤0.36%) migration of the grafted cells. We demonstrated that the use of NC is safe and feasible for TE approaches in articular cartilage repair. The repair tissue-quality generated by NC-grafts was at least comparable to that of AC-grafts, thus opening the way for clinical tests of NC as a more reproducible cell source for cartilage repair.

07.03
Ex vivo imaging and tracking of spion labelled mesenchymal stem cells and chondrocytes in orthopaedic cell based therapies

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Advances in tissue engineering play a significant role in creating novel therapies in the orthopaedic field. It is vital to evaluate these therapies. Magnetic Resonance Imaging (MRI) can be used to image and track cells labelled with superparamagnetic iron oxide nanoparticles (SPIONs) in vivo. Previous studies have focused on the development of a standardised tagging and imaging protocol for clinical use. SPIONS of various sizes and concentrations were investigated to establish minimum visibility thresholds within cartilage defects. In this study the suitability for applying this protocol to a range of orthopaedic tissues and the migration of particle-labelled cells within collagen hydrogels is investigated. Human mesenchymal stem cells and porcine chondrocytes were isolated, expanded and labelled with SPIONs. SPION-labelled cells were implanted into various orthopaedic tissues within cadaveric porcine legs and visualised using MRI. In vitro cell migration was investigated by implanting labelled cell populations into collagen hydrogels and visualised using MRI. Ex vivo MRI scans demonstrated good contrast which varied in accordance to particle size, cell dosage, and implantation site. Migratory patterns demonstrated variability in cell velocity and localisation in relation to SPION size and concentration through the collagen hydrogel. The evaluation of bio-distribution and cell survival post transplantation may allow for optimized delivery methods and dosage schemes.

07.04
Combining freshly isolated CD271$^+$ positive infrapatellar fat pad stem cells and a TGF-β3 releasing scaffold to develop a single stage therapy for cartilage repair

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Clinical translation of cell based therapies for articular cartilage (AC) repair is being impeded by the high cost and time required for stem cell (SC) culture. Adipose tissue is an attractive source of SCs because it is abundant, easy to access and it provides a high number of cells. This fact lead to the suggestion that freshly isolated adipose derived SCs can be used in one-step procedures for AC repair (Jurgens et al., 2011). The aim of this study was to investigate the use of freshly isolated CD271$^+$ infrapatellar fat pad derived cells seeded onto a TGF-β3

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releasing scaffold for one-step cartilage regeneration therapies. Cells were isolated within 3 h of harvest, enriched for $CD271^+$ and seeded onto freeze-dried agarose constructs containing TGF-β3 releasing microspheres. Fresh unsorted cells were also seeded onto scaffolds as controls. Approximately 1 million unsorted cells were seeded onto each scaffold, while around 60 000 $CD271^+$ were seeded onto each scaffold (representing the 6% of unsorted cells positive for CD271). Cells from both populations were also assayed for their tripotentiality. Significantly more sGAG accumulated in scaffolds seeded with unsorted cells, but when normalised to DNA content, sGAG synthesis was higher in $CD271^+$ constructs. This suggests that the CD271- cell population also contains a population of chondro-progenitor cells. This study demonstrated the potential of combining TGF-β3 delivery scaffolds and freshly isolated SCs for AC repair.

07.05
Biomaterial-based anti-angiogenic drug delivery system enhances the in vivo chondrogenesis of freshly seeded chondrocytes-based constructs

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Standard cartilage repair approaches consist in the implantation of freshly cell seeded-constructs whose survival and further development might be compromised by the initial host reaction. Blood vessels ingrowth and macrophages migration within the implant might indeed accelerate its resorption. Control of VEGF signal has been shown to provide great benefits to counteract these problems (Matsumoto, 2009; Zentilin, 2006). We here propose a clinically relevant fibrin/hyaluronate scaffold functionalized with an anti-angiogenic drug (Bevacizumab) – a VEGF165A monoclonal antibody – which sequestrates VEGF from the surrounding environment. We hypothesized that blocking of angiogenesis right upon ectopic implantation in nude mice might provide a better survival, as well as a superior long-term quality, of not fully developed constructs generated by human nasal chondrocytes. We demonstrated that the initial Bevacizumab release efficiently blocked vessels ingrowth, as quantified by $CD31^+$ area inside the neoformed cartilage (0.2% vs. 1.0% at 3 weeks after implant), and enhanced both the in vivo survival of the constructs (75% vs. 18% at 6 weeks after implant) and the quality of the engineered cartilage in terms of GAG and collagen II expression, with respect to the not functionalized group. The proposed approach shows a great clinical potential, as it would allow the early implantation of cartilaginous grafts, achieving their in vivo maturation while retaining control over angiogenesis.

07.06
Keynote: Biomechanics and mechanobiology of cartilage defect repair

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Focal defects of articular cartilage are found commonly in both symptomatic and asymptomatic knees, and often progress over time. Aberrant strain occurs in the articular cartilage near a focal defect during compressive loading and sliding, and such strain may contribute to cartilage remodeling and deterioration, and predispose joints to secondary osteoarthritis. A functional implant would ideally restore both the biomechanics and mechanobiology of the articular cartilage. One approach to assessing the functionality of an implant, and its influence on surrounding and opposing cartilage, is to determine the biomechanics of repaired defects in an ex vivo model. Using such a biomechanical model with an osteochondral fragment from the human femoral condyle, samples with a full-thickness cartilage defect, compared to intact samples, was found exhibit abnormal strain in the cartilage adjacent to and opposing the defect when subjected to compression. Then, when the defect was filled with an implant with appropriate mechanical properties, the strains were normalized to those approximately of normal cartilage. Thus, such an ex vivo system allows systematic analysis of certain functional aspects of repair strategies for articular cartilage cartilage defects.

07.P01
Growth and differentiation of pre-chondrogenic ATDC5 cells on bioactive self-assembled peptide nanofibers

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Healing of cartilage defects is still a problem since the current treatments are ineffective to restore full function and return the tissue to its normal state. Cartilage tissue, having slower metabolism than other tissues, cannot repair itself after damage. For this reason, developing therapies for the treatment of cartilage tissue damages occuring as a result of common joint diseases like osteoarthritis and accidents is of major importance. Regeneration of damaged cartilage tissue and complete recovery of its functionality may be possible with tissue engineering studies that hold great promise by offering novel solutions for generation of functional tissue substitutes. Heparan sulfate proteoglycan molecules are important constituents of both developing and mature cartilage ECM. Several studies indicate that action of regulator proteins of cartilage development depends on these proteoglycans. Here we explored the role of heparan sulfate mimetic self-assembling nanofibers as a scaffold in inducing chondrogenic differentiation of chondroprogenitor ATDC5 cells. Chondrogenic differentiation is defined by sulfated GAGs deposition and expression of cartilaginous ECM proteins like collagen II and aggrecan. In insulin-free medium, ATDC5 cells rapidly aggregated and formed nodules and deposited sGAGs shown by Safranin-O staining. Moreover, qRT-PCR results showed that collagen II and aggrecan expressions are highly enhanced when ATDC5 cells are cultured on heparan mimetic scaffold.

07.P02
Low friction nanocrystallino-amorphous nc-TiC/a-C coating on Ti-6Al-7Nb alloy

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Titanium alloys are widely used for components of joint prostheses due to advantageous mechanical properties and excellent resistance to corrosion. The application of titanium and its alloys as biomaterials is limited by poor tribological properties (e.g. low wear resistance, high friction coefficient and tendency to gall). Therefore, in order to improve its tribological properties, duplex surface treatment combined oxygen hardening with deposition of low friction nanocomposite nc-TiC/a-C coating was applied. A microstructure, surface topography as well as micro-mechanical and tribological properties of the nc-TiC/a-C coating deposited on oxygen hardened Ti-6Al-7Nb alloy were examined. Analytical- and high-resolution transmission electron microscopy investigation on cross-section FIB lamellas was used for determination of a microstructure and phase composition of the coated alloy. It was found

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that the coating is composed of 2–5 nm sized TiC nanocrystals embedded in an amorphous carbon matrix. The α(O) solid solution enriched by oxygen was present in the near-surface region. Oxygen hardening improved alloy hardness and wear resistance, while nanocomposite coating significantly decreased friction coefficient of the alloy. High values of the Rq, Ra and SAF measured by AFM indicate that the coating exhibited high surface development. Furthermore, the cell proliferation in vitro tests were performed to analyze the biocompatibility of coated alloy (results in evaluation).

ABSTRACT WITHDRAWN

07.P04
Preliminary study of nanoparticulate bioactive glass as a substrate for cartilage tissue engineering

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Bioactive glass is a biomaterial that has an ability to facilitate repair and rebuilding of damaged tissues, particularly hard tissue. Its good bioactivity is ascribed release of ions, which are not just critical for bond formation with the bone, but are also known to stimulate expression of several families of genes that control osteogenesis. Some reports also suggest that they stimulate angiogenesis and support the growth of chondrocytes. Therefore we performed preliminary tests to see if our formulation of bioactive glass can be used as a potential candidate for cartilage tissue engineering. Bioactive glass was prepared by particulate sol-gel method. Powder was then used to form sintered porous substrates for further tests. Rate of dissolution, released ions concentrations and pH change on a substrate surface and its surroundings were measured in order to evaluate the effect on cell growth and phenotype. The influence of bioactive glass on cell growth and proliferation was tested with cell culture cytotoxicity assays, by direct contact and with liquid extracts. Cytotoxicity tests were performed using L929cell line, primary chondrocyte, osteoblast, smooth muscle and vascular epithelium cell cultures in direct contact to evaluate cell type specific differences in cytotoxic/ conductive response. Suitability of bioactive glass as a material or component of scaffolds for cartilage tissue engineering was evaluated with analysis of chondrocyte specific gene expression profile.

07.P05
Engineering cell-cell interactions in 3D biomimetic hydrogels for cartilage tissue engineering

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Cell-cell interactions is a critical component of stem cell niche and play important roles in regulating tissue development. Conventional methods for examining cell-cell interactions utilize transwell co-culture in monolayer or mixing different cell types as a dense cell pellet. Despite the broad use of these models, they have limited control over cell density and distribution, and cell behavior in monolayer (2D) or dense pellet culture may not mimic their cell behavior in three dimensional (3D) extracellular matrices. To overcome these limitations, here we developed 3D mimetic hydrogels to examine cellular interactions between adipose-derived stem cells (ADSCs) and neonatal articular chondrocytes (ACs), for the purpose of cartilage tissue engineering. Specifically we designed various 3D co-culture models including: (i) mixed co-culture at different cell ratios; (ii) bi-layered co-culture that confined the two cell types in separate layers; and (iii) cells cultured with conditioned medium from the other cell type. By varying mixed ratios, we can control the effective distance between two cell types, which controls the local paracrine signal concentration. Our results suggest that local concentration and distribution of paracrine factors play a crucial role in mediating cell-cell crosstalk and the subsequent neo-tissue formation. This study suggests that engineering cellular interactions using 3D biomimetic hydrogels may provide a novel strategy for promoting desired tissue repair.

07.P06
Tailorable scaffold morphologies for zonal articular cartilage regeneration

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Introduction: Articular cartilage is a highly organised anisotropic tissue. Current tissue engineering solutions fail to mimic this zonal organisation and functionality of cartilage*; thus the goal of this work was to fabricate composite scaffolds using a combination of scaffold morphologies to mimic the native tissue organisation in regards to morphology and end function. Methods: Porous poly(ϵ-caprolactone) (PCL) scaffolds were produced by porogen leaching by suspending a high density of coarse (1 mm^3) or fine-grained (0.3 mm^3) salt in solubilised PCL. Upon porogen extraction, a 100 μm layer of aligned PCL fibres (ø1 μm) was electrospun onto the surface. Composite scaffold samples were assessed for mechanics (tensile/compressive/roughness) as well as for in vitro cartilage formation (biochemical/PCR) up to 4 weeks.
Results: Deposition of electrospun aligned fibres increased both tensile strength and modulus of the scaffolds, while also decreasing the surface roughness. Varying porogen size yielded scaffold systems of differing pore size with significantly different compressive moduli (15 ± 0.8 and 39 ± 3.5 kPa). In vitro assessment with bovine chondrocytes dem-

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onstrated that all scaffolds supported cell adhesion, proliferation and matrix accumulation.
Conclusion: We have fabricated a novel tailorable scaffold that incorporates combinatorial morphologies to mimic the morphological and functional organisation of articular cartilage.*Place, Evans, Stevens. Nat Mat. 2009

07.P07
Chondroitin sulfate immobilization at the surface of electrospun nanofiber meshes for cartilage regeneration

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Aiming at improving the biocompatibility of biomaterial scaffolds, surface modification presents a way to preserve their mechanical properties and to improve the surface bioactivity. In this work, chondroitin sulfate (CS) was immobilized at the surface of electrospun poly(caprolactone) nanofiber meshes (PCL NFMs). The immobilization was performed with 1-ethyl-3-(3-dimethyl- aminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS). Contact Angle, SEM, Optical Profilometry, FTIR, X-ray photoelectron spectroscopy techniques confirmed the CS-immobilization in PCL NFMs. Furthermore, CS-immobilized PCL NFMs showed lower roughness and higher hydrophilicity than the samples without CS. Human articular chondrocytes (HACs) were cultured on electrospun PCL NFMs with or without CS immobilization. It was observed that HACs proliferated through the entire time course of the experiment in both types of scaffolds. SEM observations revealed that HACs maintained their typical morphology and produced extracellular matrix. Glycosaminoglycans quantification showed increased values over time. Quantitative-PCR of cartilage-related genes revealed over-expression of Aggrecan, Collagen type II, COMP and Sox9 on both types of NFMs tested, with higher values for PCL. In conclusion, CS immobilization in PCL NFM was achieved successfully and provides a valid platform enabling further surface functionalization methods in scaffolds to be developed for cartilage tissue engineering.

07.P08
Chondrocytes from human elastic cartilage maintain their phenotype in a CHT-PVA-ECH based polymer

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Microtia is a malformation or hypoplasia of the auricle associated with hearing and cognitive deficiency. Auricular reconstruction using autologous costal cartilage is the conventional treatment with many side effects. Our purpose was to evaluate the phenotype, viability and biocompatibility of chondral cells seeded onto a CTS-PVA-ECH polymer as a scaffold to study neotissue formation after implantation in an animal model. Tissue was obtained from ear reconstruction procedures. Chondrocytes were isolated from elastic cartilage and expanded in vitro. After P1, cells were seeded onto CTS-PVA-ECH polymers and cultured for 20 days. Cell viability was assessed, constructs were analyzed by SEM and subsequently implanted in athymic mice. Matrix proteins were detected by IHC and PCR was performed to determine cartilage molecular markers. Elastic cartilage chondrocytes reached 80% confluency 3 weeks after every passage. Calcein assays revealed 85% to 87% of cell viability when seeded onto the scaffold and alter 20 days in culture. Cell adhesion and extracellular matrix formation was confirmed by SEM. PCR and IHC analysis allowed us to confirm the presence of elastic cartilage proteins such as ELN, COL2 and ACAN in a lesser extent. Cell survival and matrix formation are desirable characteristics in biomaterial for TE. According to our results, the CTS-PVA-ECH polymer could be used for further experiments in order to obtain a suitable scaffold for elastic cartilage regeneration.

07.P09
Chondrogenic medium components have distinct effects on growth factor production from adipose stem cells

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Previous studies showed that chondrogenic media (CM) decreased expression and secretion of angiogenic factors and increased expression and secretion of chondrogenic factors in adipose stem cell (ASC) cultures, making them more viable for stimulating endogenous cartilage regeneration. The objective of this study was to determine the effect different chondrogenic medium components have on ASCs in order to optimize growth factor secretion for cartilage repair. 90% confluent P1 ASCs isolated from male Sprague Dawley rats were treated for 5 days with growth medium (GM; DMEM +1 g glucose/l +10% FBS) supplemented with different combinations of 50 μg/ml ascorbic acid-2-phosphate (A2P), 100 nM dexamethasone (Dex), 10 ng/ml TGF-ß1, and 100 ng/ml BMP-6 or chondrogenic medium (CM; DMEM +4.5 g glucose/l +1% ITS+) excluding different combinations of A2P, Dex, TGF-ß1, and BMP-6. Gene expression and growth factor production over 24 h were quantified. A2P increased IGF-I and TGF-ß2 secretion and decreased fgf18 expression and VEGF-A secretion in both GM and CM. Dex increased bmp2 and fgf18 expression and decreased VEGF-A secretion in both GM and CM. TGF-ß1 increased FGF-2 and VEGF-A secretion and decreased igf1 and pthlh expression in both GM and CM. BMP-6 increased nog and fgf18 expression and TGF-ß2 secretion in both GM and CM. These results show that CM components can tailor ASC growth factor production for cartilage repair.

07.P10
The effects of an in vitro low oxygen tension preconditioning of MSC on their in vivo chondrogenic potential: application for cartilage tissue engineering

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Mesenchymal stem cell MSC-based regenerative medicine is promising for cartilage repair. Cartilage is an avascular tissue in which cells experience hypoxia. Low oxygen tension is known to promote the chondrogenic differentiation of MSC. In this context, we investigate whether hypoxia may be an instrumental tool to exploit the regenerative potential of MSC.MSC from rabbit or human adipose tissue were in vitro

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preconditioned for 3 weeks in control or chondrogenic medium in 5 or 21% O_2. Chondrogenic commitment was monitored by RT-qPCR. Preconditioned MSC were associated to Si-HPMC hydrogel and injected in nude mice subcutis for 5 weeks or in rabbit articular cartilage defects for 18 weeks. Newly formed tissue was evaluated by histological staining.5% O_2 increased the in vitro expression levels of chondrogenic markers in MSC cultured in inductive medium. Analyses of implants showed the formation of a cartilaginous tissue for cells preconditioned in chondrogenic medium in 5 or 21% O_2. These data show that MSC-based regenerative therapy could be a relevant strategy for cartilage repair. Whereas a 5% O_2 up-regulates the in vitro chondrogenic differentiation of MSC, it does not stimulate the in vivo chondrogenic regenerating potential of MSC. With respect to the putative role of oxygen tension in the control of terminal hypertrophic differentiation, we questioned whether a 5% O_2 in vitro treatment may be instrumental to control the terminal differentiation of MSC after implantation.

07.P11
Human epiphyseal chondro-progenitors provide a stable cell source for cartilage repair and regeneration

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Articular cartilage possesses a limited inherent regenerative capacity, mainly due to its low cell to tissue volume ratio and its avascular and alymphatic nature. Defining a gold standard in cell based cartilage therapies has been challenging, particularly in choosing a cell type with a balanced combination of responsiveness and stability. The work presented focuses on the reliable expansion and characterization of a clinical-grade human epiphyseal chondro-progenitor (ECP) cell bank from a single tissue donation. ECPs exhibited remarkable homogeneity in expansion with a steady proliferative potential across population doublings and a resistance to cryogenic shock. Surface marker analysis revealed no detectable contaminating subpopulation or population enrichment during prolonged culture periods. 3D micro-pellets of ECPs grown in morphogen-free conditions spontaneously deposited aggrecan and collagen type I and II. Sox9 nuclear localization was also equivalent across population doublings indicating a stable chondrogenic potential. The propensity for multilineage commitment was also investigated. Osteogenic induction was restricted across population doublings as observed by calcified matrix deposition and a notable down-regulation of classic cartilage hypertrophic markers such as Collagen X, MMP13 and ADAMTS5. The reliability, stability and responsiveness of ECPs over prolonged culture confer a major advantage in defining novel ECP based strategies for cartilage regeneration.

07.P12
Wnt3a and FGF2 administration during expansion phase of adult mesenchymal stem cells synergistically enhances proliferation and chondrogenic potential

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Bone-marrow-derived mesenchymal stem cells (BMSC) are used to repair cartilage defects. Few studies, however, have investigated the relevance of expansion medium composition in sustaining proliferation and improving cartilage formation, as is known for fibroblast growth factor-2 (FGF2). Moreover, during embryogenesis, FGFs and Wnt proteins are known to promote proliferation and maintain progenitor cells in an undifferentiated state. Here we evaluated the effect of Wnt3a (W3a)-administration during expansion and its effect on differentiation potential.BMSC from three donors were grown under four different conditions: (i) 10% fetal calf serum (FCS), (ii) FCS + FGF2, (iii) FCS + W3a, (iv) FCS + FGF2 + W3a. Cells cultured with W3a, with or without FGF2, had smaller size and polygonal shape. Similarly, FGF2 or W3a enhanced the proliferation rate compared to FCS-only and the combination FGF2 and W3a promoted cell growth even further. After expansion, chondrogenesis was assessed by pellet culture, and osteogenesis or adipogenesis were evaluated in monolayer. Combination of FGF2 and W3a displayed the best chondrogenic potential (Collagen-2 and Glycosaminoglycans), while FCS + W3a or FCS-only failed to promote chondrogenesis. Interestingly, condition FCS + W3a displayed also the lowest osteogenic and adipogenic capacity, respectively detected by VonKossa or Oil-Red staining. Our data show that during expansion Wnt and FGF signals synergistically promote proliferation and chondrogenic potential of BMSC.

07.P13
Effects of dynamic compression on chondrogenesis of bone marrow and infrapatellar fat pad derived stem cells

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The aim of this study was to investigate the effects of dynamic compression on chondrogenesis of bone marrow (BM) and infrapatellar fat pad (IFP) derived stem cells. Cells were encapsulated in agarose hydrogels and cultured in free swelling (FS) conditions in a chondrogenic medium supplemented with 10ng/ml of TGF-β3 for 21 days. Dynamic compression was then applied to samples (10% strain for 1 h/day). Control samples were maintained in FS conditions. After 42 days in culture, constructs were assessed mechanically, biochemically and histologically. Dynamic compression had no effect on sGAG accumulation for BM constructs; however it did lead to an increase in collagen content and significantly improved the mechanical properties of the engineered tissue. In contrast, loading had no effect on the mechanical or biochemical properties of cartilaginous tissues engineered using IFP derived stem cells, although these tissues were functionally superior to those generated using BM derived stem cells. Dynamic compression also appeared to suppress hypertrophy of BM derived stem cells, as evident by reduced mineralization of the engineered tissue. No evidence of mineralization was observed in IFP constructs. In conclusion, infrapatellar fat pad derived stem cells respond differently than bone marrow derived stem cells to both biochemical and biophysical cues.

07.P14
Hypoxic culture of mesenchymal stem cells for bone tissue engineering

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Introduction: The use of mesenchymal stem cells (MSC) with resorbable ceramics in bone tissue engineering is motivated by the cells' multipotency, growth factor releasing capacity and the bioceramics' ability to promote bone formation. Maintaining MSC multipotency is critical, but prolonged normoxic culture induces DNA damage reducing differentiation potential. Hypoxic culture, which mimics MSC environment in vivo, may improve proliferation and differentiation potential, although reported results are mixed. The aims are to establish MSC

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response to hypoxia and elucidate the effect of hydroxyapatite (HA) and tricalcium phosphate (TCP) substrates.
Materials/Methods: A hypoxic chamber (37 °C-5% CO_2-2% O_2) was commissioned. Baseline MSC experiments on tissue culture plastics were performed up to 21 days. Hypoxia was confirmed using a Sensordish reader and a Hypoxyprobe cell kit, cell proliferation by MTS assay, and DNA assessment by RT PCR. Experiments were repeated with MSC on HA and TCP composites. Results

An initial drop in cell number for hypoxically cultured MSC was noted; however after 7 days the proliferation rate was higher than cells in normoxia. Multipotency of the cells was maintained. Work is continuing with hypoxic cell culture on HA and TCP.
Conclusions: This work highlights the potential for hypoxic culturing of MSC for clinical applications, which may reduce cell culture times and preserve stemness. The effect of bioceramics on hypoxic cell culture will be outlined.

07.P15
Characterization of mesenchymal stem cells of patients with late osteoarthritis - towards in situ tissue engineering in arthritis

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OA leads to joint destruction and arthroplasty. Cell-based ACI/MACI or cell-free AMIC, used to treat early OA cartilage lesions, delay but not cure OA. We aim to develop an approach to treat late OA cartilage lesions. Here, unlike AMIC, where bone marrow MSC passively flow in a matrix, factor-delivery stimulates active migration of MSC into a matrix and then cartilage formation. Factors also improve cartilage metabolism. Based on FACS, end-stage OA-MSC are CD45-, $CD73^+$, $CD90^+$ and $CD105^+$. As verified by histology, antibody staining and microarray, in 3D-PLGA-culture, TGFß3 stimulation results in cartilage formation comparable to normal donor cartilage. Based on antibody staining and qPCR, they have similar chemokine receptor- and chemokine secretion profiles. In chemotaxis assays, synovial fluid, SDF1 and TECK recruited OA- and ND-MSC. After TECK stimulation, microarrays showed upregulation of genes involved in pathways related to homing (CXCL2, -3, PDE4B), cytoskeletal and membrane reorganization (IGFBP1) and movement (CXCL6, PTGS2, TGM2). We also developed a PLGA-based delivery system for SDF1 and TECK, and in vitro/in vivo (MRT) studied the effect of factor delivery on superparamagnetic iron oxide nanoparticle labeled MSC. In conclusion, we show that end-stage OA-MSC behave like ND-MSC and established key tools for active cell-free OA tissue engineering. Release of anti-inflammatory factors will allow the use during inflammation and thus, in future, other arthritis types.

07.P16
High-density stem cell systems with incorporated growth factor-releasing microspheres for cartilage tissue engineering

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Human mesenchymal stem cells from bone marrow (hMSC) and adipose tissue (hASC) are attractive cell sources for cartilage tissue engineering, as they are easily accessible and multipotent. These cells are often cultured in medium containing TGF-β1 at high density to facilitate cell-cell interactions advantageous for chondrogenesis. However, diffusion limitations of TGF-β1 can be a barrier to differentiation of cells within the construct interior and extended in vitro culture periods are tedious and costly. We report the engineering of systems of TGF-β1-releasing polymer microspheres incorporated within high-density stem cell constructs for the spatiotemporal regulation of chondrogenesis. Fast- or slow-degrading gelatin microspheres loaded with different TGF-β1 concentrations were incorporated into stem cell aggregates or self-assembled sheets in different amounts to attain varied temporal and spatial TGF-β1 delivery profiles. Microsphere-incorporated constructs formed cartilage at levels equivalent to or greater than control constructs cultured in TGF-β1-containing medium as measured by type II collagen and glycosaminoglycan production. Under some conditions, microsphere incorporation increased construct thickness, matrix uniformity, and equilibrium elastic modulus. Overall, these results demonstrate the spatiotemporal regulation of chondrogenesis via incorporated TGF-β1-releasing microspheres and may enable in vivo cartilage formation without extended prior in vitro culture.

07.P17
CD73 conjugated microspheres as targeting vehicles for mesenchymal stem cells

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Whether MSCs differentiate in the context of tissue repair or exert their therapeutic effects via trophic or paracrine factors at the site of the damaged tissue, successful engraftment and retention of the cells is prerequisite. We propose that incorporation of microspheres composed of natural components of the extracellular matrix (hyaluronan or collagen) and functionalised with MSC antibodies will help guide MSCs to sites of injury where they can elicit their therapeutic effects. This strategy may be particularly applicable to articular cartilage, since it has been previously demonstrated that MSCs when delivered to the joint engraft at sites of meniscus, synovial capsule and periosteum, yet not to fibrillated cartilage. The aim of this study was to identify microspheres that would be considered appropriate for targeting therapeutic cells. Both hyaluronan and collagen microsphere were characterised. TEM and SEM confirmed formation of microspheres and assessed their morphology and size distribution. Confocal microscopy and flow cytometry was utilized to investigate co-localisation of microspheres with hMSC and potential uptake of the microspheres by the cells. Effects of microspheres on cell viability and cell number were determined using Alamar blue test and PicoGreen dsDNA assay, respectively. Finally, the targeting ability of the antibody functionalised microspheres was assessed in vitro.

07.P18
Antagonistic activities of GDF-5: A key for cartilage maintenance?

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Joint forming interzones are suggested to be mainly responsible for the development of articular chondrocytes being essential for a proper biomechanical function of the joint throughout life. In the past years, extensive molecular and genetic studies revealed that joint development and maintenance requires a fine balanced interplay of

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gene-products with pro- and anti-chondrogenic activities. This intriguing concept becomes markedly apparent e.g. in human syndromes correlating with either gain- or loss of function mutations in growth and differentiation factor 5 (GDF-5). Since both types of mutations affect the joint forming process we assumed that GDF-5, aside of its pro-chondrogenic activities, might also antagonize signaling of other factors being expressed at the sites where joints will form such as bone morphogenetic protein 2 (BMP-2) or BMP-4. Indeed, wtGDF-5 as well as the mutant GDF-5R57A both antagonize BMP-2 mediated alkaline phosphatase (ALP) gene expression in C2C12 cells which exclusively involves type I receptor BMPR-IA signaling. The same BMP-2 antagonizing properties could be observed in vivo when implanting BMP-2 and either one of the two GDF-5 ligands simultaneously at heterotopic sites in a rat model. These findings might shed new light on GDF-5's role in limb development and cartilage maintenance. GDF-5 might inhibit certain osteogenic activities thereby keeping chondrocytes arrested in a defined differentiation stage throughout life.

07.P19
TGFbeta-depletion during expansion enhances chondrogenesis of human chondrocytes

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Autologous chondrocyte implantation is a cell-based treatment to repair cartilage defects, relying on the availability of culture expanded chondrocytes. Unfortunately, the expansion process causes phenotypical changes, requiring re-establishment of the chondrogenic phenotype to sustain proper repair. Transforming Growth Factor-beta (TGFb) stimulates chondrogenic redifferentiation of human articular chondrocytes (HACs) if provided after the expansion, but it downmodulates it if added concomitantly. Hence we investigated the effects of the depletion of TGFb present in serum or endogenously produced by HACs during the expansion phase.HACs were isolated from articular cartilage and expanded in serum-supplemented medium (FCS) or in a chemically-defined (CD) medium, with or without anti-TGFb antibody (aTGab).Cell proliferation in CD-medium was unaffected by aTGab. In FCS-medium cells expanded to a slower rate and addition of aTGab further decreased both proliferation and the activity of Smad1/5/8, protein involved in the TGFb intracellular pathway. Challenging FCS- or CD-media with aTGab during expansion enhanced chondrogenesis and inhibited hypertrophic differentiation in the subsequent pellet cultures. Interestingly, the TGFb1 level detected during expansion was correlated with collagen type-X mRNA expression after redifferentiation. These results evidence the relevance of the expansion medium composition to improve chondrogenic redifferentiation and inhibit hypertrophy.

07.P20
GDF5 induces redifferentiation without hypertrophy of human articular chondrocytes

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Introduction: Growth and differentiation factor 5 (GDF5) belongs to the BMP family and the TGFbeta superfamily. It is a key regulator of mesenchyme condensation and chondrogenic differentiation in developing joints. The aim of this project was to investigate the potency of GDF5 for the redifferentiation of human articular chondrocytes (hACs), in comparison to TGFβ1, and to define the synergistic relationship between GDF5, TGFβ1 and dexamethasone. Materials and methods

hACs were cultured in micromass cultures, in medium formulations defined using factorial design. The effects of the components for the redifferentiation of the cells were investigated using qPCR. A linear dose dependence of the GDF5 for the redifferentiation of the hACs was investigated using qPCR, histology and biochemical assays.
Results: GDF5 showed a synergistic effect with dexamethasone, but not with TGFβ1, for the redifferentiation process. GDF5 significantly increased the GAG/DNA ratio in the cultures in a dose dependent manner, with the highest ratio at the highest concentration (200 ng/ml). The GAG/DNA ratio was significantly lower than in the cultures with a TGFβ1 induced re-differentiation (-420%). GDF5 did not, as TGFβ1, induce collagen X expression. Conclusions

GDF5 can be used for the re-differentiation of hACs, does not have any synergistic effects with TGFβ1, and is less potent for the re-differentiation process. GDF5 does not, as TGFβ1, induce hypertrophic differentiation of the hACs.

07.P21
Drug-induced re-expression of the chondrogenic phenotype – actin cytoskeleton integrity and its links to PI3K-, PKC- and MAPK-signaling pathways

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Cartilage can withstand tremendous amounts of stresses and strains throughout daily activity. Upon damage however, it largely fails to heal and current repair strategies show only limited success. Cell-based therapies represent a promising approach; however, upon in vitro culture expansion on 2D substrates, the cells rapidly lose their chondrogenic phenotype and acquire a fibroblast-like appearance. Re-differentiation has been achieved by different cultivation protocols, but the mechanisms regulating this process are still not well understood. The aim of this project was to understand drug-induced re-expression of the chondrogenic phenotype and the mechanisms that are involved in the regulation thereof. Primary bovine chondrocytes were serially passaged to induce de-differentiation. Re-differentiation was induced by application of cytochalasin D or staurosporine and assayed via stainings for collagen II and glycosaminoglycans or via qRT-PCR analysis for SOX9 and collagen II. For signaling pathway analysis, specific inhibitors were added to the medium. Fibroblastic chondrocytes displayed re-differentiation upon treatments with actin disrupting agents cytochalasin D and staurosporine, but only with the latter drug re-expression of the full chondrogenic phenotype was observed. Additional treatment with specific inhibitors revealed that the actin cytoskeleton is involved in this response via its intricate links to PI3K-, PKC- and MAPK-signaling pathways.

07.P22
Biological roles of glial fibrillary acidic protein as a biomarker indicating matrix production of cultured chondrocytes in cartilage regenerative medicine

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Glial fibrillary acidic protein (GFAP) is a intermediate filament that is expressed in astrogrial cells, but is also specifically express in auricular chondrocytes, which are good cell sources of cartilage regenerative medicine. Although our group uses GFAP, as a biomarker of matrix production in the cultured auricular chondrocytes, the biological roles

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of GFAP in auricular chondrocytes remained unknown. In this study, we demonstrated biological functions of GFAP in the human and mouse derived auricles to clarify significance and role with the chondrocytes of GFAP as the purpose to provide useful information for reliably and safety regenerative medicine. We examined the cell responses to stretch stress for these chondrocytes. Although most of the human chondrocytes at passage 3 (P3) and murine chondrocytes of wild type ($Gfap^{+/+}$) maintained the cell attachment and cell viability, GFAP deficient mouse derived auricular chondrocytes ($Gfap^{-/-}$) were detached and decreased the cell viability. We also examined nuclear morphological analysis. P3 human and $Gfap^{+/+}$ nuclei were shaped columnar. In contrast, P8 human and $Gfap^{-/-}$ chondrocytes significantly showed the flattening and the irregularity of nuclei. Based on these results, GFAP seems to support the resistance to severe mechanical stress in the tissue which physiologically suffers from stretch overload, and play pivotal roles in the conservation of cell structures and functions through the maintenance of nuclear morphology.

07.P23
Chondrogenic modulation by thermogenesis

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Intervertebral discs (IVDs) as well as knee cartilage (KC) are soft tissues having viscoelastic properties. Under cyclic loadings, viscoelastic tissues dissipate mechanical loadings through production of heat. In IVD and KC, this heat might not be convected, since they are not vascularized, resulting thus in a local temperature increase. This local increase in temperature has been mostly ignored in the description of these tissues. As cells are sensitive to temperature, the increase in temperature through this thermo-mechanical process could influence their metabolism. The goal of this study is to evaluate the specific effect of temperature increase on chondrogenesis. First, we focused our work in measuring the heat generated in cartilage as a result of deformation. A custom-made calorimeter has been developed allowing a real-time quantification of entropy generated in samples subject to compression. Thus, a direct correlation between the dissipated energy revealed by hysteresis curves and the measured heat has been established. On a cellular level, the effect of thermogenesis on cell metabolism has been assessed looking at the gene expression of transcription factors involved in chondrogenesis. Human mesenchymal stem cells (MSCs) were cultured in DMEM at 34 °C, 37 ° and 39 °C. An up-regulation of sox9 as well as its co-activators can be already observed after 72 h at 39 °C. Primary results point to a thermal effect on chondrogenesis.

07.P24
Platelet lysate maintains chondrogenic potential and promotes cartilage regeneration

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Platelet-Rich Plasma (PRP), a concentrate of autologous blood platelet growth factors, obtained with a simple, low cost and minimally invasive method, has been proposed into clinical therapies to improve tissue regeneration. In our study we investigated the effect of the Platelet Lysate (PL), a PRP derivative, on primary human articular chondrocytes, with regard to the stimulation of cell proliferation and the maintenance of a cartilage commitment both in vitro and in vivo. When added to the culture medium, PL induced a strong mitogenic response in the chondrocytes. The in vitro expanded cell population maintained a chondrogenic potential as revealed by micromass culture in vitro and ectopic cartilage formation in vivo. We report that in a mimicked inflammation environment, PL is playing a role as a pro-inflammatory agent, acting synergistically with cytokine IL-1α to an initial inflammatory response, with a drastic enhancement of the synthesis of the cytokines IL-6 and IL-8 and of NGAL, a lipocalin expressed in chondrogenic lineage. Subsequently PL can contribute to the down modulation of the NF-κB signal pathway and the COX-2 expression, triggering the resolution of the inflammation and driving the cartilage regenerative process. Our findings support the concept that the platelet products could the activation and the resolution of the inflammatory process, provide a rational for their use as therapeutic agents in cartilage inflammation and damage.

07.P25
Oxygen consumption during chondrogenesis in hydrogels seeded with chondrocytes and stem cells isolated from different tissues

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The objective of this study was to quantify the local oxygen environment within cartilaginous grafts engineered using different stem cell (SC) sources and to relate this to the local biochemical composition of the resulting tissue. Agarose hydrogels were seeded with bone marrow derived mesenchymal SCs, infrapatellar fat pad derived SCs and chondrocytes (CCs) at various seeding densities and cultured in various external oxygen environments. O2 levels were measured over 24 days of culture in chondrogenic media. Constructs were separated into annuli and cores regions to determine the spatial accumulation of sGAG and collagen. O_2 levels were lower in the SC seeded constructs, implying that CCs have a lower oxygen consumption rate than SCs. For all cell types, the amount of sGAG synthesised was higher in the more hypoxic core region than in the annulus, confirming the importance of a low oxygen tension for promoting a chondrogenic phenotype. Reducing the external oxygen tension or increasing the cell seeding density (and hence the absolute level of oxygen consumption) was observed to reduce oxygen levels within the constructs, which correlated with an increase in sGAG synthesis in only the annular region. This led to development of a more homogeneous tissue with superior functional properties. In conclusion, CC and SCs have a unique oxygen consumption rate which may be related to their niche and which needs to be considered when engineering cartilaginous grafts.

07.P26
The effect of mechanical environment on repair of artificial cartilage

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The stress distribution situation in artificial and host cartilage is one of the most important factors for a successful cartilage defect repair with a tissue engineering cartilage. The objective of this study is to simulate and analyze the effect of elasticity modulus of artificial cartilage, compression rate, walking speed and defect size on stress distribution of host and artificial cartilage after repairing defect by tissue engineering. A three dimensional articular of knee finite element model was constructed under rolling – compression dynamic loads and tibia cartilage

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– hip boundary conditions. The simulation results show that under usual load and normal defect size, three times different elasticity modulus of artificial cartilage could cause 2.47 times stress variation on artificial cartilage and 4.31 times variation on host cartilage, the maximum stress presented under the defect area; six times different compression rate of artificial cartilage could cause 7.2 times stress variation on artificial cartilage and 11.9 times variation on host cartilage. The results show that the elasticity modulus of the artificial cartilage and compression rate had significant effect on stress distribution of artificial cartilage and host cartilage. The effect of walking speed and defect size are not obvious.

07.P27
Global gene array comparison between successful and failed outcome after autologous chondrocyte implantation

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Introduction: The purpose was to search the chondrocyte transcriptome for early molecular markers associated with clinical outcome after autologous chondrocyte implantation (ACI).
Methods and Materials: Five graft success patients with clinical improvement after ACI and five graft failures requiring re-intervention after ACI were included. Total RNA was extracted and analyzed using a whole transcript Affymetrix array. Hierarchical clustering analysis with Pearson-correlation as distance matrix and students T-tests were used to identify differentially expressed genes between groups.
Results: The graft success group showed up regulation of 39 genes with a fold change ≥1, 2 and down regulation of 38 genes with a fold change ≥1, 2 ($p < 0.05$). Functional annotation clustering analysis in DAVID showed the up-regulated genes to be involved in control of G-protein coupled and Beta-catenin mediated signaling and transcription regulation. Functional annotation analysis in DAVID of down-regulated genes with a fold change ≥1, 2 showed enrichment of genes involved in apoptosis.
Conclusion: This study demonstrates that chondrocytes from successful ACI treatment differ in gene expression pattern from chondrocytes used in non-successful ACI. These results may reveal new markers for quality control and evaluation of articular cartilage tissue engineering.

07.P28
ACSI:autologous chondrocyte sheet implantation for full-thickness articular cartilage defects

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Introduction: Cartilage defects can be traditionally repaired by Autologous Chondrocyte Implantation(ACI).However,it is difficult to collect intact extracellular matrix(ECM) when using ACI since it utilizes enzymes to harvest cells.To facilitate the collection of intact ECM, we used folded chondrocyte sheet to repair full-thickness articular cartilage defects.
Methods: The autologous chondrocyte sheet(ACS),harvested by forceps after the cultured cells reached confluency, was folded and implanted into the defect on rabbit. Fibrin glue was used to close the defect and fasten the sheet. A second group was conducted using Fibrin glue without cells. For comparison, we also include the ACI experiment as the third group. For all groups, the operational complexity, morphologic characteristics,chondrogenic marker genes, mechanical function were assessed.
Results: In observation period, the defect was left unrepaired in the second group. By contrast,the ACSI and the ACI groups showed good morphologic characteristics of hyaline cartilage and positive for Toluidine blue and collagen type II. There were differences between the two groups in GAGs contents and gene expression.The mechanical function in the ACSI group was better than the ACI group. The surgical time of the ACSI was significantly shorter than the ACI.
Conclusions: ACSI is as effective as ACI. Additionally, surgical procedure of ACSI is much faster than ACI and the neo-cartilage of ACSI is better than ACI in mechanical function.

07.P29
Hyaline cartilage regeneration with microfracture and long-term BMP-2 delivery

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Introduction: Microfracture of cartilage induces migration of bone-marrow-derived mesenchymal stem cells (BMMSC). However, this treatment often results in fibrocartilage regeneration. Growth factors such as Bone morphogenetic protein-2 (BMP-2) induce the differentiation of BMMSC into chondrocytes, which can be used for hyaline cartilage regeneration. Here, we tested the hypothesis that long-term delivery of BMP-2 to cartilage defects subjected to microfracture results in regeneration of high-quality hyaline-like cartilage.
Methods: Heparin-conjugated fibrin (HCF) and fibrin were used as carriers for the long- and short-term delivery of BMP-2. Rabbit articluar cartilage defects were treated with microfracture combined with one of the following: no treatment, fibrin and BMP-2 or HCF and BMP-2.
Results: Eight weeks after the treatment, histological analysis revealed that the long-term delivery of BMP-2 group (microfracture + HCF + BMP-2) showed the most staining with alcian blue. The long-term delivery of BMP-2 group had the highest expression level of collagen type? and highest GAG content.
Conclusions: Taken together, the long-term delivery of BMP-2 group resulted in regeneration of hyaline-like cartilage regeneration. Therefore, this method could be more convenient for hyaline cartilage regeneration than autologous chondrocyte implantation due to its non-invasive nature and lack of cell implantation.

07.P30
Moderate physical exercise results in increased cell activity in articular cartilage of knee joints in rats

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Introduction/aim: A moderate exercise regime has displayed minor positive effects on matrix turnover (proteoglycans) in articular cartilage, while effects on the cellular level are scarcely described. This

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study aimed to investigate effects of moderate exercise on cell proliferation in different regions of the knee joint.
Methods/materials: Eighteen rats (Sprague-Dawley) were given BrdU in drinking water(14 days) for DNA-in-vivo labelling. nine rats underwent treadmill training 50 min/day, 5 days/week (exercise group) and nine controls (non-exercise group). The animals were sacrificed after 14, 56 or 105 days and femurs/tibias collected. BrdU were visualized by IHC and $BrdU^+$ cells counted by 2 blinded observers in (i) articular cartilage(AC), (ii) stem cell niche(PN)(posterior), (iii) potential migration area(PMA)(region between PN and AC including PN) and (iv) epiphyseal cartilage (EP).
Results: Exercise-group compared to controls: $BrdU^+cells/mm^2$ in AC (tibias) was increased at day 14 ($p = 0.022$) and day 105 ($p = 0.045$) and in AC(femurs)at day105($p = 0.014$), $BrdU^+$cells were increased in PMA region of tibias($p = 0.00076$) and femurs ($p = 0.0080$) at day 105. No significant differences in $BrdU^+cells/mm^2$ were seen in PN or EP between groups when compared in each time-point.
Discussion/Conclusion: The results indicates increased cell activity in AC of the knee joint by physical exercise and add understanding of AC regeneration, which can contribute to development of treatment strategies for injured AC.

07.P31
Autologous cell carrier for autologous chondrocyte transplantation

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Aim of study: Autologous Chondrocyte Implantation has been shown to be a promising method for treating deep defects in the articular cartilage. We performed autologous chondrocyte implantation in autologous fibrinogen in 35 patients with cartilage defects in the knee.
Materials and methods: Patient's autologous chondrocytes were cultured about 20 days. 450 ml patient's own blood were collected before transplantation in Regional Blood Center of Katowice to produce autologous fibrinogen. For graft preparation chondrocytes were mixed with fibrinogen, aprotinin/tranexamic acid and thrombin resuspended in CaCl2.
Results: Between 2006 and 2012 we performed autologous chondrocytes for 134 patients. We used autologous fibrinogen as a three dimensional cell carrier in 35 cases. Average diameter of cartilage defect was 2 cm. The cell viability before transplantation was always above 85%. Gene expression profile confirmed chondrogenic phenotype of implanted cells. Average concentration of fibrinogen was 45 mg/ml. Histological evaluation of biopsy reveal round-shape chondrocytes and proteoglycan presence.
Conclusions: We considered autologous chondrocytes implanted in autologous fibrinogen is the safest one technique. Our results suggest that it's a promising and feasible method for treatment of cartilage defect. The main advantage of our fibrin carrier is good adherence within the cartilage defect and possibility of graft injection by arthroscopy.

07.P32
Repair of large osteochondral defect by 'Rib-mosaicplasty' in a rabbit model: a study of osteochondral interfacial regeneration in vivo

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This study developed a novel method, called 'Rib-mosaicplasty', to repair large osteochondral defect by multiple sliced costal cartilage, and the regenerative results of cartilage and osteochondral interface were evaluated in a rabbit model. Japanese white rabbits were used in this study. Two pieces of ribs were taken within the chondral region. After the perichondrium was removed, the ribs were sliced into segments about 3 mm. Osteochondral defects (diameter, 5 mm; depth, 3 mm) were made on the patellar groove of the distal femur, and repaired by the costal cartilage segments with or without mid-cutting longitudinously in a mosaic method, or remained empty ($n = 7$). After 1 and 3 months the defects were examined grossly by ICRS Macroscopic Score and microscopically by H&E staining and Safranin-O staining. Both after 1 and 3 months, not only the ICRS Macroscopic Score but also the ICRS Visual Histological Score were higher in mosaic group, and highest in the 3-month mosaic group. The biological integration of transplanted costal cartilage and native cancellous bed was also observed in mosaic group after 3 months. Our results suggested that the osteochondral interface could be regenerated in vivo by costal cartilage and native cancellous bone in a rabbit model. As large costal hyaline cartilage store, and less functional impairment in the donor site and easier to restore condylar contour, ' Rib-mosaicplasty ' is expected to provide an effective solution for cartilage repairment.

07.P33
Engineering zonally organized articular cartilage

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The zones of articular cartilage have differing structure,composition, and chondrocyte phenotype. Despite these differences, there is a lack of articular cartilage regeneration treatments which aim to restore the zonal organization utilizing stem or mature cell therapy. The aim of our work is to guide mesenchymal stem cell (MSC) fate into chondrocytes of zonal phenotype capable of producing zonally organized tissue. To this end, we first isolated zonal chondrocyte populations, and classified differences in gene expression and protein production between populations. From these results, and reports in the literature, it was clear one of the major differences between zones was the production of a critical lubricating protein, PRG4, in the superficial zone only. We then evaluated the potential of MSCs to differentiate into chondrocytes with two distinct phenotypes; superficial zone chondrocytes with elevated PRG4 production, and middle/deep zone chondrocytes without PRG4 expression. Results show incorporation of hyaluronic acid, a major cartilage matrix component, downregulated mRNA expression of PRG4, but enhanced retention of the protein within our scaffolds. Coculture with primary superficial zone chondrocytes upregulated expression of PRG4 in MSCs, as well as temporal growth factor delivery during chondrogenesis. In conclusion, we identified PRG4 as a marker for superficial zone cell phenotype, and identified culture conditions which upregulate its expression in MSCs.

07.P34
A tissue engineered osteochondral composite for cartilage repair: a large animal study

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Objectives: The aim of this work was to validate an osteochondral composite for the repair of an osteo-chondral lesion in adult pigs.

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Methods: An osteo-compatible cylinder was combined with a neo-cartilaginous tissue obtained by seeding chondrocytes into a collagen scaffold. Articular cartilage was harvested from the trochlea of six adult pigs; chondrocytes were isolated, expanded and then seeded onto the collagen phase of the scaffold. The seeded osteochondral scaffolds were cultured for 3 weeks in vitro in a chondrogenic medium; then, they were surgically implanted in osteo-chondral lesions of adult pigs. As control, some lesions were treated with acellular scaffolds and others were left untreated. The repair tissue was analyzed after 3 months.
Results: The histologic ICRS II scale showed higher values in chondrocytes morphology and in the superficial layer recovery in the unseeded osteochondral scaffolds with respect to the lesions treated with the seeded scaffolds or left untreated. The biochemical analysis showed a higher DNA content in the lesion repaired with cellular scaffold and a higher GAGs/DNA ratio in the lesions with the spontaneous repair.
Conclusions: This study demonstrated that the osteochondral scaffold was able to integrate with the surrounding tissue. The quality of the repair was higher when the scaffold was not seeded with chondrocytes, but filled with cells migrated from subchondral bone.

07.P35
Interaction of multipotential stromal cells (MSCs) with cartilage is enhanced by synovial fluid manipulation.

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MSCs are highly proliferative multipotential stromal cells capable of regenerating cartilage, bone and other joint tissues. Our group discovered a resident population of MSCs in synovial fluid (SF) of normal joints in humans and bovines, and have thus discovered an intrinsic pool of MSCs in the knee having direct access to superficial cartilage and other joint structures. Here we investigate the influence of SF on the interaction of MSCs with the cartilage surface. We have developed an in vitro model to investigate MSC attachment to macroscopically normal cartilage in the presence of human SF. Confocal microscopy is used to detect MSCs, tagged with Fluorescent Micron-sized Particles of Iron Oxide attached to the cartilage surface. Data show OASF to be anti-adhesive, inhibiting MSCs attachment to both tissue culture plastic and cartilage. Pre-incubation of OASF with hyaluronidase decreases this anti-adhesive property giving a 160–300% increase in MSC attachment to cartilage ($n = 3$). SF from inflamed joints is intrinsically less anti-adhesive allowing more MSCs to adhere to both plastic and cartilage. Here we show the anti-adhesive nature of SF extends beyond simply lubricating joint articulation, but to affecting the interaction between MSCs and cartilage. This effect is reduced with inflammation and with enzymatic degradation of the MSCs hyaluronan-coat. These finding suggest manipulation of the synovial environment may be an important factor when considering MSC therapy.

07.P36
Influence of cryopreservation, cultivation time and patient's age on the gene expression in cartilage transplants

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Our aim was to evaluate the impact of various parameters (cryopreservation, culture time and patient's age) on the expression of specific chondrogenic markers in cartilage transplants used for MACT. Gene expression of chondrocyte markers (Col1, Col2, aggrecan, versican, MIA and IL-1b) were analysed in cartilage biopsies ($n = 26$) and transplant samples (Hyalograft C, $n = 91$) by real-time PCR. The cells of 13 out of 91 transplant samples had been cryopreserved prior to transplantation. Correlation analyses were performed to evaluate the influence of several parameters on the gene expression level. Cryopreservation of cells was found to decrease Col2 and MIA significantly (4.7-fold, $p < 0.01$ and 2-fold, $p < 0.045$, respectively). Duration of cryopreservation had no further influence on the gene expression. No correlation was detected between cultivation time and expression level of any gene. Transplants of older patients (>35 years) exhibited a significantly higher Il-1b expression (3.7 fold, $p < 0.039$) than transplants of younger patients (≤35 years).Our data demonstrate that cryopreservation has a profound impact on chondrocyte metabolic activity by decreasing Col2 and MIA expression, independently on the duration of cryopreservation. This implicates that cryopreservation for a short time should be considered very carefully, whereas long time cryopreservation seems to be a good way of keeping the cells for future transplantations without any further negative effect.

07.P37
Attenuation of inflammation by immune modulation for cartilage repair in osteoarthritis

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Osteoarthritis (OA) is a disabling degenerative joint disease affecting synovial joints. OA is characterised by the loss or damage of articular cartilage, but the disease process affects the entire joint structure, involving changes in subchondral bone and inflammation of the synovium. Synovial inflammation is believed to contribute to both symptoms and disease progression. The release of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), and soluble mediators by inflamed synovium may accelerate cartilage matrix degradation. These cytokines have been reported to compromise cell-based cartilage repair strategies in inflamed joints, such as inhibiting chondrogenesis of mesenchymal stem cells (MSCs). The main objective of this study is to understand the role of inflammation in OA disease progression and the ability of IL-10 overexpressing MSCs to modulate this inflammation. IL-10 has been reported to modulate inflammation in a sepsis model following administration of MSCs and to attenuate symptoms in a rheumatoid arthritis model in mice using overexpressing MSCs. We have observed reduced TNF-α production by LPS stimulated monocytes in response to treatment with IL-10 conditioned media harvested from transduced MSCs. The effect of released IL-10 on macrophage polarisation state has been investigated, and the levels of other anti/pro-inflammatory molecules have been assessed by ELISA of culture supernatant.

07.P38
Two year follow up of arthroscopic implantation of matrix-encapsulated autologous chondrocytes in the knee

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Introduction: Autologous chondrocyte implantation is a cell based approach for treatment of chondral lesions to the knee. During culture passages chondrocyte phenotype is altered. In this study our objective was to determine clinical efficacy and safety of a novel method of redifferentiated matrix-encapsulated autologous chondrocytes in 10 patients after minimum 2 year follow-up.

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Methods: After biopsy chondrocytes were proliferated up to second passage and seeded tridimensionally in a collagen scaffold. Gene expression analysis for Col I, II; and SOX-9 was done with qRT-PCR. Fixation of the implants was achieved securely by means of bioabsorable anchors and PDS suture to the subchondral bone adjacent to the chondral lesion. Comparisons were tested with Wilcoxon paired samples test, p value was set at <0.05.
Results: We completed 100% of follow-up at 2 years. Mean age was 35 ± 8, twenty percent were women. Normal collagen I, II, and SOX-9 expression was obtained in the tridimemensional construct before implantation. Clinical evaluations improved at 2 years (Preoperative vs. Postoperative): Lysholm 49.60 ± 17.71 vs. 80.70 ± 11.91 ($p = 0.008$); IKDC 41.40 ± 16.03 vs. 66.30 ± 14.06 ($p = 0.028$). Our mean results with arthroscopic second look were almost normal in all cases (ICRS $11.1 + 0.87$).). We did not found adverse events.
Discussion: This novel matrix-encapsulated chondrocyte culture technique is efficacious and safe for cartilage repair.

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08. Cartilage and Joints - Cartilage Tissue Engineering

08.01
Keynote: Cartilage engineering based on adult stem cells

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Adult stem cells are an important cell source for cartilage engineering. The current studies aim to establish a series of methods for cartilage defect repair and *in vitro* cartilage construction based on adult stem cells and biodegradable polymers, including: (i) Bone Marrow Stromal Cells (BMSCs): Pig BMSCs were seeded on polylactic acid coated polyglycolic acid (PLA/PGA) for autologous articular cartilage repair *in vivo* or for cartilage engineering *in vitro*. During *in vitro* chondrogenesis, BMSCs was induced by: A. Growth factor combination, including TGFbeta1, IGF-I, and dexamethasone. B. Mixed co-culture with chondrocytes on the scaffold. C. Soluble factors secreted by chondrocytes in a trans-well separate co-culture system. (ii) Adipose-Derived Stem Cells (ASCs): Pig ASCs were seeded onto the PLA/PGA scaffold for engineered repair of autologous articular cartilage defect. For *in vitro* study, human ASCs were isolated, expanded, and sorted by MACS with CD105 as a marker. The $CD105^{+}$ and $CD105^{-}$ cells were seeded onto the PGA/PLA scaffolds respectively and induced by the above growth factors. The results demonstrate that it is completely feasible to repair articular cartilage defect or to construct cartilage tissue *in vitro* using adult stem cells and biodegradable polymers. These methods established in the current studies provide technical parameters that are crucial for clinical translation of cartilage engineering based on adult stem cells.

08.02
In vivo assessment of the regenerative potential of a novel multi-layer scaffold for osteochondral defect repair

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A healthy, functional cartilage layer is essential for smooth pain free movement of articular joints. However, cartilage has a poor ability to regenerate and success using current surgical therapies has been limited. The potential role for tissue engineering in osteochondral defect repair has been recognised of late. ChondroColl, a novel collagen based multi-layer scaffold (WO 2010/084481) designed to mimic the structure and composition of native tissue has recently been developed in our lab. This study aimed to assess the regenerative potential of ChondroColl *in vivo* in a small animal model and also long term repair in a large animal model. Short term repair was assessed in a 3 mm × 5 mm defect in a rabbit femoral condyle model. Evaluation of repair tissue using micro-CT and histological analysis at 12 weeks showed that ChondroColl enabled generation of both cartilage and subchondral bone in the defect space. Newly formed cartilage tissue was well integrated and had a hyaline-like appearance. Further *in vivo* assessment was carried out in 6 × 6 mm defects in the medial femoral condyle and lateral trochlear ridge in a caprine model, initially in a 6 week pilot study followed by a long term 6 month study. Good scaffold retention and subchondral bone formation were observed at 6 weeks. The 6 month study is ongoing. Positive results to date indicate the potential of ChondroColl over current clinical treatment options such as microfracture and osteochondral grafting.

08.03
Cartilage repair in transplanted scaffold-free chondrocyte sheets using a minipig model

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Cartilage damage is typically treated by chondrocyte transplantation, mosaicplasty or microfracture. Recent advances in tissue engineering have prompted research on techniques to repair articular cartilage damage using a variety of transplanted cells. The authors studied the repair and regeneration of cartilage damage using layered chondrocyte sheets prepared on a temperature-responsive culture dish. We previously reported achieving robust tissue repair when covering only the surface layer with layered chondrocyte sheets when researching partial-thickness defects in the articular cartilage of domestic rabbits. In this study, the use of layered chondrocyte sheets facilitated the repair and regeneration of tissue in a minipig model of full-thickness cartilaginous defects in the knee joints. Good safranin-O staining and integration with surrounding tissue was noted in the transplantation group, which achieved sufficient cartilaginous repair and regeneration. However, some animals in the transplantation group exhibited poor safranin-O staining of the repaired and regenerated tissue in the subchondral bone. Transplantation conditions and other factors must therefore be further investigated.

08.04
VEGF blockade by soluble VEGF receptor-2 induces *in vivo* chondrogenesis of human mesenchymal progenitor cells blockade by soluble VEGF receptor-2 induces *in vivo* chondrogenesis of human mesenchymal progenitor cells

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Vascular Endothelial Growth Factor (VEGF) regulates vascular invasion during cartilage growth and endochondral ossification. VEGF expression is suppressed during hyaline cartilage formation and maintenance, and blocking of angiogenesis results in improved cartilage repair in mice models (Matsumoto 2009). We hypothesized that blocking VEGF would improve *in vivo* chondrogenesis of bone marrow-derived mesenchymal stromal cells (BMSC) for tissue engineering.VEGF blockade was achieved by transducing human BMSC to express soluble

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VEGF receptor-2 (sFlk-1). Cell-based constructs were generated with either sFlk-1-expressing or control cells loaded on collagen-based scaffolds and were implanted *in vivo* in an ectopic mouse model.The released sFlk-1 effectively blocked vascular ingrowth into the implant. Chondrogenesis was observed only for sFLK-1-expressing cells. Cartilaginous extracellular matrix was stable up to 8 weeks *in vivo*. At 12 weeks after implantation a cartilage remodeling was observed, confirmed by an upregulation of type X collagen at the mRNA level in the implant generated by sFlk-1-expressing BMSC. sFlk-1 did not increase the *in vitro* chondrogenic differentiation of BMSC, excluding a cell-autonomous effect of VEGF blocking. VEGF blockade was sufficient to induce *in vivo* chondrogenesis of BMSC in the absence of other exogenous morphogens. The long-term stability of the generated cartilage requires further investigations. Funding: EU FP7 project NMP3-LA-2008-213904.

08.05
Engineering of human ear shaped cartilage by co-culturing of human microtia chondrocytes with BMSCs

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Due to the lack of clinically appropriate seed cells, previous success on engineering human ear shaped cartilage has not yet been promoted into clinical applications. It has been demonstrated that maturely developed chondrocytes in the co-culture system with bone marrow stromal cells (BMSCs) can promote the chondrogenesis of BMSCs without ectopic ossification by providing a synthetic chondrogenic niche. However, it is still uncertain whether microtia chondrocytes also possess the similar chondro-induction function and hence the feasibility to construct the ear shaped cartilage by co-culturing BMSCs with a small portion of microtia chondrocytes. This study investigated the characteristics of microtia chondrocytes and their chondrogenic influence on BMSCs as well as the feasibility of constructing a human ear shaped cartilage with the co-culture system. The results confirmed that although microtia chondrocytes proliferate robustly and can spontaneously form cartilage pellets, dedifferentiation does occur and cells after passage 3 can no longer form pellets with obvious cartilage specific markers. However, like normal chondrocytes, microtia chondrocytes also can induce BMSCs to form cartilage in PGA scaffolds. Most importantly, the human ear shaped cartilage was successfully constructed in nude mouse by co-culturing of microtia chondrocytes with BMSCs on 1 : 3 ratios. These results may provide a promising seed cell strategy for translating cartilage tissue engineering into clinic.

08.P01
Biological, biochemical and biomechanical characterisation of cartilage from the porcine, bovine and ovine hip and knee

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Osteoarthritis is the most common disease affecting joints. The progressive degeneration of articular cartilage (AC) causes pain and loss of mobility for millions worldwide. Early intervention therapies to repair initial AC defects and preserve AC function are preferential over total joint replacements for younger patients, however current therapies are ineffective. An acellular xenogenic osteochondral (OC) graft may provide an effective repair material for OC lesions. This study aims to characterise AC from the acetabulum, femoral head, groove, condyles and tibial plateau of pigs, cows and sheep. This information will allow the most optimal starting material for decellularisation to be determined. AC was analysed by histology using H&E and alcian blue staining to determine glycosaminoglycan (GAG) distribution. From the resultant images, cellularity and AC thickness were determined. GAG and collagen content were quantified. Following lyophilisation, AC water content was calculated. OC pins were compressed using an indenter to determine AC deformation. The permeability and elastic equilibrium modulus of AC were derived using a finite element method. Porcine AC had the highest concentration of GAGs, AC from the femoral knee showed higher GAG content than other joint areas. AC from younger tissues had higher cell content and was thicker, reflecting the effects of age on AC structure. AC from old sheep had a much stiffer elastic modulus and was less permeable than other species.

08.P02
Mechanisms involved in chondrogenic differentiation of mesenchymal stem cells in an osteochondral *in vitro* model

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Little is known about mechanisms involved in the formation of repair tissue after treatment of cartilage defects. Often, fibrocartilage is formed rather than articular cartilage. We have reported a model that can be used to study cartilage repair *in vitro*. Mesenchymal stem cells (MSCs) are interesting for cartilage repair strategies, based on their differentiation potential. We have studied the differentiation of MSCs in an osteochondral environment. We simulated cartilage damage in bovine osteochondral biopsies. hMSCs were suspended in alginate and polymerized in the defects. Alginate beads served as controls. For positive controls, 10 ng/ml TGFβ1 was added. An antibody against TGFβ or an inhibitor of smad2/3 phosphorylation (smad2/3p) was used to assess the role of TGFβ. Culture medium was stored weekly for ELISA. After 28 days samples were harvested for PCR, histology and biochemistry. hMSCs differentiated without addition of TGFβ in our model in contrast to culture in alginate beads. This was reflected by positive collagen type 2 staining and expression of cartilage genes. The biopsies produced 600 pg/ml TGFβ1 per 3 days. Strikingly, chondrogenesis of hMSCs was still observed when the produced TGFβ was captured or when smad2/3p was inhibited. Our findings suggest that chondrogenesis of hMSCs is not solely mediated by TGFβ in an osteochondral environment. Financial support: the European Union's 7th Framework Programme, no. NMP3-SL-2010-245993.

08.P03
Development and characterization of an *in vitro* model of cartilage degeneration

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Osteoarthritis (OA) is a dehabilitating disease affecting over 27 million people in the US alone. There is a need to investigate novel early intervention therapies for management of the disease. Novel therapies will require pre-clinical testing in physiological models of degenerated cartilage. A simple configuration pin on plate simulator was used to generate mild and moderate mechanical models of cartilage degeneration in bovine tissue. Biochemical assays were used to assess collagen and glycosaminoglycan (GAG) content in normal and degenerated cartilage. Histology and immunohistochemistry were used to visualise cartilage

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architecture and components. TEM and ESEM were used to visualise cartilage ultrastructure and the cartilage surface respectively. Mild and moderate damage models displayed features that coincided with mild and moderate degeneration grades of human OA cartilage [Osteoarthritis Research Society International Cartilage Histopathology Assessment System]. Mechanically damaged specimens showed a loss of GAG and collagen content. The cartilage surface became fibrillated and cracked. The mechanical models have produced damage to the cartilage that was representative of OA cartilage in patients. The methods developed in this project will complement future studies of whole joints in physiologically relevant simulators, which will be used for the preclinical testing of novel cartilage substitution therapies.

08.P04
A new approach to producing solvent-free scaffolds for tissue engineering

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3D porous scaffolds hold promise for tissue engineering and cell-based gene therapy. A major obstacle is maintaining adequate nutrient and oxygen diffusion to embedded cells. Our previous work on solvent-based macro/microporous scaffolds, produced by hybrid 3D-plotting/porogen-leaching technique, demonstrated the capacity of these constructs as delivery systems for treating anemia and hemophilia B. The scaffolds seeded with mesenchymal stem cells (MSCs) supported human factor IX (hFIX) delivery for over 12 weeks. While the presence of interconnected macropores enhanced cell migration, the microporosity improved cell survival and cellular microenvironment. The hybrid fabrication technique has also enabled us to design constructs mimicking the anisotropy and poroelasticity of articular cartilage. Developing a solvent-free technology to fabricate engineered scaffolds would make the technology readily translatable to clinical trials. Single- or twin-screw extruders and conventional techniques used for melt processing of scaffolds could lead to particle break-down and may adversely affect the consistency and reproducibility of the produced constructs. This work aims to develop a solvent-free scaffold fabrication technique without compromising the original particle size of the constituents (i.e., salt and polymer powder). Reproducible solvent-free scaffolds with controlled architecture and composition would advance the field of tissue engineering and cell-based gene therapy.

08.P05
Effect of gelatin microsphere size and cell/microsphere ratio on transforming growth factor-beta3-induced chondrogenesis of human mesenchymal stem cells

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Gelatin microspheres (GM) have a potential as an injectable cell carrier for cartilage repair. The effect of microspheres size and cells/microspheres ratio on the chondrogenesis of immortalized human bone marrow-derived mesenchymal stem cells (hMSCs) was evaluated. GM were fabricated by the conventional water-in-oil emulsion method, sieved and dehydrothermally crosslinked. hMSCs were then mixed with GM with sieve-sizes ranging from 20 to 32 or 53 to 75 μm at 1 : 1 or 1 : 2 ratio, and cultured as cell aggregates (CA) in chondrogenic medium for 14 days. The size of CA was monitored microscopically, while cellular proliferation and sulfated glycosaminoglycan (sGAG) production were examined using a DNA quantification and a dimethylmethylene blue assay, respectively. hMSCs in the CA formed compact spherical masses within 24 hr, while this was evident in cell-GM mixture only after 5–7 days. By day 14, mean diameters of cell-GM aggregates were 1.2–1.5 times larger than those without GM. Overall, the hMSC proliferation was significantly enhanced when cultured on GM, with the greatest extent was observed in cultures with bigger sized GM. Nonetheless, the latter attenuated sGAG/DNA content in CA by 58–73% compared with that of other groups. In contrast, cells cultured on smaller sized GM retained the ability to synthesize sGAG, resulting in the largest CA. GM size, but not cells/microspheres ratio, appears to have pronounced effects on hMSC proliferation and sGAG production in the CA culture.

08.P06
BMP-7 expressing genetically modified primary chondrocytes in cryogel scaffolds for rabbit auricular cartilage repair

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The loss of cartilage tissue due to trauma, tumour resection or congenital defects like microtia and anotia is one of the major concerns in head and neck surgery. Autologous cell transplantation and/or using various polymeric implants or silicon prosthesis to support the defect are conventional treatment methods for repairing damaged cartilage tissue. However, lack of appropriate cells and inherent drawbacks of implant's can cause problems in application. In recent years, researches are dealing with novel tissue engineering approaches. For head and neck surgery, proper cartilage tissue can be developed by the combination of tissue engineering principles with basic biological science. In this study, primary chondrocytes were genetically modified with plasmid encoding Bone Morphogenetic Protein-7 (BMP-7). It was a goal to bring in the ex-vivo transfected chondrocytes to re-synthesize BMP-7 in in-vitro like as in-vivo. Genetically modified cells were implanted into gelatin/oxide dextran scaffolds and cartilage tissue formation was investigated in 15 × 15 mm auricular cartilage defects in-vivo in New Zealand (NZ) White Rabbits for 4 months. Results were evaluated via histology and early gene expression. Results indicated that a suitable healing of auricular cartilage with BMP-7 modified (transfected) cells in comparison with non-modified (non-transfected) group and as well as the control was achieved.

Key Words: Auricular cartilage, primary chondrocytes, plasmid DNA, non-viral gene therapy.

08.P07
Chondrocytes suppress tissue reactions in tissue-engineered cartilage through induction of immune privilege

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Clear understanding of tissue reactions after transplantation of engineered tissue is imperative for clinical application of tissue engineering. In this study, we examined interaction between tissue-engineered cartilage and host cells, and explored ways to control it. Tissue-engineered

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cartilage consisting of C57BL/6 mice chondrocytes and poly-L-lactic acid scaffolds were transplanted in EGFP transgenic mice. Host-derived $EGFP^+$ cells were mostly $F4/80^+$ macrophages, which were decreased and excluded to non-cartilage areas after 2 weeks. Meanwhile, IL-1β was significantly lower in tissue-engineered cartilage than in PLLA scaffold alone, suggesting tissue reactions were suppressed in tissue-engineered cartilage. Since some immune privilege factors including FasL were expressed on chondrocytes, we cultured chondrocytes of FasL-hypomorphic mice (gld) with macrophages, demonstrating that chondrocytes of gld were less efficient in inducing apoptosis of macrophages than did wild-type. Proteome array of co-culture medium and subsequent real-time RT-PCR detected G-CSF as an inducer of FasL in chondrocytes. Furthermore, G-CSF-treated tissue-engineered cartilage underwent less infiltration of macrophages with more matured cartilage. It was therefore suggested that G-CSF secreted from macrophages could increase FasL on chondrocytes and induce immune privilege, which may in turn suppress the viability and localization of macrophages, promoting the maturation of tissue-engineered cartilage.

08.P08
Photocrosslinkable hydrogels for cartilage tissue engineering

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Articular cartilage is a highly hydrated, matrix-rich tissue that does not heal when damaged. Tissue engineering techniques involving encapsulating chondrocytes in a hydrogel to fill a cartilage defect are promising, but an optimal hydrogel for cartilage regeneration remains to be developed. Here, we evaluated the ability of four photocrosslinkable hydrogels to promote chondrogenesis in serum-free conditions. Hyaluronic acid, polyethylene glycol, alginate, and gelatin hydrogels were selected to provide a broad range of functionalities. The response of encapsulated human chondrocytes was highly dependent on the hydrogel environment. Chondrogenesis was supported in HA constructs, with high levels of collagen II and aggrecan gene expression and protein deposition, and a corresponding 4-fold increase in stiffness during culture. Gene expression and protein accumulation in PEG constructs were low. High collagen II expression in alginate did not translate to protein deposition, and constructs softened during culture. Gelatin constructs supported the highest production of glycosaminogylcans, and stiffness increased 35-fold during culture, however collagen I expression was highly upregulated. Microcomputed tomography showed a heterogeneous GAG distribution in these constructs, indicating location in the hydrogel may impact on the response of encapsulated cells. This study highlights the potential to harness cell-material interactions for enhanced cartilage regeneration.

08.P09
Injectable scaffold as noninvasive technique for cartilage tissue engineering: in vivo case study

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*Introduction*Cartilage is a tissue, with limited repair capacity and also sparse population of cells entrapped within a dense extracellular matrix (ECM), therefore; delivery of chondrocytesto site of damaged cartilage can improve the treatment. The use of injectable scaffolding materials in order to carry and keep cells in the site of injury for in vivo tissue regeneration has raised great interest. In this study, the potential of an injectable system based on injection of scaffold and cell simultaneously as noninvasive technique was investigated.
*Materials and methods*Poly(D,L-lactide-co-glycolide)(PLGH) a copolymer with a 50:50 molar ratio containing carboxyl end groups, and alginate are injectable and biodegradable scaffolds that they were mixed with chondrocytes, were isolated from the metacarpophangeal joints of new born calf, and injected for repair of cartilage in nude mice model.
*Results*Nude mice were sacrificed at 2, 4, 6, and 8 weeks and safranin-O and hematoxylin and eosin staining for histological examination was done. It was seen that the incorporation of scaffolds with adjacent tissue was excellent, and also a fibro-cartilage as immature cartilage has been formed within 2 weeks after injection.
*Conclusion*We found that PLGH/ alginate/chondrocytes as a noninvasive injectable scaffold have an excellent potential for formation of fibrocartilage for cartilage repair.

08.P10
Acellular constructs for cartilage repair

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Introduction: Strategies are emerging to meet clinical needs for repairing articular defects. Our strategy aims to create acellular constructs to meet this need. The aim of this study was to determine the effect of application of compressive loading on the quality of engineered constructs.
Materials & Methods: Bovine synoviocytes were seeded onto polyethylene terephthelate (PET) fibre scaffolds and incubated in chondrogenic media for 4 weeks. Developed constructs were compressed to an average of 20% strain by an in-house bioreactor at 1 Hz, 1 h / day for an additional 4, 8 or 12 weeks. Resulting protein, DNA and compressive engineering moduli were measured and construct histology determined.
Results: Loaded constructs had comparable protein contents to non-loaded controls throughout; whilst cellularity increased significantly in loaded samples. Compressive moduli of loaded constructs at weeks 4, 8 and 12 were on average 5.6, 45.2 and 54.6 MPa respectively. Those for non-loaded controls were 1.6, 4.5 and 11.9 MPa. Histology revealed dense inter-fibrillar deposits of Alcian Blue staining matrix in the loaded samples at the later time points.
Conclusion: Culture of synoviocytes with PET scaffolds in chondrogenic media followed by compressive loading produced constructs with compressive moduli and matrix staining similar to native cartilage. Future work will focus on construct decelluarisation to eliminate an immune response for their potential use in cartilage defect repair.

08.P11
Development of a collagen-glycosaminoglycan scaffold for cartilage defect repair applications

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Articular cartilage has a limited capacity for self-repair. However, the use of tissue engineering (TE) biomaterials has shown significant promise in healing defects (Cheng et al., 2010). The aim of this study was to develop collagen-glycosaminoglycan scaffolds with properties optimised for cartilage repair. We investigated the effect of (i) glycosaminoglycan (GAG) type (chondroitin sulphate (CS) or hyaluronic acid (HyA)), (ii) mean pore size (130 μm and 300 μm) and (iii) scaffold stiffness (0.5, 1.0 and 1.5 kPa), on mesenchymal stem cell (MSC) differentiation and cartilage matrix production in vitro. Collagen-CS (CCS) and collagen-HyA (CHyA) scaffolds were fabricated by freeze-drying (O'Brien et al., 2005) and subsequently cultured with rat MSCs in chondrogenic medium for 28 days. It was evident that CHyA

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scaffolds facilitated greater cell infiltration and sulphated GAG distribution than CCS scaffolds. Interestingly, scaffolds with large mean pore sizes (300 μm) exhibited significantly higher (2-fold) sulphated GAG content than scaffolds with smaller mean pore sizes (130 μm). Furthermore, scaffolds with low stiffness (0.5 kPa) exhibited higher chondrogenic gene expression (SOX9) than scaffolds with higher stiffness. In summary, we have developed a novel scaffold with optimised properties (composition, architecture and stiffness) that favour MSC infiltration, chondrogenesis and matrix distribution, thus exhibiting great implications for cartilage defect repair applications.

08.P12
Porcine auricle perichondral cells may serve as cell source for in vitro cartilage differentiation

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Cartilage has a limited regeneration capacity and its replacement requires a suitable cell source with the following attributes: stable phenotype without hypertrophic cartilage development, broad availability, and high proliferative potential. Thus, we analyzed proliferation and chondrogenic differentiation capacity of porcine perichondral progenitor cells from ear cartilage (ePPC) and tracheal cartilage (tPPC).Proliferation of both cell types was analyzed by measurement of doubling time. PPC-pellets were cultured in chondrogenic differentiation medium for 4 weeks. Chondrogenic differentiation was determined by gene expression of cartilage markers (collagen II, cartilage oligomeric matrix potein (COMP), aggrecan, precartilage marker collagen I, hypertrophic cartilage marker collagen X), immunohistological and histological stainings. ePPC exhibited a higher proliferative capacity than tPPC. Both cell types expressed collagen II, aggrecan, which was more pronounced in tPPC, COMP and collagen I after chondrogenic stimulation. No collagen X could be detected. Histology showed that both cell types produced cartilage-like extracellular matrix components, whereas no production of elastic fibers could be observed. Perichondral progenitor cells represent a promising source for cartilage TE. ePPC seem to be more convenient than tPPC caused by their high proliferative capacity and accessibility, whilst showing a similar chondrogenic differentiation potential.

08.P13
A modified three dimensional polyelectrolyte scaffold for cartilage tissue engineering

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Although cartilage tissue engineering using stem cells holds great potential, it is yet to provide satisfactory quality of repair. Recent insights from tissue engineering paradigm underscore the need for cells to interact and remodel their extracellular matrix (ECM) for optimal tissue regeneration. Providing an instructive, permissive three-dimensional (3D) matrix for cartilage regeneration is critical. To this end, a chitin-alginate based 3D scaffold was developed through interfacial polyelectrolyte complexation (IPC), for encapsulation and chondrogenic differentiation of human bone marrow derived mesenchymal stem cells (MSC). An IPC based approach was employed for fabricating instructive matrices as it offered great control in varying ECM cues within the 3D niche of encapsulated cells. MSC were encapsulated in IPC scaffolds with and without type I collagen (Col-I) incorporation, to study the effects of Col-I on MSC chondrogenesis. Chondrogenic differentiation was analysed by qualitative histochemical staining and quantitative measurement of the components of cartilaginous ECM formation. Our results show that Col-I incorporation promoted superior chondrogenic differentiation of MSC. It also caused significant changes in cell morphology and cell-cell interactions as observed by F-actin and N-cadherin staining. Thus IPC based scaffolds offer an attractive platform to study the effect of various ECM cues on MSC chondrogenesis, pertaining to the functional tissue outcome.

08.P14
Engineering functional cartilaginous grafts using chondrocyte-agarose constructs supported by a superficial layer of stem cells

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During early postnatal bone development progenitor cells in the superficial region of articular cartilage drive the rapid growth of the tissue and later direct the formation of mature hyaline cartilage. These developmental processes may provide directions for the optimal structuring of co-cultured chondrocytes (CC) and stem cells required for engineering cartilaginous grafts. The objective of this study was to engineer such grafts by recapitulating aspects of cartilage growth where a pool of superficial cells drives tissue development. Infrapatellar fat pad derived mesenchymal stem cells (FPSC) were self assembled (SA) on top of CC laden hydrogels. Control constructs were formed using CC SA on top of FPSC laden hydrogels. In order to determine the effect of these structured co-cultures, FPSC and CC SA constructs, as well as FPSC and CC laden hydrogels, were cultured independently. After 4 weeks in culture, a structured co-culture consisting of FPSC SA onto CC hydrogels accumulated more sGAG and collagen than all other groups. When separated at the interface, we found that CC in the hydrogel layer of the structured co-culture proliferated faster than isolated CC embedded into control hydrogels, leading to greater levels of matrix accumulation. In conclusion, assembling FPSC onto CC laden hydrogels enhances tissue development. It appears that the superficial layer of stem cells is driving CC proliferation beneath it, mimicking certain aspects of developing articular cartilage.

08.P15
Increase of chondrogenic potential in adipose-derived stromal cells by co-delivery of specific genes

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Stem cell therapy has been developing rapidly as a potential cure for repairing or regenerating the functions of diseased organs and tissues. Adipose-derived stromal cells (ASCs) are an attractive cell source for stem cell therapy because they can be isolated easily from fat tissue in significant numbers and exhibit multiple differentiation potential under appropriate in vitro culture conditions. However, ASCs derived from individual donors can show wide variations in differentiation potential. In addition, the regulatory mechanisms underlying stem cell differentiation remain unclear. Bone morphogenic protein 2 (BMP-2) is an important signal for up-regulation of osteogenesis and chondrogenesis of stem cells. SRY-related HMG-box gene 9 (SOX-9) has also been reported as one of the key transcription factors for chondrogenesis. Meanwhile, transforming growth factor β (TGFβ) is a well-known ASC chondrogenic differentiation factor that stimulates ASC signaling pathways by activating transmembrane type I and type II receptors. We hypothesized that co-delivery of those genes would result in improved efficiency of recovery of normal chondrogenic properties in dedifferentiated chondrocytes.

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08.P16
Combining extracellular cartilage matrix and mesenchymal stem cells for cartilage tissue engineering

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Extracellular matrix (ECM)-derived implants are clinically used to replace damaged tissues, including skin and heart valves. Bioactive cues present within the natural ECM are thought to drive regeneration and repair. Within the field of orthopedics, cartilage repair remains a challenge. Therefore, this study aimed at using ECM-derived scaffolds to drive cartilage formation by chondrocytes and MSCs in vitro.Cartilage and chondrocytes were harvested from equine knees ($n = 3$; 3–8 years). Equine MSCs (eMSCs) were harvested from the bone marrow of mature healthy horses ($n = 3$). Cartilage for scaffold production was decellularized using mechanical and enzymatic treatments. Chondrocytes and eMSCs were seeded on the scaffolds and harvested after either 2, 4 or 6 weeks of culture. Samples were analyzed histologically for cells, glycosaminoglycans (GAGs), and collagen type I, II, and VI, and biochemically for GAG content using DMMB. Histology of the generated scaffolds showed successful decellularization (no cells present). Interestingly, eMSCs outperformed chondrocytes in producing new cartilage-like ECM. This was confirmed by positive stainings for GAG and collagen type II, as well as an approximately 7 times increased GAG content (0.57 ± 0.29 vs 3.95 ± 1.58 μg GAG/mg scaffold, $P < 0.01$). Cartilage ECM-derived scaffolds show potential in inducing cartilage matrix formation by chondrocytes and MSCs. Moreover, MSCs are more potent in producing new cartilage-ECM than chondrocytes.

08.P17
Dynamic compression improves biosynthesis of human zonal chondrocytes from osteoarthritis patients

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*Objective*We hypothesize that chondrocytes from osteoarthritis (OA) patients can be used to engineer cartilaginous tissues and that their biosynthesis would be enhanced from compressive stimulation. In this study, we aimed to determine the effect of dynamic compression on superficial (S) and middle/deep (MD) zone chondrocytes from OA patients in alginate model.
*Design*Human S and MD chondrocytes from OA joints were encapsulated in 2% alginate, pre-cultured, and subjected to compression with varying dynamic strain (5, 15, 50% at 1 Hz) and loading duration (1, 3, 12 h). Chondrocyte responses to loading were evaluated following shorter compression (qRT-PCR), and long-term compression (GAG, compressive modulus, immunofluorescence).
*Results*Compressive stimulation significantly induced ACAN, COL2A1, COL1A1, PRG4, and COL10A1 gene expression after 2 h of unloading, in a zone-dependent manner ($P < 0.05$). ACAN and PRG4 mRNA levels depended on strain and load duration, with 50% and 3 h loading resulting in highest levels ($P < 0.05$). Long-term compression increased collagen type II and aggrecan immunostaining and total GAG ($P < 0.05$), but only S constructs showed more PRG4 stain, retained more GAG ($P < 0.01$), and developed higher compressive moduli than non-loaded controls.
*Conclusions*The biosynthetic activity of zonal chondrocytes from OA joints can be enhanced with selected compression regimes, indicating the potential for cartilage tissue engineering applications.

08.P18
Injectable cell/microsphere aggregates for cartilage regeneration

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Injectable delivery systems have been attractive in tissue engineering, as they can deliver cells into the body in a minimally invasive manner. We hypothesized that microspheres modified with cell adhesion ligands could be useful to form shear-reversible cell aggregates as an injectable. Alginate is a natural polymer that is broadly used in many biomedical applications, due to its good biocompatibility, low toxicity, and mild gelation conditions to form a cross-linked structure. Alginate microspheres were prepared by the water-in-oil emulsion method and solidified with a use of calcium chloride (mean diameter = 16 μm), followed by modification with RGD peptides using carbodiimide chemistry. RGD-alginate microspheres formed a solid-like, aggregated structure when mixed with either ATDC5 cells or primary chondrocytes without additional cross-linking agents, in which microspheres and cells were uniformly dispersed and closely packed. The aggregation behavior was shear-reversible, which was confirmed by a rotational rheometer. However, non-modified alginate microspheres formed a phase separated structure, due to the lack of specific interactions between microspheres and cells. RGD-alginate microspheres/chondrocytes mixtures were injected into the dorsal region of mice and the efficacy of the mixtures in cartilage regeneration was tested in vivo. These cell/microsphere aggregates may find useful applications in tissue engineering as an injectable system.

08.P19
Overexpression of PTHrP-related miRNA enhances chondrogenesis and inhibits hypertrophy in human bone marrow derived stem cells

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MicroRNAs (miRNAs) are 20–25-nucleotide-long endogenous non-coding RNAs that are involved in multiple biological pathways in a variety of animals. miRNA can regulate target gene expression through translational repression and/or target mRNA degradation in a sequence-dependent manner. In this study, we performed microRNA microarray analysis with total RNAs from PTHrP-treated BMSCs. Four novel microRNAs were detected by microarray analysis, we confirmed that one of them increased the chondrogenic potential of BMSCs. The miRNA microarray data showed that miR-im6, 7, 8 and 9 were significantly up-regulated at 4 weeks following PTHrP-treated chondrogenic induction, with expression levels that were 1.5-fold greater than those in PTHrP untreated conditions. Lentivirus-mediated overexpression of has-miR-im6 increased GAG/DNA content and metachromasia in Safranin-O staining. Western blot analysis also revealed a significant decrease in alkaline phosphatase (ALP) expression with overexpression of has-miR-im6. We report that miR-im6 acts as a positive regulator of chondrogenic differentiation as well as a hypertrophy blocker in BMSCs by decreasing the ALP expression through unknown mechanism. To our knowledge, this is the first report showing that a specific microRNA, miR-im6, plays a crucial role in the course of chondrogenesis.

08.P20
Chondroprotective effect of sulforaphane and PLGA-based delivery system

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*Introduction*Recent study suggests that sulforaphane(SFN) suppresses MMP production from chondrocytes stimulated with either IL-1 or

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TNF-a and that SFN inhibits NF-KB and JNK activation in pro-inflammatory cytokine-stimulated chondrocytes. In this study, chondrocytes derived from OA patients were used for in vitro model to investigate the protective effect of SFN on inflammatory damage induced by LPS. In addition, we devised SFN delivery system using biodegradable PLGA and demonstrated that the system has enduring chondroprotective effect on inflammatory damage induced by LPS.
*Materials & methods*The cells were pretreated with 1 μg/ml LPS for 6 h and subsequently with 0–20 μM SFN for 18 h. Effect of PLGA-based SFN delivery system on cartilage protection was examined by the experiments using transwell insert.
*Results*SFN was not toxic to chondrocytes upto the dose of 5 μM. However, SFN at 5 μM significantly inhibited mRNA expression and protein level of ADAMTS-5 and MMP-2 without cytotoxicity. In addition, slow release SFN delivery system (SFN-PLGA) inhibited mRNA and protein level of COX-2, ADAMTS-5 and MMP-2 by LPS. TIMP-2 mRNA expression and protein level were increased by SFN-PLGA system.
*Conclusions*These results indicated that SFN has chondroprotective effect on inflammatory damage induced by LPS. PLGA-based delivery system may provide an effective tool for the controlled release of SFN.

08.P21
Towards a chondromimetic scaffold for cartilage repair

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Over the last 20 years, there have been many tissue engineering and cell therapies developed for the repair of damaged or diseased cartilage. Despite the fact that no optimal modality has been identified, mesenchymal stem cells (MSC) have shown tremendous potential. To deliver these cells to the site of injury, two scaffolds were selected; a monophasic polyethylene oxide terephthalate/polybutylene terephthalate (PEOT/PBT) scaffold and a biphasic PGA/PLGA+CaS construct (Trufit™). Rabbit MSC were isolated, cultured and characterized, where it was shown that 2% rabbit serum was required for optimal colony formation, characteristic MSC morphology, growth, proliferation and tri-lineage differentiation. Subsequently, optimal cell seeding density and attachment conditions were evaluated and found to be 25-million cells/ml and 50 μg/ml fibronectin for both scaffolds. Cartilage repair was examined in an osteochondral defect in a rabbit model, with test groups including, empty defect, PEOT/PBT scaffold, PEOT/PBT+MSC, Trufit™ and Trufit™ + MSC. Tissue repair was assessed in terms of toluidine blue staining. A modified ICRS scoring system was used to evaluate defect fill, surface continuity, architecture, integration, cellular morphology and matrix staining. In combination, the staining and scoring system revealed that for both scaffolds, the presence of the MSC enhanced repair and that bioinspired architectural cues are promising strategies in the design of future delivery vehicles.

08.P22
Tissue engineering cartilage from multilayer extracellular matrix materials

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Successful treatment of articular cartilage lesions remains a major clinical challenge. Naturally derived extracellular matrix material such as urinary bladder matrix (UBM) and small intestine submucosa (SIS) have potential in cartilage tissue engineering as they can be actively remodelled by cells and contain growth factors such as transforming growth factor-β1 (TGF-β1). This study investigates the 3D culture of chondrocytes in multi-layered scaffolds of UBM, SIS and electrospun poly-L-lactide acid (PLLA) for tissue engineered cartilage. PLLA fibrous constructs were fabricated by electrospinning. SIS and UBM were fabricated through a decellurisation process. Bovine chondrocytes were isolated and seeded on the scaffolds, specifically five individual layers were seeded (200K cells/layer), assembled into multi-layered scaffolds, and cultured for 4 weeks. All groups were cultured with or without TGF-β1. Glycosaminoglycans (GAG) and DNA analysis showed significant differences between the multi-layered scaffolds with the SIS scaffolds showing the highest level (350 $\pm$ 67 μg). The lowest production of GAG was seen in the PLLA scaffolds. The mechanical properties of the SIS were significantly higher in the TGF-β1 group (98 $\pm$ 21 kPa) at 4 weeks of culture compared to without (40 $\pm$ 5 kPa) and displayed similar properties to that of native cartilage. This study displays how the use of ECM materials in their native configuration is a promising possibility in tissue engineered cartilage.

08.P23
Chemical and physical cues to regulate the development of cartilaginous tissues engineered using osteoarthritic human infrapatellar fat pad derived stem cells

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Stem cells from adipose tissue can potentially be used in cell-based therapies for cartilage repair. We have previously explored the potential of infrapatellar fat pad derived stem cells (FPSCs) isolated from osteoarthritic patients for engineering functional cartilaginous tissues, however the mechanical properties of the grafts were significantly lower than normal articular cartilage. The aim of this study was to identify alternative biochemical and biophysical cues for engineering functional cartilaginous tissues using FPSCs. Firstly, we systematically investigated how the addition of different growth factors, specifically BMP-6 and FGF-2, to a chondrogenic media containing TGF-β3 would influence the development tissues engineered using FPSCs embedded into agarose hydrogels. It was observed that continuous supplementation with TGF-β3 and the presence of dexamethasone is required for robust chondrogenesis of diseased human FPSCs. Supplementation with FGF-2 suppressed matrix synthesis, with BMP-6 supplementation enhancing matrix synthesis in a cell seeding density dependent manner. Having identified the most beneficial growth factor supplementation regime, we further stimulated the constructs with cyclic hydrostatic pressure, which was observed to further enhance the functional development of the engineered tissue. In conclusion, the functionality of cartilaginous tissues engineered with human FPSCs can be improved by employing specific biochemical and biophysical cues.

08.P24
Increased viscosity media for use in bioreactor culture to prepare tissue engineered cartilage constructs for tribo-mechanical testing

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Tissue engineering (TE) techniques have the potential to provide a patient-specific treatment for the repair and regeneration of articular cartilage. We showed previously that small TE cartilage pins (5 mm Ø) with a coefficient of friction comparable to native tissue can be made in a rotating wall vessel (RWV) bioreactor. To be more physiologically representative in tribological tests, however, large ($10 \times 15 \times 1$ mm) TE 'plates' must be engineered, and existing methodological approaches have been shown to be unsuitable due to limitations of the

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RWV bioreactor. The aim of this research was to develop a high viscosity media using dextran to enable culture of large hyaline cartilage constructs in the RWV bioreactor. Large constructs were engineered using PGA scaffolds and P2 bovine articular chondrocytes in semi-static culture and in an RWV bioreactor using normal and high viscosity media, biological quality was assessed using histology and biochemical analysis. In standard viscosity medium large constructs proved unsupportable due to their weight, shear stresses caused by the subsequent agitation resulted in low glycosaminoglycan (GAG) and collagen II levels. However construct integrity was improved compared to static and semi-static controls. TE cartilage cultured in a high viscosity, low shear stress environment in the RWV bioreactor could demonstrate low friction properties in common with native tissue and superior to those shown by tissue cultured under standard conditions.

08.P25
Functionalized biomimetic poly (ethylene glycol) hydrogels utilizing GFOGER peptides for cartilage tissue engineering

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Chondrocytes cultured in 3D hydrogels have been shown to maintain the cartilage phenotype. Synthetic gels such as poly (ethylene glycol) (PEG) are attractive for 3D culture of chondrocytes, as they can be functionalized with specific adhesion sequences. Recently the GFOGER peptide was identified as the sequence responsible for chondrocyte adhesion to collagen type 2. We hypothesized that chondrocytes encapsulated in GFOGER functionalized PEG gels would have a better chondrogenic activity compared to commonly used RGD-modified gels. Passage 3 chondrocytes were encapsulated in 1% alginate beads, non-modified PEG, PEG modified with 70 μM RGD, 155 μM RGD, 70 μM GFOGER and 155 μM GFOGER peptide. Disks of 30 μl volume were cast in a QGel caster at 6 × 106 cells/ml. Cells were also cultured on tissue culture plastic at 1000 and 5000 cells/ml as controls. Samples were collected at days 1, 4 and 7 for gene expression and cytoskeletal analysis. Cell spreading was observed in all modified PEG gels after 24 h of encapsulation but only minimally in the 0 μM peptide PEG or 1% alginate gels. GFOGER modified gels improved cell spreading in a dose dependent manner. Gene expression of Col2 and Sox9 was highest in the 1% alginate and 0 μM peptide gels over 7 days of culture. The 70 μM GFOGER modified PEG gels maintained the highest expression of Col2 and Sox9 among peptide modified gels at 4 and 7 days. This novel system provides a better cartilage mimic and might be beneficial in cartilage engineering.

08.P26
Selecting the best candidate to treat cartilage and bone defects in the knee: differentiative potential of MSCs from infrapatellar and subcutaneous fat

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The identification of specific features in different mesenchymal stem cells (MSCs) populations may help to select the most suitable candidate for a specific application. Infrapatellar and subcutaneous fat are easily harvestable during knee surgery, which makes MSCs resident in these sites (IFP-MSCs and ASCs) appealing candidates for orthopaedic applications. We compared osteogenic and chondrogenic potential of IFP-MSCs and ASCs and we performed autologous coculture mixing them with articular chondrocytes (ACs) to evaluate the combined use of MSCs and ACs to treat chondral lesions.Cells were isolated from 25 donors with osteoarthrosis. Surface marker expression was assessed by cytofluorimetry. After osteo- and chondro-induction, the expression of specific markers was determined by biochemical and gene expression analysis. In coculture pellets 75% IFP-MSCs or ASCs were combined with 25% ACs.IFP-MSCs and ASCs displayed surface markers typical of MSCs. Following osteo-differentiation, Alkaline Phosphatase and Collagen I were significantly more expressed in ASCs. On the contrary, IFP-MSCs pellets were characterized by a significantly greater glycosamminoglycans content and higher expression of chondrogenic genes compared to ASCs. In coculture pellets chondrogenic genes were upregulated, with IFP-MSCs pellets performing better than ASCs pellets. The dissimilarities in IFP-MSCs and ASCs differentiative potential suggest their use to treat respectively cartilage and bone defects.

08.P27
TiO2-coating promotes human mesenchymal stem cell proliferation without losing the capacity of the chondrogenic differentiation

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Human mesenchymal stem cells (hMSCs) have wide area of applications, which require large amount of cells. However, the proliferation rate of hMSCs has certain limitations during monolayer expansion. We studied whether TiO2-coating on cell culture dishes could promote hMSCs proliferation without losing the chondrogenic capacity.Thin TiO2-coating layer was deposited onto the dishes or coverslips by ultrashort pulsed laser ablation. Human MSCs from 3 donors were expanded on them until 95 % confluence. The cells were evaluated by morphology, immunocytochemistry and qRT-PCR. The chondrogenic differentiation was also performed after cultivation on TiO2-coated dishes. After chondrogenesis, the pellets were evaluated by histological staining of proteoglycans (PGs), collagen II, and qRT-PCR.Human MSC specific markers Stro-1, CD44, CD90, CD73, CD105 and CD146 did not change after expansion on TiO2-coated coverslips as compared to non-coated ones. However, their proliferation increased by 46%. Importantly, TiO2 coating did not cause significant differences in the PG and collagen II staining of the pellets, or the expression of chondrocyte-specific genes in the chondrogenesis assay. The TiO2 thin film didn't reduce the transparency of culture dishes significantly allowing optical transmission microscopy.We conclude that the proliferation of hMSCs could be significantly increased when cultured on TiO2-coated dishes without weakening their chondrogenic differentiation capacity.

08.P28
Engineering zonal cartilaginous tissue using infrapatellar fat pad derived stem cells

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The depth dependant structure and composition of articular cartilage (AC) are critical to its biomechanical function. We have previously demonstrated that bone marrow derived stem cells can be used to engineer cartilaginous grafts that mimic certain aspects of the zonal composition of AC by modulating the local environment through the depth of the construct, although native levels of matrix accumulation were not reached. The aim of this study was to explore if infrapatellar fat pad (IFP) derived stem cells can be used to engineer cartilaginous grafts with near native levels of matrix accumulation and distribution. Agarose hydrogels were seeded with IFP derived stem cells and then

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radially confined to half their thickness and subjected to dynamic compression (DC). Overall, sGAG and collagen accumulation reached approximately 2% and 1.5% of construct wet weight respectively. In unconfined free swelling constructs, similar levels of sGAG and collagen synthesis were observed in the top and bottom regions of the construct. In contrast, sGAG synthesis was greater in the bottom of confined constructs, with the combination of confinement and DC leading to greater levels of collagen synthesis in the top of engineered tissues. The bottom of engineered constructs had a higher equilibrium modulus. In conclusion, cartilaginous grafts with improved composition and organization can be engineered by modulating the environment within hydrogels seeded with IFP derived stem cells.

08.P29
Collagen-hydroxyapatite tubular scaffold with radially oriented porosity for osteochondral defect replacement

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When the cartilage damages, sometimes the subchondral bone is involved becoming pathologically altered. An alternative therapeutic approach to surgery for osteochondral defect replacement may consist in the use of innovative scaffolds able to be colonized by cells in vivo. A key role in the scaffold colonization by cells is played by its vascularization. Aim of the present study is to develop a hydroxyapatite-collagen (HA-Coll) tubular scaffold with a radially oriented porosity in order to enhance the angiogenesis within the scaffold structure. HA crystals in collagen matrix composites 70wt% Coll - 30wt% HA were prepared by direct nucleation of HA using Ca(OH)2 and H3PO4 as calcium and phosphorus precursors; tubular scaffolds were produced by a spin-casting technique followed by a standard freeze-drying and dehydrothermal crosslinking. XRD and EDS spectrum revealed the formation of HA crystals on the collagen matrix. SEM analysis of tubular scaffold confirmed the formation of nano-sized apatite crystals on the fibrous collagen with radial oriented pores. FTIR diagram evidenced the chemical interaction between HA crystals and collagen. In vitro/in vivo tests have been used to evaluate the ability of the scaffolds to be colonized by cells, enhance angiogenesis, and sustain the osteochondral matrix formation.

08.P30
Dual growth factor delivery from bilayered composite hydrogels for osteochondral tissue repair in a rabbit model

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Bilayered oligo(poly(ethylene glycol) fumarate (OPF) hydrogels encapsulating gelatin microparticles (GMP) have been utilized as growth factor (GF) delivery vehicles for spatially defined osteochondrogenesis. It is hypothesized that (1) the dual release of insulin-like growth factor-1 (IGF-1) and transforming growth factor-β3 (TGF-β3) from OPF hydrogels will act in combination to improve the quality of osteochondral tissue repair compared to IGF-1 alone in vivo and (2) modulating the incorporation method of TGF-β3 can change the release profile of TGF-β3 and influence the degree of articular cartilage regeneration. To assess GF release kinetics, bilayered hydrogels were prepared with the top layer containing 125I-labeled GF-loaded GMPs and the bottom layer containing blank GMPs. Group 1 contained only IGF-1; TGF-β3 was additionally incorporated in the gel phase in Group 2; Group 3 contained both IGF-1 and TGF-β3 loaded in GMPs. The radioactivity of released GFs was counted over 28 days in vitro. A sustained and controlled release of IGF-1 and TGF-β3 from bilayered OPF hydrogels was achieved and the release kinetics of TGF-β3 were significantly changed by different loading methods. For in vivo evaluation, GF-loaded hydrogels were implanted in a full-thickness defect in rabbit femoral condyles for histological evaluation at 12 weeks. Ongoing histological analysis will show the effects of dual GF release and varying TGF-β3 release kinetics on osteochondral tissue regeneration.

08.P31
Combinational effects of BMP9, Raloxifene and Pluronic F-68 on OA-like chondrocytes

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The aim of this study was to assess combinational effects of BMP9, Raloxifene (Ral) and Pluronic F-68 (PLF-68) on osteoarthritis (OA)-like chondrocytes and develop a biomechanical model to further evaluate the results. To trigger OA-like changes, rat articular chondrocytes in agarose discs were exposed to 5-azacytidine (Aza-C). The chondrocytes were then treated with BMP9-Ral, BMP9-PLF68, Ral-PLF68 and BMP9-Ral-PLF68 for 10 days. Doses used for BMP9, Ral and PLF68 were 5 ng/ml, 5 μM and 12 mg/ml, respectively. For 30 min/day (5 days/week), dynamic hydrostatic pressure (max 0.2 MPa, 0.1 Hz) was applied to the discs. After 10 day-treatment, sGAG and collagen amounts in the discs were measured and unconfined compression stress relaxation test was performed to the discs. To analyze viscoelastic behaviour of the deposited matrix in the discs, the Kelvin model was fit to the relaxation data. Material parameters-namely relaxation time constant, τ?, instantaneous modulus, Eo and relaxed modulus, E∞ were estimated with the model. Once the model paramaters were estimated, the recovery effects were discussed both in static and dynamic loading cases through simulations. The combination groups positively affected matrix deposition by OA-like chondrocytes. Except BMP9-Ral group, the positive effects were also reflected to the material parameters. In conclusion, it can be suggested that combination groups of BMP9, Ral and PLF68 are potential candidates for restoration from OA-like changes.

08.P32
Synergistic effects of growth factors with diffusion in scaffold-free cartilage tissue under shear flow conditions

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The relationships between tissue growth, oxygen/nutrient supply, phenotypic modulation and necrosis formation is not yet clear. The balance between tissue size and the provided oxygen/nutrient supply may be crucial for life-or-death decisions for organs formed by tissue engineering-based technologies. Our purpose is to verify this hypothesis by using a scaffold-free cartilage model cultured under shear flow conditions. Since BMP-2 has a strong potential in promoting matrix production by chondrocytes, it is likely to be an important factor in determining the size of tissue engineered cartilage. Rotational shear flow culture supplies oxygen and nutrients in a simple manner; therefore, we focused on an investigation on the synergetic effect of BMP-2 with dynamic flow conditions on a scaffold-free cartilage model. The addition of BMP-2 under static conditions induced a flared and distended plate formation containing dead cells and cell-debris. However, rotational culture with BMP-2 resulted in living tissues that were flat without any flapping or distention, and also had increased chondrogenic matrix production and enhanced compressive mechanical properties. In conclusion, our findings suggest that the balance between

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tissue development by growth factors such as BMP-2 and nutrient/oxygen supply and demand levels by dynamic cultures should be considered for tissue formation in regenerative medicine.

o8.P33
The interplay between chondrocyte redifferentiation pellet size and oxygen concentration

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Autologous chondrocyte implantation and similar cartilage repair strategies require ex vivo expansion of the donor chondrocytes in order to generate sufficient cell numbers. Monolayer expansion of chondrocytes is known to result in dedifferentiation. Formation of a cell aggregate is commonly used to restore chondrogenic phenotype following monolayer expansion. Traditional pellets contain approximately 200 000 cells and have diameters of 1–2 mm. Such length scales result in the formation of diffusion gradients causing in a heterogeneous microenvironment within the pellet. We hypothesized that a reduction in pellet dimensions would minimize diffusion artifacts and result in more homogeneous tissue formation. Our studies indicate that redifferentiating chondrocytes in micropellets of 166 cells rather than in macropellets of 200 000 cells does indeed result in more homogeneous and richer matrix formation. Further, we show that these enhanced outcomes are dependent on the provision of a hypoxic atmosphere. In subsequent studies, we have demonstrated that these potent micropellets can be assembled into macrotissues. The ability to generate larger homogeneous tissues from micropellets may make this a useful platform for cartilage defect repair, either through the assembly of macrotissues in vitro or the in situ assembly of a repair tissue within cartilage defects.

o8.P34
High-hydrostatic pressurization decellularized bovine articular cartilage scaffolds for cartilage tissue engineering

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Tissue engineering is emerging as a promising method to treat cartilage diseases. Decellularized articular cartilage is an ideal scaffold for cartilage tissue engineering as it is biomimetic in both the biochemical composition and morphology. In our study, bovine articular cartilage was decellularized using a high-hydrostatic pressurization (HHP) method for use as a scaffold for cartilage tissue engineering. Under HHP, the cells in the cartilage are disrupted and these cellular components are removed with a simple PBS wash for one or 2 weeks. This method is ideal as it uses no detergents, so cytotoxicity effects are negligible. The resulting scaffolds were critical-point dried and treated with EOG gas prior to reseeding with dedifferentiated articular bovine chondrocytes. Histological and DNA analysis of HHP-treated cartilage confirmed the absence of visible cells and genomic DNA from the matrices after 2 weeks of washing, with a 93% removal after 1 week. Cells were successfully reseeded into the decellularized scaffolds. These results indicate that HHP-decelluarized scaffolds could be a useful model for the treatment of osteochondral defects through cartilage tissue engineering.

o8.P35
Stem cell-based cartilage repair in isolated articular cartilage lesions and arthritic conditions

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Mesenchymal stem cells (MSCs), nonhematopoietic progenitor cells found in various adult tissues, are characterized by their ease of isolation and rapid growth in vitro while maintaining their differentiation potential, allowing culture expansion to obtain large quantities for therapeutic use. These properties make MSCs an ideal cell type as building blocks for tissue engineering efforts to regenerate tissues and repair damaged structures encountered in isolated cartilage lesions and arthritic conditions. Promising results have been introduced in animal experiments about treatment of cartilage defects with autologous MSC implantation (AMSCI) but there are only a few case reports in humans. The purpose of this study was evaluating the effect of AMSCI on cartilage healing, in patients with isolated cartilage lesion or degenerative joint disease as a cell based treatment. MSCs impregnated collagen scaffolds were implanted into the defects in 12 patients with isolated cartilage lesion in knee or ankle. MSCs prepared as an injectable solution were injected into the joint in 12 patients with arthritic condition in knee or ankle. Functional and radiographic evaluation revealed moderate and good results at short to midterm follow up. The study revealed that autologous MSC is a promising method for stem cell-based cartilage repair.

o8.P36
Elastomeric PLCL/chitosan scaffold for cartilage repair with mesenchymal stem cells under dynamic compression

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Articular cartilage is a connective tissue which has limited self-repairing capacity. Cell-based cartilage repair, e.g. autologous mesenchymal stem cell (MSC) via scaffolds implantation has been developed for decades. However, the neocartilage could not perfectly replace the original articular cartilage for it indicates fibrocartilaginous tissue and unsatisfactory mechanical properties. Thus, an ideal scaffold for cartilage tissue engineering should exhibit appropriate mechanical responses as native cartilage does. This study focuses on an implantable porous scaffold, Poly (L-lactide-co-ϵ-caprolactone) (PLCL) for its characteristics of elasticity and biodegradation. Our previous results have shown that chitosan modified PLCL scaffolds promote biodegradation and indicate a strong ability of absorbency. Due to better MSC proliferation, extracellular matrix (ECM) deposition and mechanical strength of PLCL/chitosan constructs, we continue to use these scaffolds in a dynamic compressive cultural condition. In this study, we optimized the compressive parameters, i.e. 0.5 Hz, 5%strain, 2 h per day, and created a MSC-inducing PLCL/chitosan construct with better cell morphology and differentiation, ECM deposition and mechanical properties in vitro, which could highly meet the standard of native cartilage characteristics. Further studies will include mechanism analysis on cell adhesion and migration by examining cytoskeleton distribution, and intracellular signaling cascades.

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09. Peripheral Nerve Repair

09.01
Nerve guides using a three-dimensionally structured chitosan core with an electrospun poly-caprolactone shell for peripheral nerve reconstruction

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Clinical standard for treatment of large scale peripheral nerve defects is nerve autotransplantation. But availability of autologous nerves is limited, needs further surgical interventions and functional recovery is often limited. Here we present the development of a nerve construct consisting of an inner chitosan core having axially oriented, continuous pores made using directional solidification, with an outer shell of electrospun poly(caprolactone) (PCL) fibres. The nerve guide construct had a diameter of 1.3 mm. The chitosan core had a diameter of 0.7 mm with a pore size of 40 μm. Thickness of the electrospun wall was 0.3 mm with a fibre diameter of 3–4 μm. Pore size of the chitosan core, as well as wall thickness and fibre diameter of the electrospun shell, can be tailored as required by changing the process parameters. Adult female Wistar rats ($n = 8$) underwent unilateral 10 mm gap sciatic nerve reconstruction by either autotransplantation, transplantation of the compound construct or of the chitosan core alone in the epineurial sheath. Over 12 weeks motor nerve conduction velocity was recorded biweekly in anaesthetized animals using subcutaneously inserted needle-electrodes. Weekly, footprint analysis (Static Siatic Index, SSI) and evaluation of sensory recovery (pinch test) was performed. Direct nerve electrophysiology and lower limb muscle weight analysis will be followed by nerve morphometry to comprehensively analyse the quantity and quality of axonal regeneration.

09.02
End-to-side neurorrhaphy using an electrospun PCL/collagen nerve conduit for complex peripheral motor nerve regeneration

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In cases of complex neuromuscular defects, finding the proximal stump of a transected nerve in order to restore innervations to damaged muscle is often impossible. In this study we investigated whether a neighboring uninjured nerve and a biomaterial-based nerve conduit could reinnervate the denervated muscle while still preserving the donor muscle function. Tubular nerve conduits were fabricated by electrospinning a polymer blend consisting of poly(ϵ-caprolactone) (PCL) and type I collagen. Using a rat model of common peroneal injury, the proximal end of the nerve conduit was connected to the side of the adjacent uninjured tibial branch (TB) of the sciatic nerve after partial axotomy, and the distal end of the conduit was connected to the distal stump of the common peroneal nerve (CPN). The axonal continuity recovered through the nerve conduit at 8 weeks after surgery. Recovery of denervated muscle function was achieved, and simultaneously, the donor muscle, which was innervated by the axotomized TB also recovered at 20 weeks after surgery. Therefore, this end-to-side neurorrhaphy (ETS) technique using the electrospun PCL/collagen conduit appears to be clinically feasible and would be a useful alternative in instances where autologous nerve grafts or an adequate proximal nerve stump is unavailable.

09.03
Rescue of injured lumbar motorneurons after ventral root avulsion by grafted neuroectodermal stem cells

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Plexus injuries are based on avulsion of one or more ventral roots from the spinal cord. Such lesions lead to death of the majority of affected motoneurons. In this study we investigated whether immortalized clonal neuroectodermal stem cells applied to the injured cord in various ways posses effective neuroprotective effect on motoneurons destined to die.
Methods: The lumbar 4 ventral root of Sprague-Dawley rats was avulsed and reimplanted ventrolaterally into the injured cord. NE stem cells were injected immediately following avulsion into the L4 segment, into the reimplanted ventral root or were placed in fibrin clot around the reimplanted root. Three months after the primary surgery the L4 motoneuron pool was retrogradely labelled with Fast blue and the numbers of reinnervating motoneurons were determined.
Results: Animals that received intraspinal stem cell grafts or Riluzole treatment, had approx. 70% of their L4 motoneurons regenerated into the vacated endoneural sheaths of the reimplanted root. Intraradicular neural stem cell grafting (transplantation into the reimplanted root) resulted in moderate morphological and functional reinnervation, while both negative controls and animals with perineural stem cell treatment showed poor motor recovery.
Conclusion: Significant numbers of motoneurons can be rescued both by Riluzole treatment and stem cell grafting. Intraradicular stem cell transplantation appears to be a promising procedure to rescue damaged motoneurons.

09.04
Transitioning to a non-human primate nerve defect model for peripheral nerve repair

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Peripheral nerve injuries can occur due to trauma (including battlefield injuries, violence and vehicular accidents), as well as tumor removal or disease states. While there are several hollow tube nerve guides on the market, none are cleared for nerve gaps >3 cm in humans. The use of an autograft is currently the gold standard, but there are several disadvantages with the use of an autograft, including potential lack of sufficient tissue and mismatch of cable diameter leading to underwhelming functional recovery. We have developed a biodegradable conduit containing neurotrophic factors to enhance repair. The neurotrophic factors are encapsulated within double-walled polymeric microspheres and embedded within the walls of the nerve guide. After demonstrat-

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ing success in a critical-sized rat sciatic nerve defect model, our next step is to examine the nerve guide in a non-human primate model. After identifying the appropriate gap size of 5 cm in the median nerve, extensive animal training begins. Functionality assessment also includes electrophysiology measurements, including somatosensory evoked potentials and nerve conduction velocity. Histological analysis is conducted to determine the degree of axonal regeneration. In summary, we are currently examining the novel nerve guides in a non-human primate median nerve defect model, and we will present our initial results and describe the numerous requirements necessary to establish such a model.

09.05
Immune response to the acellular nerve xenograft laden with allogeneic adipose derived stem cells in rhesus monkey

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Introduction: Tissue-engineered nerves constructed with acellular nerve xenograft and autologous adipose-derived stem cells (ADSCs) have showed promising outcomes in rats. The aim of this study was to test the hypothesis that acellular nerve xenograft laden with allogeneic ADSCs would not elicit rejection response in rhesus monkeys.
Materials and methods: Ten adult rhesus monkeys were divided into Group A and Group B. A 25 mm-length radial nerve defect was created and repaired with acellular porcine nerve laden with allogeneic ADSCs (Group A), or acellular porcine nerve(Group B). The count of lymphocytes and T cells, percentage of $CD4^+$ and $CD8^+$ T cells, and the ratio of $CD4^+/CD8^+$ in peripheral blood was measured preoperatively and on the postoperation day (POD) 14, 60 and 90. The grafts were harvested 5 months after surgery and underwent histological examination.
Results: Lymphocyte analysis pre- and post-operation showed no statistical differences except the percentage of $CD4^+$ T cells on POD-14 in Group A was lowered. No necrosis or fibrosis was found in the grafts 5 months after surgery, only scattered $CD68^+$ and $CD163^+$ cells were revealed. The positive rate of CD68 and CD163 stain were comparable in both groups.
Conclusion: Transplantation of acellular nerve xenograft elicits neither systemic nor local immune rejection response in rhesus monkeys. Implantation of allogeneic ADSCs might result in transient depression of CD4 T cells proliferation early after surgery.

09.06
Outcomes with the use of human acellular nerve graft for repair of digital nerve defects: a prospective, multicenter, controlled clinical trial

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Peripheral nerve defects usually require nerve graft implantation. We developed a human acellular nerve graft (hANG) as an alternative to autogenous nerve and herein we report its safety and efficacy. This trial was designed as a non-inferiority study. There were 72 patients that received digital nerve repair with hANG and 81 that received conventional direct suture repair of the nerve defect under conditions of no tension. The efficacy of the treatment was evaluated by static 2-point discrimination (s2PD) and Semmes-Weinstein (SW) monofilaments testing. Safety was evaluated by local wound response and laboratory testing. Results showed that hANG group's efficacy, and the non-inferiority hypothesis was proven. The excellent and good rate of s2PD in the test group was 65.28% and 95% CI was 51.98–78.93%. During follow-up, the vital signs in all patients who were followed-up were stable with II/A healing for contaminated wounds and I/A healing for clean wounds. All results of blood tests were within the normal range. No serious adverse event or product-related event was reported. The results of this study indicate that hANG is clinically safe and effective. It was capable of returning adequate sensation in nerve defects ranging from 1 to 5 cm.

09.P01
Investigating maleic anhydride and acrylic acid plasma polymers as coatings for peripheral nerve guide conduits

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Nerve guide conduits for peripheral nerve repair are used clinically and whilst most degrade at an appropriate rate, the maximum distance for re-innervation is limited to approximately 20 mm. Coating existing FDA approved materials may be a way of increasing nerve regeneration across conduits. The aim of the present work used plasma polymerisation of acrylic acid and maleic anhydride to investigate if these surface coatings improved neural attachment, growth and differentiation. Plasma polymerisation was carried out and X-ray photoelectron spectroscopy (XPS), contact angle and time of flight secondary mass spectroscopy (ToF-SIMS) used to confirm the functional group presence. NG108-15 neuronal cells were cultured on surfaces for 4 days, fixed and stained with β-tubulin III / DAPI. Epifluorescence microscopy identified total cell number, percentage of neurons with neurites, number of neurites per neuron and length of longest neurite. XPS and ToF-SIMS demonstrated that the desired chemical functionalities had been deposited. Contact angle measurements were 58̊ for acrylic acid and 46̊ for maleic anhydride. Maleic anhydride surfaces stimulated the longest neurite lengths compared to all other surfaces, including laminin. Viability was highest on maleic anhydride and laminin surfaces. Maleic anhydride surfaces stimulate neurite formation and could be promising as coatings for nerve guide conduits, especially as the coating technique can be applied to any material.

09.P02
Amine functionalised nanodiamond for neuronal cell culture

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The unique combination of properties that diamond possesses such as its biocompatibility, high strength and tuneable electrical characteristics make it an emerging biomaterial in clinical applications such as retinal prostheses and biosensors. The ability to functionalise diamond further allows its properties to be tuned to enhance cellular adhesion, proliferation or differentiation. In this study we report the manufacture of a specialised surface based on nanodiamond particles for the culture of neuronal cells which improves cellular dendricity. Currently poly-L-lysine is used as a substrate coating to support neuronal cell culture. However its apparent cytotoxicity and animal-derived origin pose problems for *in vivo* use in applications such as nerve guidance conduits (NGCs). To achieve a nanodiamond-based neuronal substrate surface,

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hydrogenated nanodiamond was photochemically functionalised with trifluoroacetic acid protected 10-amino-dec-1-ene (TFAAD). Upon deprotection, amine-coated particles were produced which electrostatically adhered to plasma-polymerised acrylic acid coated glass, forming a coherent surface layer. Over a period of 7 days *in vitro* culture, NG108-15 cells cultured upon this surface exhibited significantly longer dendrites compared to glass and proliferated at a faster rate than upon poly-L-lysine surfaces. Primary cells (dorsal root ganglia and Schwann cells) were also successfully cultured illustrating the coating's versatility.

09.P03
Enhanced guided axonal growth through fibrin microchannels

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The ability of bioscaffolds to improve the process of neuronal regeneration of peripheral nerves was evaluated using microchannels in transparent fibrin that direct the regenerating axonal growth to penetrate through the nerve guide conduit and bridges over the nerve injury site. Fibrinogen (100 mg/ml) and thrombin (4 I.E./ml) were injected into 1.5 mm inner diameter silicone tubes and polymerized inside the tubes at 37 °C. Polymerized material was taken out from the tubes, and 20 microchannels were photoablated into the bioscaffold using a focal laser microdissector (P.A.L.M.). After the ablation fibrin constructs were washed carefully and returned into silicone tubes and covered with PBS containing 1.500 U/ml aprotinin until implantation. All was prepared under sterile conditions. Female Sprague-Dawley rats were operated under anesthesia. The sciatic nerve was exposed and sharply transected (8 mm gap), two nerve stumps were pulled into the silicone tube and tightly sutured to the bioscaffold material. After 6 and 12 weeks of healing samples were explanted and prepared for H&E and staining for S100, NF and CAB. Immunohistological evaluation showed nearly a 70% regeneration of the sciatic nerve after 12 weeks compared to the healthy site and the controls using an empty silicone tube. First results suggest an improvement of axonal growth regeneration through guided fibrin microchannels. The project was supported by the EU FP7-NMP-2007 Angioscaff' project.

09.P04
Nerve guides – the role of topography on monocyte differentiation

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Introduction: Nerve regeneration can be impaired by an uncontrolled inflammatory reaction. The key role of macrophages in the inflammatory response led us to study their cellular behavior when differentiated on distinct topographical cues.
Methods: Poly(trimethylene carbonate-co-e-caprolactone) (P(TMC-CL)) (11 mol% of TMC, Mn = 8.2E4, Mw/Mn = 1.6) solutions of 10% and 6% (w/v) in dichloromethane were used to prepare electrospun fibers and solvent-cast films, respectively. Human monocytes were isolated from Buffy coats and seeded (1E5 cells/cm^2), 24 h after, on P(TMC-CL) substrates sterilized by gamma irradiation (25 kGy), or on glass coverslips. Cells were cultured in supplemented RPMI (10% FBS) during 9 days. Cell morphology (F-actin and α-tubulin) was evaluated by confocal microscopy. Total protein was quantified from cell lysates. Secreted IL6 and IL10 were quantified by ELISA.
Results: P(TMC-CL) substrates favored monocyte adhesion, as suggested by the high protein content of cell lysates. Macrophages cultured on P(TMC-CL) fibers (1.1 ± 0.1 μm) developed an elongated morphology, parallel to fiber orientation, and accompanied by lamellipodia-like structures at the cell edge. On solvent-cast films cells are rounded, with actin-enriched podosomes-like structures at cell periphery. On fibers, macrophages release less IL6 suggesting that such substrates can minimize the pro-inflammatory response, creating a regenerative niche at the lesion site. Authors thank FEDER, COMPETE and FCT

09.P05
Multi-walled carbon nanotubes-coated phosphate glass fibers enhance the regeneration of peripheral nerve following injury; *in vitro* and *in vivo* studies

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Peripheral nerve injury is frequently seen in the clinical setting; however current medical and surgical managements are not sufficient to regenerate completely in the most cases. We developed aligned phosphate glass microfibers (PGf) which could provide a direction for the regenerating axons and enhance early functional improvements in previous studies. Carbon nanotubes (CNT) are known to promote neurite outgrowth of neuronal cells. In this study, we aimed to get any synergistic effects on the regeneration of injured peripheral nerves by applying the combination of PGf and multi-walled CNT. For *in vitro* study, dorsal root ganglion (DRG) cells from young adult rats were placed on multi-walled CNT coated PGf. For *in vivo* study, three-dimensional scaffold of CNT-coated PG fibers (CNT-PGf) was developed and inserted into completely transected sciatic nerve of rats, and PGf scaffold without CNT and collagen scaffold were also made for controls. We found that the maximal length of neurite outgrowth of DRG cells, which were cultured on CNT-PGf, were significantly higher than controls. In CNT-PGf scaffold implanted rats, the number of axons crossing transection site and distal stump of injured sciatic nerve and the cross-sectional area of plantar muscles were increased and the sensory and motor functions were recovered more than controls at 4 weeks post-implantation. As conclusion, multi-walled CNT and PGf have the synergistic effect on the regeneration of injured peripheral nerve.

09.P06
A biosynthetic hydrogel matrix used for neuronal guidance in peripheral nerve repair

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To overcome a limited regenerative capacity of peripheral nerve injuries, engineering strategies have aimed to produce nerve guidance conduits (NGCs) that can promote nerve regeneration. In this research we explored the use of semi-synthetic hydrogels as NGCs that provide precisely controlled environmental stimuli to activate neuronal and glial cell repair. The nerve conduit was comprised of proteins, including fibrinogen, gelatin, or albumin, which were conjugated to a synthetic polymer, PEG. By changing the relative amounts of protein and PEG, we achieved the desired protein composition, mechanical properties, degradation rate, and structural features of the NGC matrix. We demonstrated the capability of the PEG-Fib, PEG-Gel, and PEG-Alb hydrogels to support neurite and Schwann cell invasion from encapsulated DRGs using an *in vitro* assay. Altering the matrix properties resulted in

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different invasion kinetics of the DRG cells into the various hydrogel compositions. Accelerated directional outgrowth of neuritis was further attained by creating laser-ablated microchannels in these hydrogels. Finally, the PEG-Fib hydrogels were used to accelerate *in vivo* nerve regeneration in an 8-mm rat sciatic nerve resection model. We conclude that the structural versatility and bioactivity of this semi-synthetic NGC material is important in providing the means to control and enhance natural repair processes of injured peripheral nerves.

09.P07
In vivo evaluation of axonal regeneration using fibrin-agarose artificial nerve conduits

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Introduction: In this work, we evaluated the nerve regeneration and remyelination capability of a fibrin-agarose collagen conduit (FAC).
Materials and methods: Ten millimetre of the sciatic nerve were excised in a group of Wistar rats. Then, we surgically implanted the FAC between both nerve stumps and the animals were followed for 12 weeks. Then, tissues were extracted for histological analysis using the MCOLL histochemical method and S-100 and GAP-43 immunohistochemistry.
Results: Our results revealed that FAC promotes peripheral nerve regeneration with the formation of a thick regeneration cone accompanied by remyelination of the axonal sprouting, surrounded by collagen fibers forming fascicles. The regeneration cone and the axonal sprouting were positive for S-100 and GAP-43, thus confirming the involvement of the Schwann cells and axonal regeneration in the process of nerve regeneration.
Conclusion: Our results demonstrate that the FAC model promotes peripheral nerve regeneration and remyelination induced by the Schwann cells. In addition, fibrin-agarose hydrogels have promising results during this process, although further analyses are in need to identify the clinical usefulness of this model.
Acknowledgements: Supported by grant IPT-2011-0742-900000 from the Spanish Ministry of Science and Innovation.

09.P08
Bacterial nanocellulose scaffolds; a novel three-dimensional *in vitro* model for neuronal cell culture

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Neurodegenerative diseases, including Alzheimer's disease and Parkinson's disease, are characterized by loss of synapses and neurons. To be able to achieve a deeper understanding of the mechanisms behind neurodegenerative diseases, advanced and reliable *in vitro* models, preferable based on human cells, are needed. This project focuses on the development of an *in vitro* artificial 3D neuronal network model based on neuronal cells seeded on nano-cellulose scaffold. The viability and maturity of the neuronal cells grown on the scaffolds have been evaluated using electron microscopy, immunohistochemical- and electrophysiological methods. We have investigated the possibility to use bacterial nanocellulose (BNC) as an extracellular matrix mimic for neural cell culture. To further enhance cell attachment on the BNC we used different chemical surface modifications (THMAP and CDAP-treatment) and protein coatings such as Collagen type 1. The human neuroblastoma cell line SH-SY5Y, was used as a neuronal cell model due to its ability to be differentiated into mature neurons. With electron microscopy the cells were visualized on the material, showing that the cells were well integrated with the BNC and showed good proliferation and viability. Whole cell patch clamp recordings showed that it is possible to differentiate the SH-SY5Y cells to mature neuronal cells on the BNC, as demonstrated with an ability of the cells to produce mature action potentials.

09.P09
Functionalised degrable photocurable polymers for peripheral nerve repair

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Current Nerve Guidance Conduits (NGCs) have limited regenerative capacity, mainly due to the absence of physical guidance cues and poor support for nerve cell growth. The aim of this work is to develop NGCs with improved bulk materials, physical design and surface chemistry. Here the use of biodegradable, peptide functionalised, photocurable pre-polymers for 3D structuring via laser stereolithography is reported.Caprolactone and trimethylene carbonate pre-polymers were microwave synthesised, methacrylate functionalised and characterised by THF-GPC, MALDI-TOF, MS and NMR. Pre-polymers were UV cured into 2D sheets and 3D structures via stereolithography. Surfaces where then modified by addition of RGD / IKVAV peptides and characterised by XPS / SEM. Characterisation revealed an accurate reliable production method. *In vitro* testing included culturing, cell viability testing and immuno-fluorescence labelling of neuronal cells, rat-derived primary Schwann cells and dorsal root ganglia, demonstrating cellular adhesion and neurite outgrowth on these materials. Degradable, functionalised, photocurable pre-polymers for 3D structuring have considerable potential for the manufacture of a new generation of NGCs, combining improved physical and biochemical properties. This technique is highly adaptable and is not restricted to NGCs - with potential for a range of applications where accurately fabricated 3D scaffolds for medical device or tissue construct formation is required.

09.P10
RGD functionalised highly branched poly(N-isopropylacrylamide) (HB-p(NIPAM)) semi interpenetrating polymer networks (semi-IPNs) for peripheral nerve repair

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This work introduces highly branched HB-p(NIPAM) materials as a platform for adding peptide functionality to clinically approved polymer systems. Peptide functionalised HB-p(NIPAM) was shown previously to have minimal cytotoxicity and will lift cells from tissue culture plastic. Here we show how it can be used to modify synthetic hydrogel systems, initially with RGD functionality, to be used for nerve repair. HB-p(NIPAM) was synthesised and functionalised with RGD based peptides. Microfabrication of the HB-p(NIPAM) in clinically approved monomers using 470 nm laser and digital micromirror device (DMD) to create structures of semi-IPN. 2D sheets and 3D channels were made for *in vitro* testing with rat derived primary Schwann cells and dorsal root ganglia.It was shown that even with rigorous extraction, most of the HB-p(NIPAM) cannot be extracted from the semi-IPN. FTIR demonstrated that the LCST behaviour characteristic of p(NIPAM) was maintained when entrapped within a semi-IPN. In 2D and 3D neuronal cell culture it was shown that introduction of the RGD peptide functiona-

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lised HB-p(NIPAM) increased cell adhesion compared to the control with no RGD peptide functionalised HB-p(NIPAM).RGD-HB-p(NIPAM) can be used to increase biofunctionality in preformulated systems - in the present work as improved materials for nerve guides. The entrapped HB-p(NIPAM) cannot be fully extracted from the semi IPN and maintains it's LCST behaviour when entrapped.

09.P11
Naturally-derived hydrogels for growth factors release in peripheral nerve tissue engineering

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Introduction: Naturally-derived hydrogels are often used as localized drug depots. In this work, biocompatible and biodegradable hydrogels based on blends of natural polymers were developed for the loading and the release of growth factors (GFs) to accelerate the nerve regeneration process. Vascular endothelial growth factor (VEGF) was loaded into the hydrogels allowing the localized release of chemotactic cues.
Methods: Agar (A) and gelatin (GL) were dissolved in phosphate buffered saline (PBS) obtaining a 2% wt./vol. solution (A/GL 20/80 wt./wt.). Genipin (GP) was added as a crosslinker. Finally, VEGF was incorporated into the solution in mild conditions prior to gelification. Physicochemical, rheological and biological properties of hydrogels were evaluated. The VEGF release was quantified by Enzyme-linked Immunosorbent Assay (ELISA) and its biological activity was examined by Western Blot analysis.
Results: The developed hydrogels were found to promote glial-like cells adhesion, proliferation and to allow their migration in the inner part of the hydrogels. VEGF was successfully loaded into the hydrogels and the release of bioactive VEGF was observed until 30 days.
Conclusions: The developed hydrogels allowed the release of bioactive VEGF and can be easily inserted into a syringe and then injected into nerve guides during *in vivo* implantation. Acknowledgements:

This work was supported by BICONERVE (Regional Project, Regione Piemonte) and MOVAG (Compagnia SanPaolo).

09.P12
Bioengineered nerve conduits co-delivering synergistic neurotrophic factors with controlled release kinetics for axonal regeneration

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Repair and regeneration of injured peripheral nerves remains critical due to lack of appropriate delivery systems for controlled co-release of multiple neurotrophic factors (NTFs). We have demonstrated the synergistic function of nerve growth factor (NGF) and glial cell-line derived neurotrophic factor (GDNF) on axonal growth. To avoid unwanted branching effect and exploit the synergistic function on axonal elongation, NGF should be released at lower rate (<1 ng) and amount than GDNF (>1 ng). We have developed eight different nerve conduits (NC) loaded with both NGF and GDNF by gel spinning of collagen (Col) or silk fibroin (SF) individually or layer-by-layer (Hybrid NC) or using blends (blended NC). All NC types showed sustained release of bioactive NTFs over 28 days. SF NC exhibited significantly slower release than Col NC, although release kinetics for GDNF and NGF appeared similar. Interestingly, all hybrid and blended NC showed significantly different kinetics, and NGF was released at lower rates than GDNF, as was desired. In case of blended NC, GDNF showed a trend towards decreased release with increase in SF content. These results were further supported by porosity and swelling and thermal behaviour of NC scaffolds. SF/Col-based bioengineered NC scaffoldings provides an adaptable delivery system for co-delivery of several growth factors with predefined release kinetics and holds potential for nerve regeneration and possibly for other tissue engineering applications.

09.P13
Adipose-derived stem cells in rodents enhance early peripheral nerve regeneration

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Introduction: The aim of this study was to investigate if human or rodent adipose-derived stem cells (ADSCs) injected into a fibrin conduit enhance early peripheral nerve regeneration in an immunocompetent rat model.
Methods: ADSCs from the neck and perineural region of adult female Sprague Dawley rats and human ADSCs from deep and superficial layers of abdominal fat were isolated. In 28 female Sprague Dawley rats (four groups of seven animals, 8 weeks old) the left sciatic nerve was transected and the fibrin conduit with either culture medium alone, rat ADSCs or human ADSCs (1×106 cells) was connected to the nerve stumps. After 2 weeks, the nerves were harvested and morphological (immunohistochemistry) and imaging analysis (MRI) were performed.
Results: The isolated ADSCs were positively characterised by immunocytochemistry. Immunohistochemical analysis (S100, PGP 9.5) showed a significant longer growth cone in rat ADSCs compared to culture medium alone and human ADSCs from the superficial layer. Using a clinical 3T MRI scanner with human wrist coils we were able to visualize the graft as a small black outline and a small hyperintensity indicating the growth cone.
Conclusions: Rat ADSCs had a significant better regenerative capacity than culture medium alone or human ADSCs from the superficial layer. Also, MRI with human approved equipment was able to visualize the fibrin conduit and growth cone which can be a valuable clinical tool to measure nerve regrowth.

09.P14
Experimental use of hyaluronic acid tubes filled with 'adipose-derived adult stem cells-ADAS' to enhance peripheral nerve regeneration in lesions up to 2 cm: a preliminary report

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Introduction: Recent studies identified stem adipose-derived adult stem cells (ADAS) characterized as multipotent adult stem cells. New tubular conduits (hyaluronic acid) used after a lesion as peripheral nerve regeneration guide were filled with ADAS trying to enhance this process.
Methods: November 2009–January 2011: 30 Wistar, mean weight 180 g, divided in two Groups (control and study). Group 1: 15 rats (control) with an inverted autologous sciatic nerve graft at the left leg and hyaluronic acid tubular conduit at the right leg (study). Group 2: 15 rats (control) with an inverted autologous sciatic nerve graft at the left leg and hyaluronic acid tubular conduit addicted with autologous

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fat taken from the groin region at the right leg (study). At 7, 30, 60 days motor function was evaluated (walking test and sciatic functional index) and objectified throught a grading in four levels (poor-moderate-good-complete). At 60 days morphohistological (E/E) and immunohistochemical (s-100) studies were carried out on central and lateral sciatic nerve sections.
Results: Hyaluronic acid nerve guide allows an appreciable nerve regeneration. Our data show that further investigations are still necessary to evaluate the role of ADAS in enhancing nerve regeneration inside hyaluronic acid grow chamber.
Conclusions: Hyaluronic acid tubes are a valid option for nerve lesions up to 2 cm in length. The enrichment with ADAS, thus the wellknown regenerative properties, is nevertheless not justified.

09.P15
Human dental pulp stem cells have a positive influence on neural regeneration

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Stem cell-based therapies can be a future novel strategy to repair peripheral nerve injury, based on their multilineage differentiation potential and ability to produce and secrete (neuro)trophic factors. Recently, a possible transdifferentiation of MSC into SC have been demonstrated which had a positive effect on neuronal survival and neurite outgrowth. In this study, dental pulp stem cells (DPSC) are differentiated toward SC via a mix of growth factors. In addition the influence of secreted neurotrophic factors by DPSC and SC-DPSC on neuronal outgrowth and survival is investigated. After 21 days of differentiation, the expression of SC-markers GFAP, p75 and S100 was observed together with a decreased expression of nestin and Stro-1. Ultrastructurally, SC-DPSC displayed a spindle-shaped bipolar morphology with numerous organelles spread throughout the cell cytoplasm. Furthermore, collagen fibers were observed in the extracellular matrix. DPSC and SC-DPSC produced and secreted several neurotrophic factors which promoted the survival and neurite outgrowth in DRG cultures, with SC-DPSC yielding a significantly better effect than control DPSC. The results of this study indicate that DPSC are capable of differentiating towards SC. Moreover, differentiated DPSC had a better neuroprotective and neurotrophic effect than naïve stem cells suggesting that DPSC can be good candidates for cell-based therapies as treatment for peripheral nerve injuries.

09.P16
Effect of neurotrophic factors on Schwann cell differentiation prior to transplantation

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Schwann cells (SCs) secrete neurotrophic factors (NTs) that promote neuronal survival and guide axons during regeneration. The addition of SCs to nerve grafts is a promising strategy for enhancing peripheral nerve regeneration. To obtain a sufficient number of cells for transplant, SCs must be expanded *in vitro*. However, in our lab, we have shown that after long-term *in vitro* expansion SCs de-differentiate into an immature state. *In vivo* as axons grow, cues from the environment guide SC differentiation into mature myelinating SCs to support functional recovery. The goal of this study was to determine the effect of NTs on SC differentiation. SCs were harvested from motor and sensory branches of rat femoral nerves and expanded in culture until the cells were confluent (~30 days). Cells were then seeded in media with 0, 50, or 100 ng/ml of nerve growth factor (NGF) or glial-derived neurotrophic factor (GDNF). SC differentiation was evaluated by qRT-PCR to determine the levels of S100 (mature SC) and nestin (dedifferentiated SC) compared to fresh nerve and SCs that were freshly passaged (Day 0). The addition of NGF or GDNF increased S100 and decreased nestin expression in motor and sensory-derived SCs at 3 and 7 days. This change in gene expression suggests that NTs may provide cues to guide SC differentiation. Culturing SCs with NTs prior to transplantation may promote differentiation into mature SCs, which may increase myelination of nerves to restore function more quickly.

09.P17
Antioxidant and neuroregenerative properties of chondrotin sulfate-coated scaffolds in an *in vitro* oligodendroglial cell model

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Radical oxygen species are metabolism waste products that in excess lead to oxidative stress involved in several neurodegenerative diseases. In the CNS, oligodendrocytes are especially vulnerable to ROS. Here we studied the potential of chondroitin sulfate as antioxidant and neuroregenerative biomaterial for nerve tissue regeneration. HOG cells reach mature phenotype by replacing growth media with differentiation media. Cells are cultured and differentiated on CS- coated scaffolds or other ECM components. Oxidative stress is H_2O_2. Cell proliferation was analysed by spectrophotometry, viability and apoptosis by annexin staining and flow cytometry. ORAC method reported antioxidant capacity. HOG cells maintain their proliferative capacity up to moderate levels of oxidative stress but once differentiated moderate concentrations of H_2O_2 affect viability. Oligodendroglial cells cultured on ECM components-coated surfaces have differential cell proliferation profile which shows that GAGs facilitate cell proliferation over ECM proteins. ORAC test reports that CS has antioxidant properties lasting longer than ascorbic acid. Finally, CS exerts immediate mild neuroprotective effect on HOG cells and also promotes proliferation up to 48 h. CS presents relevant properties for oligodendrocyte protection and regeneration. This GAG acts as antioxidant and enhances HOG cell proliferation. Therefore, CS has potential value as a component of a biomaterial designed to promote remyelination.

09.P18
Extracellular-matrix mimetic peptide nanofibers for neural regenerative medicine

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Regenerative medicine studies rely on mimicking the natural extracellular matrix for promoting new tissue formation by host cells. Extracellular matrix contains an abundant variety of signals that are received by cell surface receptors contributing to cell fate, via regulation of cellular activities such as proliferation, migration and differentiation. Neural extracellular matrix (ECM) is rich in axonal growth inducer proteins and by mimicking these permissive elements in the cellular environment, neural differentiation as well as neurite outgrowth can be induced. In this study, we used a synthetic peptide nanofiber system that can mimic not only the activity of laminin, an axonal growth promoting constituent of the neural ECM, but also the activity of heparan sulfate proteoglycans in order to induce neuritogenesis. Heparan sulfate mimetic groups that were utilized in our system have affinity to several growth factors including NGF and enhance the neuroregenerative effect of laminin mimetic nanofibers. The self-assembled nanofibers with heparan sulfate mimetic and laminin derived epitopes significantly promoted neurite outgrowth by PC-12 cells. In addition,

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these nanofiber systems were even effective in the presence of chondroitin sulfate proteoglycans (CSPG), which are the major inhibitory component of central nervous system. In the presence of these nanofibers, cells could overcome CSPG inhibitory effect and extend neurites on peptide nanofiber scaffolds.

09.P19
Design of PCL and PCL/gelatin electrospun conduits for *in vivo* evaluation in rat sciatic nerve model

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Material choice plays a crucial role in ensuring the success of neural tissue engineering strategies, minimizing inflammatory response and providing the required support and guidance to regenerating axons. Here, poly-(ϵ-caprolactone) (PCL) or PCL and gelatin solutions were processed by electrospinning to fabricate fibrous conduits as artificial grafts for sciatic nerve repair after transection. We demonstrated that the integration of gelatin in PCL fibers reduced the characteristic network size scale – average diameter of 0.59 ± 0.15 μm - compared to micrometric PCL fibers (5.61 ± 0.80 μm), interacting more effectively with cells *in vitro*, due to the higher scaffold surface area. The presence of gelatin also affected the stiffness of conduits, with PCL/gelatin conduits having a smaller compressive modulus than PCL conduits in longitudinal compression tests. Conduits were also compared *in vivo* when implanted in the 5 mm rat sciatic nerve defect in an 18-week study. Animals implanted with PCL conduits showed better recovery of the injured muscle weight and electrophysiological signal, as well as more mature nerve morphology as compared to PCL/gelatin conduits. *In vivo* data suggested that bioactive signals induced by gelatin are countered by unfavorable mechanical properties of PCL/gelatin conduits. Hence, PCL electrospun conduit appears to be the more promising device to support the *in vivo* regeneration of peripheral nerve.

09.P20
Intraluminal fiber based scaffolds: a platform for cell migration and axonal regrowth and increased targeted nerve regeneration

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Introduction: This study investigates the ability of intraluminal collagen fibres (ICFs) to improve existing nerve guidance conduits (NGCs). It is hypothesized ICFs increase the surface area available for cell adhesion and migration, and provide structural cues to regenerating axons for improved nerve repair.
Method: SICFs were produced in a multi-step process and their surfaces characterised using SEM-FIB analysis. Neuronal interaction and cell migration were assessed *in vitro* using both neural and migratory cells. ICFs were enclosed within a hollow NGC, and implanted in a rat sciatic nerve model for 16 weeks. After 16 weeks, retrograde tracing and nerve morphometric analyses were carried out.
Results: In vitro assessment on the ICFs, showed a significant increase in neurite length and higher alignment versus controls, with cells successfully migrating across the fibres. After implantation, all ICF groups regenerated across a 10 mm nerve gap. ICFs showed a significant decrease in the number of misdirected axons versus autograft treatment. Conclusion:

NICFs increase aligned nerve growth and act as a platform for cell migration *in vitro*. *In vivo* ICFs significantly reduce the numbers of axons incorrectly re-innervating distal targets versus autograft repair.

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09.P21
Activated Schwann-like cells guided by fibrin structures enhance Axonal Regeneration

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Introduction: The gold standard in peripheral nerve regeneration is the autologous nerve transplant, which is limited by source. New approaches include not only nanoscaled guiding structures but also activated Schwann cells (SC) forming bands of Bungner for enhanced axonal outgrowth. In this study we characterize rat Schwann-like cells (SCLs) differentiated from adipose derived stem cells (ASCs) concerning SC markers for the use *in vivo*.
Materials and methods: SCLs were evaluated morphologically, with flow cytometry (P75, S100, MAG, P0), PCR (ATF3, cJun, PAX3) and Western Blot (ATF3, cJun, PAX3) concerning differentiation and activation status. SCLs were cultured with/without forskolin to trigger both, proliferation and potential myelination. Seeded on an electrospun fibrin matrix, SCLs were used to bridge an 8 mm dissected rat sciatic nerve.
Results: Cultured with forskolin SCLs appeared spindle-like and expressed P75, S100, ATF3 and cJun- indicating an activated status. Cultured without forskolin SCLs appeared more flat and round, expressing S100, MAG, P0 and PAX3 - indicating a promyelinating status. Grafting a sciatic nerve *in vivo*, animals treated with SCLs showed enhanced axonal regeneration.
Conclusion: It is possible, to trigger SCLs in activated or promyelinating status, similar to native SC. Activated SCLs seeded on fibrin guided structures are promising for axonal regeneration. Financial support from FFG (#818412) and City of Vienna is gratefully acknowledged.

09.P22
A living replacement tissue for peripheral nerve that can enhance regeneration *in vitro* and *in vivo*

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Introduction: A peripheral nerve repair device with the ability to enhance regeneration would be a promising alternative to nerve autograft repair. The growth of axons across a lesion is most effective when supported by columns of aligned Schwann cells that provide cell-level guidance, as found in an autograft. Here we report the development and testing of engineered neural tissue (ENT): aligned Schwann cells in a 3D collagen environment, which supports and guides neuronal growth.
Methods: Collagen gels containing F7 Schwann cells were tethered for 24 h to permit cellular self-alignment and then stabilised by rapid removal of interstitial fluid. This process generates sheets of ENT, which are stable tissue-like gels with cells organised within a 3D matrix. Cell alignment was monitored before and after stabilisation. Dissociated dorsal root ganglia neurons were cultured on the surface of the material for 3 days and neurite growth assessed. Sheets of ENT were rolled into columns and packed together to form the core of a

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repair device. Various column formats were tested in a rat sciatic nerve model.
Results: Chains of aligned Schwann cells formed within the collagen matrix and persisted following stabilisation to form a robust, aligned cellular biomaterial that promoted and guided neuronal growth *in vitro* and *in vivo*.
Conclusion: Engineered neural tissue containing aligned Schwann cells can form the basis of a functional conduit for peripheral nerve repair.

09.P23
Microanatomy of the separable length of cervical nerve 7

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Abstract not available.

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10. Axonal Regeneration and Repair in the Peripheral Nervous System

10.01
Keynote: New concepts in peripheral nerve's tissue engineering

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Although peripheral nerves have an intrinsic potential for spontaneously regenerating after trauma or disease, the clinical outcome in patients who suffered for a severe nerve lesion is often unsatisfactory. Recently much interest has been dedicated to the perspective of improving peripheral nerve repair and regeneration by means of tissue engineering and, similarly to many other fields of regenerative medicine, great expectations have risen within the general public. However, in spite of the scientific advancements, applications to the patients is still very limited and it appears that to optimize the strategy for the tissue engineering of the peripheral nerves in the clinical view, more basic science research is needed and neuroscientists have to strive for a new level of innovation which will bring together (in a multi-translational approach) different regenerative approaches. In this presentation, the basic concepts of repair and regeneration of peripheral nerves are introduced. In addition, a brief overview of the today's key strategies to peripheral nerve tissue engineering is carried out in order to throw a light on the most promising future perspectives in combining the different approaches for improving posttraumatic recovery in patients. Finally, examples of successful translational research in tissue engineering of peripheral nerves are provided together with a critical discussion on some of the pitfalls which may arise in this intriguing scientific field.

10.02
Keynote: New molecules and biohybrid templates to increase peripheral nerve regeneration

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This paper will first present two molecules as new candidates to increase peripheral nerve regeneration. At first, the 26 amino-acid-fragment, derived from the Clostridium botulinum C3 exoenzyme, which has shown good potential as therapeutic agent for the topical treatment of peripheral nerve repair sites. The 2nd molecule, the Growth/Differentiation Factor-15, GDF-15, has been found to positively influence regeneration when exogenous GDF-15 was substituted into 10 mm sciatic nerve gaps in adult rats. The paper will further introduce the BIOHYBRID consortium of eight academic partners and three small-medium-enterprises (SMEs) from Europe and Israel. Based on the on extensive basic and clinical experience within the consortium, the main objective of the BIOHYBRID project is the development of a regenerative therapy using an innovative biohybrid artificial nerve device with the goal of repairing damaged nerve trunks. The work program includes an integrated experimental approach combining: (i) reconstructive microsurgery, (ii) regenerative scaffolds and (iii) transplantation. This approach will allow the biological pre-fabrication of biohybrid nerve devices, their transplantation into nerve gaps in various animal models and the comprehensive evaluation of the regenerative outcome. A key objective of the BIOHYBRID project will be to generate a protocol that can serve as a template for future clinical trials in the regenerative therapy of damaged peripheral nerves.

10.03
Keynote: Novel approaches to accelerate axon elongation by growth factors

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Peripheral nerve lesions cause functional deficits with often serious clinical consequences. Therefore, improvement of long-distance axon growth is required for fast regeneration of axons to their targets which atrophy in the absence of reinnervation. Fibroblast growth factors (FGFs) play an important role in axon growth during brain development and regeneration in the adult nervous system. FGF-2 has been shown to promote neurite outgrowth mainly via activation of FGF receptor type 1 (FGFR1). Our laboratory focusses on the signaling pathways activated by FGFR1 to exert neurotrophic effects. FGFR1 overexpression and inhibition of receptor degradation strongly stimulate the neuronal ERK pathway and promote elongative axon growth of adult sensory neurons. Sprouty proteins act as negative feedback inhibitors of the ERK pathway. Down-regulation of Sprouty2 via transfection of shRNA promotes elongative axon growth by peripheral and central primary neurons. In response to Sprouty2 knockdown, enhanced FGF-induced activation of ERK and Ras is observed, but phosphorylation of Akt and p38 remains unaffected. Moreover, Sprouty2-knockout mice reveal improved axonal elongation *in vitro* and *in vivo*. Our results imply that Sprouty2 is highly expressed in adult peripheral neurons and its down-regulation strongly promotes elongative axon growth by activation of the Ras/Raf/ERK pathway suggesting novel therapeutic strategies to promote peripheral nerve regeneration.

10.04
Keynote: The influence of nanostructures on cellular processes during differentiation of neuronal stem cells

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Stem cell differentiation can be driven by the material /cell interface which allows for a chemistry independent manipulation of stem cells. Identification of polymers and surfaces that modulate stem cell fate have remained elusive. It was our aim, to identify specific polymers and nanostructures that modulate proliferation and/or migration of neuronal stem and that allow for a selection of functional high qualtiy stem cells for transplantational approaches. The neuronal stem cell line Ntera-2 was cultured on COP, PMMA, PS, PP, PLA, PC for 4 days. Adhe-

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sion and viability of Nteras was significantly increased when cells were cultured on corona or plasma treated COP and PMMA as compared to commercially available PS culture dishes from Nunc or Costar (242.72%, $p < 0.001$; 227.78%, $p < 0.0001$ respectively). Based on these results corona treated COP and PMMA culture plates were further processed for nanostructure studies such as pillars and ridges. A significant increase of adhesion of undifferentiated neuronal stem cells was only observed on ridges with dimensions of 1 μm width, spacing and depth. Furthermore, Nteras were found to align with the striations at these dimensions, exhibiting an elongated dipolar phenotyp. On pillars with dimensions of 1 μm width, spacing and depth cell morphologies are comparable to cells grown on smooth surfaces. Nanostructure induced morphological changes are currently being quantified with a specific imaging software.

10.05
Keynote: Extracorporeal shock wave treatment induces improved rate of functional recovery in the rat

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De-focused low energy extracorporeal shock wave therapy (ESWT) has been widely used in various clinical models such as epicondylitis and also bone and wound healing. Little is known about its effects on nervous tissue. We aimed to investigate whether ESWT improves the regeneration of injured nerves in an experimental rat model. Sprague-Dawley rats received an 8 mm long homotopic nerve autograft into the right sciatic nerve. Two experimental groups were set up: group 1 animals received ESWT (300 impulses, 3 Hz) immediately after surgery, group 2(control) animals received only autografts. Serial functional, electrophysiological and morphological investigations were carried out. Survival time was either 3 weeks or 3 months. At 6–8 weeks of survival the ESWT group of animals showed a significantly improved functional recovery relative to the controls. Electrophysiological studies at 3 weeks after surgery revealed marked values of amplitude and compound nerve action potential in the ESWT group, but there were no detectable amplitudes in the control group. Significantly greater numbers of myelinated nerve fibres in the middle of the graft and in the distal stump of ESWT animals relative to the controls 3 weeks after surgery could be found. Three months after surgery, no significant differences were observed in the functional, morphological and electrophysiological data. These results suggest that ESWT induces an improved rate of axonal regeneration and an improved rate of functional recovery.

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11. Spinal Cord Injury - Neuroreconstruction of the Acutely and Chronically Injured Spinal Cord

11.01
Keynote: Stem cells and biomaterials for the treatment of spinal cord injury

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Stem cells have been investigated for their therapeutic potential in spinal cord injury (SCI). We compared human mesenchymal stem cells (MSC) from bone marrow and a conditionally immortalized human stem cell line from fetal spinal cord (SPC-01) for their capacity to migrate towards lesion sites, differentiate and induce better regeneration. We used a balloon-induced compression lesion in rats, followed by the transplantation of MSC or SPC-01 labeled in culture with iron-oxide nanoparticles for MRI tracking. Electrophysiology was used to study the properties of SPC-01-derived neurons *in vitro*. Animals were tested using the BBB (motor) and plantar (sensory) tests for up to 6 months after acute (7 days post-injury) or chronic (5 weeks post-injury) transplantation. Animals with chronic injury were implanted with a PHPMA hydrogel seeded *in vitro* with cells. Ca^{2+} imaging on single SPC-01 cells revealed voltage-activated Ca^{2+} channels, typically observed in neurons. *In vivo* MRI proved that both MSC and SPC-01 migrated into the lesion and survived for several months. Animals implanted with MSC or SPC-01 showed functional improvement; MSC rarely differentiated into neurons, while SPC-01 implantation resulted in greater improvement and many implanted cells differentiated into motoneurons. Improved motor and sensory scores in chronic SCI were found after the implantation of biomaterials seeded with MSC or SPC-01. A clinical trial using hydrogels seeded with MSC is under consideration.

11.02
Keynote: Biomaterials for cell transplantation and drug delivery after spinal cord injury

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The development of biomaterials to serve as scaffolds for wound healing and tissue repair is crucial for successful tissue engineering. My research focuses on developing biomaterials that promote cell survival and/or differentiation after transplantation for the treatment of nerve injury. The lab has developed heparin-binding affinity-based drug delivery systems that sequester growth factors within scaffolds and release growth factors in response to cell in-growth during tissue regeneration. More recently we have combined these scaffolds with embryonic stem cell-derived neural progenitor cells and shown that the combination of fibrin scaffolds and growth factor delivery can enhance cell survival and differentiation of neural progenitor cells transplanted after spinal cord injury. Furthermore, we demonstrated this approach enhanced functional recovery after spinal cord injury, as assessed by gridwalk. In conclusion, fibrin scaffold containing our drug delivery system can serve as a platform for cell transplantation for many applications in regenerative medicine by tailoring the choice of growth factors and the cell type used. This study was supported by the National Institutes of Health, USA.

11.03
Keynote: PEG-based improvement of axon regeneration and functional outcome in chronic spinal cord trauma

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We have established a chronic spinal cord injury (SCI) and scar resection model in rat in order to identify suitable biomatrices to support axon regeneration and improve functional outcome. Five weeks after SCI the scar was resected via aspiration and the resulting cavity was filled with one of the following materials: Matrigel^{TM}, polyethylene glycol (PEG) and alginate hydrogel. The polymers were compared with respect to tissue compatibility and tested for axon regeneration promoting features. We observed remarkable polymer-specific differences regarding cell invasion, revascularization, fibroblast invasion and extracellular matrix formation. Astroglial, Schwann cell and endothelial cell invasion was increased in the PEG-grafted area, whereas at the same time fibroblast invasion and scarring was reduced. We provide the first direct comparison of these polymer matrices in chronic SCI ranking the biopolymers in the following order for their supporting effects: PEG > alginate hydrogel > MatrigelTM. PEG was superior to alginate hydrogel and MatrigelTM with respect to axon regeneration. MatrigelTM did not support axon growth in our SCI model. The PEG bridge allowed regenerative long-distance axon growth into and beyond the grafted area. The remarkable beneficial effects of PEG implantation in chronic SCI were accompanied by long-lasting functional improvement. Supported partly by the Medical Faculty of the University of Düsseldorf and the German Paraplegia Foundation (DSQ).

11.04
Keynote: Bridging the spinal cord –a step towards functionalisation

A Pandit
Network of Excellence for Functional Biomaterials, National University of Ireland, Ireland

The development of nerve guidance conduits is constantly evolving as the need arises for therapies for spinal cord injury. In addition to providing a path for regrowing axons to reconnect with their appropriate targets, the structural and biochemical cues provided by these conduits need to be permissive for directional neurite outgrowth and be protective against inhibition in the vicinity of the injury site. We have developed biomimetic substrates by exploiting iso-electric focusing to drive the alignment of supramolecular fibrils into self-assembled hydrogels (300 μm diameter), and tested these hydrogels for the ability to direct and enhance the migration of neurites. Structural characterization revealed anisotropic alignment of nanofibrillar aggregates, arranged in micron-scale bundles similar to the hierarchical size scales observed in native tissues. Neurite outgrowth extended bidirectionally along the axes of aligned hydrogels. Furthermore, it was shown that, as opposed to poly-D-lysine, neurite outgrowth on aligned hydrogels is not inhibited in the presence of myelin-associated glycoprotein ($p > 0.05$). These results highlight for the first time a structural and biochemical role for aligned hydrogels in controlling neuronal growth, and indicate

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that the short-term signaling associated with these hydrogels can be used in adjunct therapy following injury to the spinal cord.

11.05
Altering secondary injury: How hydrogel scaffolds influence the inflammatory response in the spinal cord

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The treatment of spinal cord injury has proved resistant to therapeutic strategies due to the complex inflammatory reaction and inhibitory factors to regeneration. Secondary injury, which increases the region of functional deficit and defines the inhibitory boundary of the astrocytic scar is largely driven by excitotoxicity, ischemia and inflammatory processes. This study compares short time points to gather insights into the innate immune reaction associated with implanting a foreign material into the spinal cord. Within the injury parenchyma and scaffold microenvironment, the recruitment of neutrophils into the non-degradable pHEMA hydrogel was rapid and after 24 h the scaffold was filled exclusively with these cells. Migration of activated macrophages into the scaffold was apparent after 3d, with many of these cells seen in the highly vascularised grey matter. Significantly greater gene expression of TGF-b after 6d occurs with scaffolds implanted in both the grey and white matter. TGF-b is recognised as an important inflammatory mediator in this injury scenario, and influences the remodelling of ECM after injury. Importantly, small injury models could distinguish the molecular environment attributed to TE scaffold implantation. Whilst similar levels of neutrophils and macrophages invade the TE scaffold and lesion controls, a differing molecular environment is observed surrounding cell-invasive scaffolds implanted in the spinal cord that has implications on secondary injury.

11.P01
Therapeutic effects of time window for human umbilical cord blood-derived mesenchymal stem cells with methylprednisolone treatment in the contused rat spinal cord

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Methylprednisolnone (MP), a glucocorticoid steroid, has an anti-inflammatory action and seems to inhibit the formation of oxygen free radicals produced during lipid peroxidation in a spinal cord injury (SCI). Currently MP is the standard therapy after acute SCI on reported neurological improvements. The combination therapeutic effect of human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) for transplantation time (1d, 7d, and 30d) after MP treatment on the axonal regeneration and on the behavioral improvement in SCI were studied in the rat. The spinal cord was injured by contusion using a weight-drop at the level of T9 and MP (30 mg/kg, i.m., 10 min and 4 h) was acute administered after injury. hUCB-MSCs were labeled GFP and our study was performed the efficacy for transplantation time (1d, 7d, and 30d) of hUCB-MSCs into the boundary zone of injured site. Efficacy was determined by histology, anterograde and retrograde tracing, and behavioral test. We found that hUCB-MSCs with MP treatment exerted a significant beneficial effect by neuroprotection and reducing cavity volume. Also the transplantation of hUCB-MSCs with MP treatment was significantly improved functional recovery. Combined transplantation at 7d after SCI provided significantly greater efficiency than combined transplantation at 1d and 30d. These results suggest that transplantation time window of the hUCB-MSCs with MP treatment give rise to an earlier neuron protection strategy and effect of cell.

11.P02
Therapeutic effects of brain-derived neurotrophic factor-expressing human umbilical cord blood-derived mesenchymal stem cells in the contused rat spinal cord

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Mesenchymal stem cells (MSCs) transplantation has been proposed as a therapeutic strategy for spinal cord injury (SCI) repair. The aim of this study was to evaluate the efficacy in adult rat contused spinal cord of adenovirus vector-mediated brain-derived neurotrophic factor (BDNF) gene transfer to human umbilical cord blood (hUCB)–derived MSCs (UCB-MSCs). We efficiently were engineered UCB-MSCs secreted of BDNF (MSC-BDNF) via adenoviral transduction mediated by cell-permeable peptides. SCI was induced by contusion using a weight-drop at the level of T9 and then the MSC-BDNF (3×10^5) were transplanted into the boundary zone of injured site at 1 week after injury. BDNF production by MSC-BDNF was greater than seen in uninfected MSCs. MSC-BDNF showed significantly more functional recovery than did control rats following SCI. MSC-BDNF at 6 weeks after transplantation were found to survive and preferentially localize to near the injury site. Also these cells were differentiated neurons and astrocytes. These data suggest that MSC-BDNF may be useful in the treatment of SCI and may represent a new strategy for the treatment of SCI.

11.P03
Transplantation of human mesenchymal stem cells and syngeneic macrophages into mice with contusive spinal cord injury

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Transplantation of a variety of cell types induce favorable effects in animals with experimental spinal cord injury (SCI). Here we demonstrate that so does transplantation of human placenta mesenchymal stem cells (hpMSC) or syngeneic macrophages directly to the injury site in mouse contusive SCI model. SCI was produced by the impact of a load falling onto the open dorsal surface of the spinal cord causing limited tissue necrosis, demyelination, inflammation, and scar formation. Functional damage included paraplegia and pelvic problems. Transplantation of hpMSC or activated murine macrophages attenuated SCI-induced neurological deficit. Some hpMSC remained at the injury spot, but most cells migrated within the spinal cord and, surprisingly, to certain remote brain locations including hippocampus. Only a small fraction of hpMSC could be detected at the injury spot and throughout spinal cord and brain 45 days after transplantation. Some of them expressed neuronal or astroglial markers but never showed typical neuronal or glial morphology. Hence, beneficial effects of hpMSC transplantation are unlikely to be associated with substitution of damaged cells. It is likely that human placenta cells facilitate regrowth of axons through the injury site and renovation of the pools of host's neurons and glia. Activated macrophages probably participate in the reshuffling of scar tissue facilitating axon regrowth. Com-

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bined transplantation of MSC and macrophages may have clinical potential.

11.P04
Combination of human eyelid adipose stem cells implantation and 17-beta-estradiol administration improve spinal cord injury repair

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Stem cell transplantation and pharmaceutical administration represent two promising strategies for the repair of spinal cord injury (SCI). However, the synergistic effect of the combination of these two approaches has yet to be evaluated. Here, we report that 17-β-estradiol (E2) administration together with human eyelid adipose-derived stem cells (hEASCs) transplantation synergistically improved the functional repair of SCI. Our results showed that E2 boosted the survival of grafted hEASCs by reducing apoptosis through regulating the expression of caspase-3 and bcl-2. E2 also increased the secretion of growth factors by hEASCs. The survived hEASCs preferentially differentiated into neurons instead of glias. Furthermore, E2 combined with hEASCs transplantation synergistically reduced cavity formation and promoted axon remyelination as well as motor function recovery. Our study demonstrated that combination of estrogen administration and hEASCs transplantation improved functional recovery of SCI. It provides a promising chemo-cell cocktail strategy for the treatment of incurable neurodegenerative diseases.

11.P05
Biomodified hydrogel scaffolds for neural differentiation and the treatment of spinal cord injury

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Spinal cord injury (SCI) is accompanied by tissue damage and the formation of physical and biochemical barriers that prevent axons from regenerating. To create scaffolds that would bridge the area of injury and promote neural tissue regeneration, poly(2-hydroxyethyl methacrylate) (PHEMA) hydrogels modified with laminin-derived peptide sequence IKVAV (Ile-Lys-Val-Ala-Val) have been developed. In an *in vitro* study, IKVAV-modified hydrogels were shown to support cell attachment and proliferation, as well as the differentiation of rat adipose-derived stromal cells into a neuronal phenotype. In an *in vivo* study, hydrogels with parallel oriented channels were implanted to bridge the spinal cord lesion cavity. Histological evaluation was done 1–6 months after implantation and revealed the good incorporation of the implanted hydrogel into the surrounding tissue and the ingrowth of neurofilaments and blood vessels into the hydrogel channels. The parallel orientation of the hydrogel channels promoted axonal sprouting across the lesion; however, the number of axons observed within the scaffolds 2 months after implantation decreased after 4 and 6 months. The results show that IKVAV-modified PHEMA hydrogels are able to bridge a spinal cord lesion. Nevertheless, the combination of scaffold bridging with further therapeutic approaches is essential to achieve effective spinal cord repair. Supported by: P304/11/0731, P304/11/0653, P304/11/P633, IAA500390902, 10810/1560, KAN200520804.

11.P06
Multiple drug delivery hydrogel system for spinal cord injury repair strategy

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Spinal cord injury (SCI) remains one of the most devastating conditions among neurological diseases, due to the acute and long-term health effects, the reduced quality of life and the high economic impact on society. The primary target for neuroscientists is to obviate the mechanisms of secondary injury to minimize its pathological consequences. This issue can be addressed by using polymeric smart drug delivery systems, providing local multiple administration able to reduce systemic side effects of drugs and synergize treatment efficacy. The *in vivo* testing in animal SCI models was approached with the aim to demonstrate the complete neutrality of this gel with respect to the host tissue. Biocompatibility was assessed by examining different time of gel injection, in according with a translational clinical approach and no significant differences were shown in terms of behavior and histological analyses. Furthermore, to achieve multiple release profiles, the combination of fast diffusion-controlled release of low steric hindrance molecules and slow drug release of high steric hindrance ones was here investigated *in vitro*, ex vivo and *in vivo*. Release studies showed an independent combination of fast diffusion-controlled kinetics for small molecules (mimicking drugs typically used for short term neuroprotection purposes), together with slow ones for compound with high steric hindrance (e.g. antibodies), such as those used for long term neuroregeneration approaches.

11.P07
Functional recovery of spinal cord injured rats after implantation of a novel biodegradable 3D scaffold

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Spinal cord injury represents a major world health problem. In this sense, the objective of this work was the development and characterization of a new range of 3D hybrid structures, based on rapid prototyped starch scaffolds, aimed at offering spine stabilization, and gellan gum hydrogels, aimed at inducing the regeneration within SCI sites. Six different 3D scaffold designs were developed and characterized. The histomorphometric analysis showed a interconnected network of pores with porosity ranging from 70% to 85%. Cytotoxicity assays revealed that the hybrid SPCL/Gellan Gum scaffolds displayed a nontoxic behaviour. Moreover, to enhance cell adhesion, the gellan gum was modified using click chemistry with a fibronectin-derived peptide (GRGDS). Amino acid analysis demonstrated that 328 nmol/ml of GRGDS was immobilized to the gel. Moreover, this modification had a profound influence on NSCs and MSCs growth, morphology and secretome profile. Afterwards, the *in vivo* evaluation revealed a good integration and an absence of inflammatory response to the scaffolds implantation. Furthermore, we show a correlation between spine stabilization and motor

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recovery after SCI in rats. Moreover, the peptide modification in the hydrogel demonstrated to highly improve its capacity to promote neuronal repair. Animals showed significant improvements on BBB scale, rearing activity, and motor coordination. These results indicate that this strategy may have therapeutic benefit for SCI repair.

11.P08
Biodistribution and preliminary therapeutic potential of CMCht/PAMAM dendrimer nanoparticles administration in rats

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The development of drug delivery systems (DDS) for targeted intracellular delivery of therapeutic agents has been attracting great deal of attention. In traumatic central nervous system conditions, where therapies have been revealing to be highly inneficient and non-specific, these targeted DDS could be highly beneficial. We have previously shown *in vitro* studies where the carboxymethylchitosan (CMCht)/ poly(amidoamine) (PAMAM) nanoparticles (NPs) were able to be uptaken by different cell types (neurons and glial cells), while not evidencing any cytotoxicity. In the present study, *in vivo* biodistribution of the CMCht/PAMAM NPs was investigated. Following intravenous injection in adult male Wistar rats, the NPs showed to be stable in circulation and able to be internalized by cells from different tissues (e.g., brain, liver, kidney and lung). Afterwards, methylprednisolone (MP)-loaded fluorescently-labelled NPs were administered in the cerebrospinal fluid of the cisterna magna of adult male Wistar rats. Upon the intracisternal injection, NPs were detected throughout the cortical and parenchimal areas of the brain, namely in the prefrontal cortex, hippocampus and periventricular areas after 24 h. More recently, ongoing studies are focusing on the therapeutic value of these methylprednisolone-loaded NPs administered following a spinal cord lesion in rats. Significant differences in the BBB locomotory test were found in MP-NPs treated rats 1 month after injury.

11.P09
CLMA scaffold and FM19G11 for a proper spinal cord-derived neural progenitor niche

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Spinal cord injury (SCI) is a major cause of paralysis. Currently, there are no effective therapies to reverse this disabling condition. Recently we have shown that acute transplantation of ependymal stem/progenitor cells (epSPC) –spinal cord-derived neural precursors- rescue lost neurological function after SCI in rodents. However, in a chronic scenario, with axon repulsive reactive scar, a combinatorial approach is necessary. The integration of biomaterial scaffolds for a physical support with the cell-replacement therapy and pharmacological treatments to protect and induce neuronal survival offers a good formula for chronic SCI regeneration. Here we show a new application of caprolactone 2-(methacryloyloxy)ethyl ester (CLMA) as porous scaffolds. The epSPC can growth and expand into the scaffolds in the presence of EGF and FGF, however significant reduction on the cell population was achieved after 6 days *in vitro* (DIV). FM19G11, first described as a HIFa protein inhibitor, which is able to allow progenitor cells to differentiate under hypoxia, under normoxic conditions induces self-renewal. epSPC growth in CLMA scaffolds in the presence of FM19G11 significantly divide more at 6 DIV than the ones treated with vehicle alone. Stronger protein expression of oligodendrocyte precursor cells occurs by FM19G11 treatment. Overall, epSPC seeded in CLMA scaffolds and activated by FM19G11 would offer a good combination for the chronic SCI treatments. Work supported by FISS PI10/01683.

11.P10
Influence of porosity and surface modification of biocompatible hydrogels on mesenchymal stem cell survival and tissue repair in a model of spinal cord injury

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Combined therapy using hydrogels and stem cells seems to be a promising approach to restoring damaged tissue following spinal cord injury (SCI). Mesenchymal stem cells (MSC) were seeded on 4 different hydrogels: hydroxy propyl methacrylate-RGD prepared by heterophase separation (HPMA-RGD-HS) and 3 other hydrogels polymerized in the presence of a solid porogen: HPMA-SP, HPMA-RGD-SP and hydroxy ethyl methacrylate [2-(methacryloyloxy)ethyl] trimethylammonium chloride (HEMA-MOETACl). Cell adhesion and survival were evaluated 1, 7 and 14 days after the seeding of MSCs on the hydrogel scaffolds. HEMA-MOETACl and HPMA-RGD-SP hydrogels were superior in the number of cells attached *in vitro*. Cell-polymer scaffolds were then implanted into hemisected rat spinal cords for 1 month. The best survival of MSCs was found in HEMA-MOETACl hydrogels; however, only a small ingrowth of blood vessels and axons was observed. Both HPMA-SP hydrogels showed better survival of MSCs compared to the HPMA-RGD-HS hydrogel. Both RGD-attached hydrogels contained significantly higher numbers of blood vessels. Further, both HPMA-SP hydrogels promoted a significantly greater ingrowth of axons into the implant. Our results demonstrate that the physical and chemical properties of HPMA-RGD-SP hydrogels result in the best combined effect in serving as a stem cell carrier and a bridging material for the treatment of SCI.Supported by: AV0Z50390703, IAA 500390902, P304/11/P633 and P108/10/1560

11.P11
Development of 3D co-culture of aligned glial cells in nanofibre scaffolds for implantation into neural injury sites

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Research into the utility of nanofibres in neural tissue engineering has overwhelmingly relied on the use of basic 2D culture models employing cell lines and monocultures. This 'proof-of-principle' study reports the fabrication of 3D nanofibre-hydrogel constructs for alignment and co-culture of the major glial cell subpopulations (viz. astrocytes, OPCs/oligodendrocytes and microglia). Electrospun aligned, fluorescent PLA nanofibres were transferred to portable acetate frames that permitted their layering onto hydrogels. A single nanofibre layer was used to quantify the elongation (aspect ratios) of individual cells from pure, primary astrocyte, OPC cultures, or their co-cultures, compared with control hydrogel constructs. Astrocytes showed attachment and alignment on nanofibres and displayed healthy nuclei with evidence of cell proliferation. OPCs showed poor survival on hydrogels alone or those containing nanofibres. However, the survival, proliferation and elongation of OPCs was pronounced in co-cultures with astrocytes, with differentiation into oligodendrocytes after an extended culture period.

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Preliminary feasibility of addition of microglia to astrocyte-OPC co-cultures is demonstrated, along with the alignment of astrocytes in multi-nanofiber layers. This approach provides evidence of the feasibility of developing a portable, functionalised nanofibre construct, of enhanced cellular complexity with potential for implantation into neural injury sites such as spinal cord.

11.P12
Olfactory Ensheathing Cells (OEC) plus fibroblast growth factor (a-FGF) and fibrin glue (FG) implantation in a model of transected spinal cord in adult rats.

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The olfactory system is well known as one of the places where neurogenesis in the nervous system takes place in adult mammals. Different studies of spinal cordlesions report how the use of these cells has been found to help with axonal regeneration and mobility improvement. Bulb and Laminar olfactory cells, combined with a-FGF and FG, from male adult rats with spinal cords transection lesions were used in this study where three groups are compared: a control one, another where only cells were transplanted and a third one where the cells combined with a-FGF and FG were transplanted and signs of locomotor functional recovery were examined using the Basso Bresnahan and Beattie (BBB) scale, adding the analysis of the track left on paper in each walk. Results obtained from the BBB scale show that the combination of ensheathing olfactory cells and a-FGF plus FG significantly improves (12.50 ± 3.51) the locomotors function when compared with the other experimental groups. (OEC: 6.75 ± 0.957 and a-FGF plusFG: 6.00 ± 1.414). Histological cuts suggest a reconnection of the two ends of the transection. The study confirms that olfactory ensheathing cells improve functional locomotor recovery in paraplegic rats and suggests that a-FGF plus FG can increase the effect of these cells, possibly because they stimulate cellular proliferation. The results of the combination of olfactory ensheathing cells and a-FGF plus FG as a therapeutic strategy.

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12. Spinal Cord Injury (in coop. Wings for Life)

12.01
Keynote: A novel self-assembling peptide attenuates glial scarring and promotes neurological recovery following spinal cord injury

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The pathophysiology of spinal cord injury (SCI) involves post-traumatic inflammation and glial scarring which interfere with repair and recovery. Self-assembling peptides (SAP) are new molecules designed for tissue engineering; they form biocompatible nanofibers to support cell growth. Here, we tested the ability of QL6, a novel SAP to attenuate inflammation and glial scarring and to facilitate functional recovery. We successfully injected QL6 into the rat spinal cords 24 h after SCI. GFAP, Iba1 antibodies and TUNEL immunostaining were used to evaluate astrogliosis, inflammation and apoptosis, respectively. QL6 led to a significant reduction in post-traumatic apoptosis, inflammation and astrogliosis. It also resulted in significant tissue preservation as determined by quantitative histomorphometry. Furthermore, QL6 promoted axonal preservation, demonstrated by BDA anterograde and Fluorogold retrograde tracing. *In vitro* and *in vivo* electrophysiology confirmed that QL6 led to significant functional improvement of axons, including increased conduction velocity, reduced refractoriness, and enhanced high frequency conduction. These neuroanatomical and electrophysiological improvements were associated with significant neurobehavioral recovery as assessed by the BBB technique. As the first detailed examination of the pathophysiological bases of QL6 in SCI, this work highlights the therapeutic potentials of the SAP in this devastating disorder and other forms of CNS injury.

12.02
Keynote: Combined treatments to repair the injured spinal cord

K Fouad
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Following spinal cord injury in adult mammals axonal regeneration is restricted by multiple factors. These factors include myelin-associated inhibitors, a dense scar tissue surrounding the lesion site, cavitation, intracellular changes, and the lack of growth promoting neurotrophic factors. Thus, it appears intuitive that addressing only a single factor results in only very limited axonal regeneration. Consequently, combined approaches addressing various growth limiting factors in one treatment promise to be more effective and should result in enhanced functional recovery. However, such approaches have been proven to be challenging. Here we will present results from a combination of cell grafts and various growth promoting treatments following complete and incomplete spinal lesions in adult rats. Our results demonstrate that especially a subset of axonal populations can be encouraged to grow into and beyond a cellular graft and that this regeneration is accompanied by moderate functional recovery. However, our results also indicate that some treatment components that maybe beneficial on their own, can interfere with recovery when combined with others. Thus, in the future more of such combinations have to be tested and detailed studies with a large amount of control groups are needed to develop safe treatments for individuals with spinal cord injury.

12.03
Keynote: Axonal regeneration across cellular bridges in the lesioned spinal cord – challenges on the other side

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Over the last decade, there has been substantial progress in enhancing axon regeneration in animal models of spinal cord injury. Several experimental approaches have targeted multiple mechanisms of axon regeneration failure to maximize the intrinsic growth capacity of injured neurons, to overcome inhibitory influences in the injured spinal cord and to stimulate growth at the injured axon tip. We have investigated in detail regeneration of dorsal column sensory axons stimulated by gradients of neurotrophin-3 and enhancement of the intrinsic regenerative capacity by conditioning lesions and increases in cellular cAMP levels. Using such combinatorial approaches, axons can extend for several mm beyond a lesion site in the acutely and chronically injured spinal cord and reinnervate their original target nucleus in the medulla. However, despite the formation of new synapses, electrophysiological recovery is not observed, possibly due to a lack of remyelination. Regenerated axons that do not form axodendritic synapses also appear to be highly dependent on the continuous presence of neurotrophic factors. Taken together, these data highlight the complexity of axon regeneration to result in functional recovery after spinal cord injury.

12.04
Keynote: CNS remyelination - can we wrap it up? Regulators of oligodendrocyte precursor cells in the process of myelin regeneration

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The process of myelin regeneration (also termed 'remyelination') in the CNS is mediated by a pluripotent adult stem-precursor cell population commonly referred to as oligodendrocyte precursor cells (OPCs). Successful remyelination relies on the recruitment of OPCs into areas of demyelination, their subsequent engagement of denuded axons and their differentiation into mature oligodendrocytes. Although spontaneous remyelination can occur in the adult CNS it often fails leaving axons denuded and thus prone to chronic degeneration. Enhancing remyelination in the central nervous system by stimulating the differentiation of OPCs has been recognised as an important strategy to ameliorate the devastating consequences of a number of neurological diseases, including spinal cord injury. An important strategy to promote CNS remyelination is to enhance the differentiation of the endogenous OPC population into oligodendrocytes. Here a series of experiments will be presented demonstrating that environmental factors in the CNS exert inhibitory effects on OPC differentiation. The identification of lesion associated inhibitory factors enables to neutralise of effects and to enhance OPC differentiation in vitro. Furthermore, overcoming the inhibitory effects in vivo is able to accelerate the differentiation of OPCs into oligodendrocytes and promote CNS remyelination.

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13. Skin Regeneration

13.01
Keynote: A versatile, biodegradable polyurethane scaffold for tissue repair and drug delivery

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We are investigating the basis of regeneration in the MRL/MpJ mouse (MRL) through gene expression profiling, proteomics, stem cell biology, scaffold development, and drug delivery. The scaffold for validation is a biocompatible polyurethane foam (PUR), tunable for mechanical, drug delivery, porosity, and biodegradation properties. The PUR scaffold can also be formed in situ as an injectable material. PUR peptide delivery is effective in soft and hard tissue, making the foams versatile, inductive tissue templates for tissue repair. MRL mouse mesenchymal stem cells (MSC) enhance wound healing, due in part to over expression of a Wnt inhibitor, as shown by gene transfer and mimicked by a small molecule, pyrvinium. Cell- and drug-based strategies reveal the usefulness of PUR scaffolds for delivery of repair agents that recapitulate the MRL regenerative phenotype. The PUR foam has been validated as a useful dermal scaffold in the mouse, rat, and pig, although the macrophage-driven degradation kinetics varies among species. PUR scaffold is capable of robust (>80–90%), long-term (>3 weeks) suppression of target genes *in vivo* through delivery of interfering RNA. Synthetic scaffolds offer a chemically defined means of creating a cellular microenviroment that can be tuned to a wide variety of tissue repair applications. Supported by the NIAMS (JMD), Department of Veterans Affairs (JMD,PPY), NIDDK (CLD), Vanderbilt Discovery Grant (CLD), NHLBI (PPY), NIBIB (CLD), NSF (SAG), and AFIRM (SAG).

13.02
Development of a vascularized skin construct using adipose derived stem cells from debrided burn skin

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Current skin substitutes used to treat large surface area burns are limited due to the availability of autologous cells and tissue. The standard of care involves surgical debridement of necrotic tissue along with the removal of subcutaneous layers. We hypothesize resident stem cells within the subcutaneous adipose tissue survive and used as a cell source to develop a tissue-engineered skin equivalent. Debrided skin was collected from severely burned patients (HSC20080290N) and Adipose Stem Cells (dsASCs) isolated and characterized. The skin equivalent contained a collagen–polyethylene glycol fibrin (FPEG)-based bilayer hydrogel which differentiate dsASCs to form a vascularized dermis and epithelial layer. RT-PCR and immunocytochemistry of lineage specific markers confirmed the dsASCs differentiation into stromal, vascular and epithelial phenotypes. The dsASCs proliferated and remained spindle-shaped in collagen, developed a tubular microvascular network in FPEG and, when induced, a cuboidal keratinocyte-like morphology on collagen. RT-PCR and immunocytochemical analysis showed a matrix and time-dependent change in the expression of differentiated cell markers. dsASCs isolated from debrided skin can be used as a single autologous cell source to develop a vascularized skin construct, without the addition of exogenous growth factors, in a matrix dependent fashion. This technique provides an approach to treat extensive burn skin injuries and potentially enhance graft success.

13.03
Allogeneic living cell spray: a new concept in the treatment of wounds

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Currently available cell-based therapies have been successfully used to treat a variety of injuries. Despite interesting results, new approaches continue to emerge, such as cell spray treatments which are a potentially viable improvement to standard grafting procedures. An allogenic living cell spray (ALCS) has been developed for the treatment of chronic venous ulcers. It consists of two components: growth arrested allogeneic keratinocytes and fibroblasts suspended in thrombin and cryoprotectant and a solution of fibrinogen. The product is stored frozen at -80 °C until use. The ALCS technology is a novel concept in the treatment of hard to heal ulcer: (i) the fibrin produces a biocompatible matrix allowing the embedded cells to secrete and locally deliver growth factors and ECM components critical to the wound healing process, (ii) the cells are a key component to the efficacy of this product, optimal proportion and quantity has been defined, (iii) the growth arrest allow a controlled dose of secreted growth factors by preventing cell proliferation, (iv) the freezing process allow a long term storage of the product with consistent properties, (v) the spray provide an easy handling and delivering to the wound. A multicentre randomised dosing trial using ALCS has been completed showing a strong healing stimulation of healing for chronic VLU when compared to placebo. Future challenges and opportunities for improving wound care using this technology will be addressed.

13.04
Skin tissue engineering in burns

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Skin grafts are still the gold standard in the treatment of deep skin defects. However, in patients with more than 50% burned skin area, donor sites are limited. Hence the need for cost effective and user friendly synthetic or engineered skin grafts, which can serve for acute and chronic wounds and which can be also used in critically ill patients, is at hand. During the last 30 years a huge number of biological and synthetic skin graft materials and products based on the patient's own cells were launched on the market. Researchers and clinicians are constantly working on further improvements. One possibility is the engi-

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neering of skin grafts in vitro, which have to be integrated into the wound bed after transplantation. Another approach is the fabrication of biocompatible and bioresorbable matrices, which can attract host cells and stimulate a wound-healing process without scars. However, the skin graft materials available today cannot yet replace skin grafts completely because of their inherent limitations. Thus researchers in the field of skin tissue engineering are still working on the final goal of developing a skin graft which has all the features of healthy human skin and is capable of replacing human skin completely. This presentation gives on overview of the currently available solutions and products in the field of skin tissue engineering.

13.05
Development, validation and testing of a human tissue engineered hypertrophic scar model

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A major problem in burn wound care is the quality of the scar. Development of new therapies is hindered by a lack of relevant models. We developed an *in vitro* tissue engineered human hypertrophic scar (HTS) model derived from a reconstructed epidermis on a dermal matrix containing adipose mesenchymal stem cells (ASC) for testing therapeutics. Similar to HTS the model showed increased thickness of the dermis, collagen 1 secretion, contraction and number of epidermal cell layers and decreased degree of epithelialisation, IL-6 and CXCL8 secretion. The HTS model was first validated with known scar therapeutics: 5-fluorouracil (5FU) and triamcinolone (TC), and a therapeutic known not to be effective in scar reduction: 1, 25-dihydroxy vitamin D3 (VitD3). Both treatment with 5FU or TC partially normalized two different parameters: contraction, epidermal thickness or collagen 1 secretion. VitD3 did not correct scar phenotype in the model. Potential novel scar therapeutic atorvastatin normalized the abnormal thickness of the dermis, epidermal thickness and CXCL8 secretion. In line with the clinical experience, 5FU and TC were not able to totally restore HTS to normal skin. Each therapeutic selectively effected a different combination of parameters. As expected, VitD3 was not effective in scar reduction. Atorvastatin may be a new scar therapeutic. Our results suggest that the *in vitro* HTS model can be used to test new anti-scar therapeutics and test combinations of existing therapeutics

13.06
Wnt signalling as a target for skin regeneration

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Aberrant or incomplete wound healing in the skin impart significant economic and social burdens on the health of human populations. Therapies that expedite wound healing or stimulate the regeneration of functional skin would alleviate many of these problems. We hypothesize that the Wnt signalling pathway is a target for such therapies. To determine whether elevated Wnt signalling promotes wound healing and tissue regeneration in the skin, we created full-thickness skin wounds in the back skin of mice heterozygous or homozygous for the replacement of Axin2, a negative regulator of Wnt signalling, with LacZ (Axin2LacZ/+ or Axin2LacZ/LacZ respectively). We also investigated the therapeutic potential of Wnt elevation at injury sites by delivering exogenous liposomally-packaged Wnt3a to skin wounds at various time-points post-injury. We found that following wounding, Axin2 promoter activity was increased in cells of both the dermis and epidermis at the wound periphery, and in cells of the granulation tissue. There were no significant differences in the rate of re-epithelialization between groups but we found a significant increase in hair follicle neogenesis in Axin2LacZ/LacZ compared to Axin2LacZ/+ mice, or following injection of Wnt3a liposomes. We conclude that while increased Wnt signaling does not stimulate re-epithelialization of wounds in healthy mice, it promotes hair follicle neogenesis. This suggests Wnt signalling is an attractive target for skin regenerative therapies.

13.P01
Isolation and culture of human keratinocytes and epidermal stem cells for clinical purposes

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Keratinocytes and epidermal stem cells have been under extensive investigation due to their increasing potential and application in medicine and biotechnology. Cultured epidermal cells are used in treatment of chronic wounds, as well as they became a target for gene therapy and toxicological studies. In this paper we present methods of cell isolation and culture with the following technique of implementation of cultured cells to the patient. The applied enzymatic method, allows to obtain an average of $1.4 \pm 0.28 \times 10^6$ cells from 1 cm^2 of skin, with cell viability over 96%. Routinely, cell for patients were cultured in medium KBM/KGM (without serum and bovine pituitary extract), until the second passage. These cells were characterized by low differentiation status (high expression of beta-1 and alpha-6-integrins and low expression of involucrin), without features of apoptosis. Additionally, these cells displayed high mitotic activity (cells in the cell cycle phase S + G2/M = 32.4 ± 2.3%, Ki67+ = 41% ± 3.1%). In presented clinical case, cultured autologous cells were given to the patient twice, on burn non-healing wounds. Our method included fibrin gel, which components were combined directly during application on the wound. An increase in the wound healing parameters was obtained and the wound was healed completely within 14 days after cells application. Summarizing, the presented technique allows effective isolation, cell culture and application of cells on non-healing wounds.

13.P02
The efficacy of Adipose-Derived Adult Stem Cells (ADSC) in improving the evolution of scars

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Introduction: Autologous adipose tissue grafting (lipofilling) is currently used for clinical applications such as volume correction due to malformations, pathological scars and after oncologic and/or aesthetical procedures. Many protocols have been tried for treatment of pathological scars but there are not yet precise guidelines. Purpose of this study was to investigate if autologous fat grafting is a good strategy for the management of these scars.
Materials and methods: Twenty five patients, from January 2006 to June 2010 underwent lipofilling according to Coleman's technique (16F,9M, range 13–58, mean age 36 yrs) for pathological scars of face

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(6 cases), breast (8), thorax (4), abdomen (4), upper and inferior limbs (3). Always short-term profilaxis. Mean hospital stay: 3.8(3–5 days). Follow up performed at 3, 6 and 12 months.
Results: Using the Vancouver scale we observed a significant improvement of four variables: vascularity, thickness, pliability and pigmentation. Patients also reported a remarkable reduction of unpleasant sensations. No complications excepted the necessity to repeat the lipofilling (a mean of 40–50% of injected adipose tissue was resorbed after 6–12 months).
Conclusions: 'Lipostructuring' appears to be a reliable, safe, easy, repeatable technique able to improve tissue healing, cells regeneration, bothersome symptoms. Identification of ADSC in the grafted adipose tissue, clarify why skin trophism and healing processes improve dramatically after lipostructuring.

13.P04
Optimization of auto-assembled tissue-engineered autologous bilamellar skin substitute reconstructed from a decellularised dermal matrix

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Introduction: The Laboratoire d'Organogenese Experimentale (LOEX) uses a unique autologous bilamellar auto-assembly skin reconstruction technique to treat severely burned patients. Current research aims to shorten the eight weeks production delay in order to reduce patient morbidity. A novel approach with a dermal matrix is used to prevent epidermal cysts and achieve a structure similar to native human skin.
Materials and Methods: Dermal matrices are reconstructed by the auto-assembly technique then decellularised, dehydrated and frozen. At need, matrices are thawed and seeded with fibroblasts to restore matrix cellular population. The dermis is further seeded with different keratinocytes concentrations and matured with an air-liquid interface on plexiglas or nylon support.
Results: Absence of epidermal cysts is achieved with reduced keratinocytes concentration and nylon support. Continuous basal collagen IV and laminin with keratin 10, transglutaminases, involucrin and fillagrin support adequate skin architecture. Basal keratin 19 and Ki67 suggest the presence of stem cells in the substitute. This pattern is similar to native human skin and suggests a permanent grafting potential.
Conclusion: We propose a novel faster technique for producing autologous bilamellar auto-assembled skin substitutes using a dermal matrix. This process requires 5 instead of 8 weeks for complete skin reconstruction. This could be an important clinical gain, thus paving the way for future clinical trials.

13.P05
Autologous tissue-engineered skin preserving epithelial stem cells applied to the permanent coverage of large burn wounds and to skin reconstruction

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Stem cells are at the forefront of tissue engineering of self-renewing tissue such as skin, since their presence is required to ensure the long-term renewal of the epidermis. Here, the presence and functionality of stem cells within tissue-engineered skin substitutes (TES) elaborated by the self-assembly approach of tissue engineering were investigated. A subset of basal cells exhibiting the stem-cell associated slow-cycling property were identified using 5-bromo-2'-deoxyuridine (BrdU) within the epidermis of TES cultivated *in vitro*. Immunofluorescence and flow cytometry analysis revealed that an important proportion of the slow-cycling cells highly expressed keratin (K) 19. In addition, keratinocytes harvested from TES gave rise to stem cell-like colonies in secondary monolayer subcultures. TES were used as autologous full-thickness grafts for the permanent coverage of full-thickness burn wounds of few patients. The integrity of the transplanted TES persisted over time with low occurrence of scarring. More than 5 years after grafting, no epidermal regeneration defect was observed confirming the presence of functional stem cells. These results indicate that the microenvironment supports epithelial stem cell survival and function within TES *in vitro* and after grafting in patient *in vivo*.

13.P06
In vitro and *in vivo* evaluation of an artificial skin model based on fibrin-agarose biomaterials

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Introduction: In this work, we describe a novel human dermo-epidermal substitute based on fibrin-agarose biomaterials and we evaluated the sequential changes that take place during its *in vivo* maturation and integration.
Materials and Methods: Primary cultures of human keratinocytes and fibroblasts were generated and a human skin substitute was developed using fibrin-agarose biomaterials. After 4 weeks of *in vitro* development, the skin substitute was grafted in immunodeficient mice, and evaluated after 10, 20, 30 and 40 days. Collagen fibers and proteoglycans were evaluated by histochemistry and electron microscopy. The expression of cytokeratins 1 and 5 was analyzed by immunofluorescence.
Results: Analysis of the epidermis revealed a progressive epithelial stratification with positive reaction for CK1 and CK5 after *in vivo* grafting. A progressive increase and organization of collagen fibers and proteoglycans was found from day 10 to 40. Well-developed desmosomes were found at the epithelium, with a mature basement membrane at the dermal junction.
Conclusion: Our results confirm the progressive *in vivo* maturation and integration of the human dermo-epidermal substitute, with structural and functional characteristics similar to native human skin. For all these reasons, we hypothesize that this human dermo-epidermal substitute based on fibrin-agarose biomaterials might be useful for clinical trials.
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13.P07
Autologous cell therapy for facial rejuvenation

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Introduction: The dermis is mainly conformed by fibroblasts which produce collagen and elastin to provide mechanical support to the epidermis. Based on a previous assayed cell therapy product consisting of a suspension of cultured autologous fibroblasts, that demonstrated its

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efficacy on nasolabial fold wrinkles treatment, we proposed a modified protocol in order to improve the described product.
Methods: Patients approved the procedure by written consent. A 5 mm punch biopsy was collected from behind the ear. Tissue was received in sterile DMEM/pen-strep, and seeded as explants on culture flasks. After 15 days, the cells were expanded to obtain sufficient quantity for treatment, and cryopreserved until the patient could be scheduled for cell application. The injection was prepared by thawing and packaged to be administered within 6 hours of product release. We tested the treatment on nasolabial, periocular and forehead wrinkles. Photos were taken before/after treatment and subjected to qualitative comparison by physician, patient and a blind observer. Patient's satisfaction data were obtained through a questionnaire.
Results: Ten men and 40 women with one or two applications were involved. No adverse effects were observed. Average cell viability was 85 ± 12%, mean cell concentration was $8 \times 10^6 \pm 2$, 7×10^6. Most patients felt satisfied and ask for another application.
Conclusion: This is an attractive, efficient and economic therapy for facial rejuvenation.

13.P08
Degradable gelatin microcarriers for cell delivery to cutaneous wounds and enhanced wound healing

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Cell delivery with the aid of spherical gelatin micro carriers seeded with keratinocytes was previously found to accelerate healing of epidermis in an *in vivo* mouse cutaneous wound model. Additionally, gelatin spheres were recolonized by surrounding fibroblasts under the newly formed epidermal cell layer. In an *in vitro* human excised full thickness skin wound healing model we have investigated the effect of keratinocyte introduction to the wound and the improved milieu for dermal regeneration provided by the gelatin microcarriers prior to degradation. Wounds were cultured in the presence of gelatin microcarriers only or microcarriers seeded with human primary keratinocytes and rate of wound healing was monitored by paraffin embedding at at set days of culture and hematoxylin and eosin stain. Collagen deposition by colonizing fibroblasts was visualized with Masson's thrichrome stain. We show effects of the microcarriers on wound healing in our *in vitro* human wound healing model.

13.P09
Genomic alterations in cultured epithelial autograft (CEA) associated with the development of squamous cell carcinoma

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Introduction: Autologous stem-cell derived products are applied to the wounds of severely burned patients when split-thickness skin autograft is not possible due to the lack of donor material. Cultured Epithelial Autograft is composed of cultured dermal keratinocytes from autologous tissue. CEA is ideal for use on patients with limited donor sites. CEA restores the epidermal barrier in patients with limited donor sites, providing an environment that is optimal for wound healing
Methods: Techniques used to create CEA may select for highly proliferative cells with impairment of regulatory processes that restrict cell growth or cell death. Keratinocytes are also treated with a cell immortalizing agent and sheets of this cell product are grown and applied to the patient. Recent detection of a new squamous cell carcinoma in three patients who received CEA coverage has raised concerns that the clonal amplification of cells selected for rapid and aggressive growth - which are then grafted onto patients with chronic systemic inflammation may have introduced genetic alterations which now result in malignancy.
Results: Three cases of squamous cell carcinoma have developed in patients treated covered in this manner. We have examined one of the carcinomas which have curious genetic alterations.
Conclusion: The potential for alterations at the genetic and genomic levels, which may alter the behavior of the cells must be considered when utilizing stem cell products such as these.

13.P10
Effects of surface chemistry and topography on fibroblasts

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Healthy skin has a vital role in survival of an organism, and transplantation is often the best treatment for extensive chronic wounds and burn injuries. However, complications frequently occur in skin-transfer and the elaboration of improved engineered solutions for normally functioning artificial skin is an ongoing challenge. It has been shown that cells are mainly affected not only by the chemistry, mechanical properties and microstructure of the surrounding environment, but also by the nanostructure of the substrate. Carbon nanotubes (CNT) with excellent properties are widely explored as potential applications in regenerative medicine. Furthermore, conductivity of CNTs can also have a significant role as electrical stimulation has been shown to enhance wound healing by various mechanisms. CNT-microfibers can be potentially used in scaffolds as they can be engineered to mimic the natural collagen-fiber network in extracellular matrix (ECM). We have studied the chemical and topographic interactions between fibroblasts and sol-gel patterned surfaces as well as CNT composites with different surface structure and functionality. Laminin, a component of ECM in natural skin, was also included. Primary human dermal fibroblasts were used to evaluate cell viability, adhesion, migration and spreading on prepared surfaces by fluorescence and scanning electron microscopy analysis.

13.P11
In situ bioprinting of autologous skin cells accelerates skin regeneration

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Conventional skin grafts are often limited in providing immediate coverage for large wounds. We developed a skin bio printer that accurately delivers skin cells and biomaterials to stabilize large wounds. We investigated whether large full thickness wounds could be repaired using the skin bioprinter in a porcine model. Four full thickness excisional wounds of 10 × 10 cm in size were created on the back of a pig model ($n = 6$). Autologous and allogeneic dermal fibroblasts and keratinocytes, suspended in fibrinogen/collagen solution, were sequentially printed directly on two wounds. The remaining two wounds received gel only or left untreated. The animals were followed for up to 8 weeks

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and analyzed for wound healing, re-epithelialization, contracture, new tissue formation and angiogenesis. Animals treated with autologous cells showed faster wound healing as compared to the other treatment groups. Autologous cell treated wounds showed 95% reduction in wound size, 90% re-epithelialization and 20% contracture of original wound size within the first 3 weeks. Animals treated with allogeneic cells showed comparable wound size, re- epithelialization and wound contracture to the controls and had complete healing and re-epithelialization in 6 weeks. This study indicates that skin bio printer may be a preferred approach to rapidly cover extensive skin defects such as burn.

13.P12
How fibroblasts respond to different biomaterials: comparative adhesion and proliferation studies

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Biomaterials from different categories have been thoroughly investigated regarding their use in scaffolds for skin tissue engineering. However, a direct comparison between polymers of different natures is seldom reported. Therefore, we decided to study the performance of three different polymers in promoting the adhesion and proliferation of fibroblasts grown on electrospun nanofibers. A polyester (polycaprolactone), a polysaccharide (chitosan) and a protein (gelatin from cold water fish skin) were used. Characterization of the electrospun mats was conducted by SEM, porosity evaluation and mechanical testing. *In vitro* tests were made by culturing 3T3 fibroblasts up to 7 days. Cell viability was determined using the WST-8 assay. Morphology was observed using SEM. Cells were stained for confocal microscopy observation of the nuclei and cytoskeleton. Gelatin scaffolds had the best overall performance: cells attached and spread well, their number equalled that of the controls and they exhibited the expected morphology with polygonal shapes. Cells then proliferated until confluence. Adhesion to chitosan nanofibers was less efficient: cell number decreased significantly but then initial seeding density was reached by day 3. In addition, we observed that cells tended to grow in clumps. PCL showed to be the least suitable substrate in terms of cell adhesion: a high number of cells died; however, those that attached proliferated and the initial seeding density was reached by day 6.

13.P13
Superficial and deep dermal fibroblasts differentially interact with keratinocytes: implications for development of the basement membrane.

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Dermal fibroblasts are heterogeneous in size and biochemical properties, and based on location are known as superficial (SF) and deep dermal fibroblasts (DF).We recently identified that DF differentially remodel collagen-glycosaminoglycan (C-GAG) matrices and play a major role in hypertrophic scarring (HTS). Further characterization of SF and DF, and their interaction with keratinocytes (K) would enable development of novel skin substitutes and effective treatment strategies for HTS. This work aimed at studying SF, DF and their interaction with K using C-GAG matrices as 3D substrate, assessing biomechanical differences and proteoglycan composition. SF, DF and K from female abdominoplasty patients were cultured on C-GAG matrices separately and co-cultured, and used in assays (7, 14, 21 days). Cell viability was assessed by MTT and matrix contraction by Image J. Gene expression of basement membrane and extracellular matrix proteins nidogen, keratin-5, laminin-5, E-cadherin, fibronectin, collagen IV and VII was analyzed by RT-qPCR. Expression of proteoglycans: decorin, versican and fibromodulin were also assessed. Viability of cells increased with time. Contraction of the matrices followed the order: DF > DF + K > SF > SF + K. Matrices with DF and K had higher levels of laminin-5, E-cadherin, fibronectin and versican, but lower levels of decorin and fibromodulin. Our results show differential interaction of SF and DF with K, and their differential role in basement membrane formation and HTS.

13.P14
Human STSG storage: changes in graft quality and performance.

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Introduction: Storage of new and utilization of previously harvested split-thickness skin grafts (STSGs) is part of the clinical routine in burn surgery. This study determines the quality of stored skin and examines the performance of preserved human STSGs *in vivo*.
Materials & Methods: A modified dorsal skinfold chamber was performed in SCID mice ($n = 15$). Human STSGs harvested from healthy patients undergoing abdominoplasty were transplanted immediately or after 3 and 7 days of storage in saline-soaked gauze at 4 °C. Intravital microscopy (IVM) was performed. Apoptosis (TUNEL assay) and proliferation staining (Ki67) were performed on paraffin-embedded samples taken 0, 3, 5 and 7 days after harvesting to assess graft quality. Metabolic activity was measured in a MTT assay.
Results: STSGs manifested an increase of proliferating cells (25% of total cells) on day 3 of storage which was significantly dropping to a level of 7% on day 7. The fraction of apoptotic cells slightly increased on day 5 (25%) and remained constant until day 7. Metabolic activity decreased by 30% on day 7 of storage. Capillary widening was only observed via IVM after fresh STSG placement. The host wound bed reacted with an early angiogenic response on day 3 after grafting of fresh STSGs. Preserved STSGs exhibit a delayed but markedly increased response on day 5.
Conclusion: Storage influences wound bed angiogenesis and has a significant effect on the proliferation and metabolic activity of graft cells.

13.P15
Creation of a novel wound dressing consisting of collagen vitrigel combined with plastic film

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The skin's primary role is as a barrier separating an organism's inner from outer space. This barrier inhibits undesirable water losses and

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defends the body against infection. This barrier function depends on the physical character of epidermis and dermis: tightly packed epidermis reduces the gap in the barrier, and collagen fiber shaping dermal fibroblast supports the barrier's biomechanical strength and the skin's biological microenvironment. To mimic the basic function of the skin, we created a novel wound dressing comprising collagen vitrigel membrane made up of high density collagen fibrils and plastic film (CVP). This alternative skin replicates the physical barrier and provides extracellular matrix.CVP was evaluated as a wound dressing: full-thickness transcutaneous dermal wounds induced in C57BL6J mice were treated with CVP. Macroscopic monitoring
Results: The number of deaths due to severe dehydration decreased, and the wound bed was far smaller, in animals of the CVP group than in control group. Histology revealed no granulomatous inflammatory reaction or foreign-body type reaction in skin lesions with CVP. These findings support the local and systemic histocompatibility of the biomaterial. The present data suggest that this biomaterial may enhance regeneration of an extensive skin wound.

13.P16
Modified collagen-PCL scaffolds enhance skin cell ingrowth and promote skin regeneration during wound healing

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Introduction: Burn injuries are among the most physically and psychologically traumatic injuries. Globally six million patients suffer from severe burns each year. Therefore, development of skin equivalents to promote wound healing is of clinical importance.
Methods: Microporous collagen-polycaprolactone (PCL) scaffolds are produced by modified electrospining. The surface morphology was characterized by SEM. Human fibroblasts were co-cultured with keratinocytes on the scaffolds to reconstruct skin equivalents. Cells growth rate was monitored by counting DAPI stained cells. Biopsies were also collected and stained with H&E staining. A mouse model was established to assess the bio-safety of the skin equivalents. Wound healing rate were determined and biopsies were collected for histology analysis.
Results: The 3D collagen-PCL scaffolds were developed with optimised internal structure. In cell culture, fibroblasts and keratinocytes colonized efficiently on the surface over 21 days. SEM and histology analysis showed enhanced cells attachment and proliferation. Animal study demonstrated that the scaffolds promoted wound healing with wound size reduced approximately 50% by day 7. H&E and Masson's thricome staining showed excellent cells ingrowth and formation of a collagen layer. Early stages of angiogenesis were evidenced by small capillaries in neo-dermis. Our study will provide significant information on development of skin equivalents in wound healing and skin regeneration.

13.P17
A novel antimicrobial bacterial cellulose membrane for repair and reconstruction of skin wound healing

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Bacterial cellulose membranes have attracted a great deal of attention as novel wound-dressing materials, unfortunately, without antimicrobial activity. In this work, we developed a novel method to synthesize silver nanoparticles *in situ* of bacterial cellulose membranes (*n*-Ag/BC) through the reaction of Tollens' reagent with aldehydes. Synthesis processing of silver nanoparticles in the nano-network of BC was investigated. *In vitro* Ag releasing property of *n*-Ag/BC was tested in PBS buffer solution and the circulation of PBS buffer solution. The determination of their antibacterial activity using Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa is presented in this article. Co-cultivation of epidermal cells was designed to investigate possible effects of *n*-Ag/BC on epidermal growth. The experiments include observation of epidermal cells of fetal rats' fusion on membranes, the determination of MTT curves and cell growth curves. The results show that *n*-Ag/BC is a useful antimicrobial membrane without toxicity, allowing attachment and growth of the epidermal cells. Thus, we anticipate that *n*-Ag/BC might have a beneficial contribution to future applications of bacterial cellulose with not only antibacterial properties but also abilities to promote skin growth as a wound dressing. This study is supported by Beijing Municipal Science and Technology Plan Projects (No. Z111103066611005) and the Royal Society-NSFC international joint project (51111130207).

13.P18
Comparisons of biocompatible scaffolds for human and mouse hair regeneration study

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Hair follicle is known for distinct organ of epithelial mesenchymal interaction. Since regenerative medicine start to use biocompatible scaffold, fiber sheet and sponge type of scaffold are commonly used for tissue regeneration. Once the cells are out for growth *in vitro*, they tend to lose the character they have *in vivo*. The three-dimensional cell cultivation can enhance the natural property of the cell and restore the functional feature of them. In this study, we tried several scaffold for making functionally working epithelial structure for human and mouse hair regeneration study. The bladder-submucosal sponge (BSM hybrid sponge) could be applicable to the study of epithelial mesenchymal signaling pathway. Fiber sheet has less porous as well as thinner than BSM sponge and has advantage for cell attachments. The human outer root sheath keratinocytes did make cluster onto the mouse newborn epithelial skin scaffold (NESS) unevenly due to the physical damage during preparation. This study speculated the possibility of using scaffold for hair regeneration study as point of epithelial cells. The present findings could be relevant model for epithelial cells to advance our understanding of the hair regeneration and cure for hair loss.

13.P19
Revcel, a resorbable biomaterial as wound dressing

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Chronic wounds of the skin, if at all, generally take very long to heal. Stagnation of wound healing usually results in excessive extracellular matrix (ECM) degradation, which is however crucial for tissue regeneration (growth factor reservoir and matrix for cell ingrowth). Current treatment systems are relatively expensive and often contain collagen which has the potency to transmit pathogens. The aim of this project was to develop a biocompatible, degradable artificial ECM based on polyurethane for wound healing. The Revcel materials were produced

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by nolax in a discontinuous procedure by combining polyol and isocyanate. Potential adverse effects of Revcel extracts and degradation products were assessed by according ISO10993-5 measuring total protein content as well as metabolic activity. Cell adhesion was evaluated by cultivating 3T3 mouse fibroblast cells on the material. Revcel is a synthetic, soft, foam-like scaffold with tunable degradation time yet form-stable and that offers optimal pore size for angiogenesis. With the base formulation, extracts showed small adverse effects and cells formed clusters when seeded on foams. Modified formulations affected protein levels to a lesser extent, and exhibited good cell attachment and spreading. Products formed by an *in vivo* like degradation were found to be non-toxic. The results therefore suggest that Revcel has the potential to support wound healing *in vivo*. We acknowledge support by the CTI (project 11874.1 PFLS-LS)

13.P20
Evaluation of the potential of polyhydroxybutyrate-co-hydroxyvalerate bi-layered scaffolds for skin tissue engineering

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Polyhydroxybutyrate-co-hydroxyvalerate (PHB-HV) is natural-based polyester produced by microorganisms under unbalanced growth conditions. This polymer is known for its biocompatibility and biodegradability and has been explored for tissue engineering approaches. The aim of this work was to create a bi-layered construct, based on PHB-HV, for skin tissue replacement. The PHB-HV bi-layered scaffold was produced taking into consideration the two main skin strata, the epidermis and the dermis. For the epidermal fraction, a membrane was developed using a solvent cast approach, while freeze-drying was used to obtain the dermal fraction. The two structures were combined to obtain a scaffold that has a thin compact surface (epidermal) and a porous structure (dermis), as confirmed by scanning electron microscopy (SEM) and micro-CT analysis. Each layer of the scaffold, epidermal and dermal, was respectively seeded with human keratinocytes (Kc) and human dermal fibroblasts (Fb). The characterization by SEM confirmed the typical morphology of Kc and Fb on the expected surfaces. Calcein-AM and DNA quantification showed that both type of cells remained viable along the experiment and were able to attach and proliferate on the respective seeding layers. In conclusion, the developed PHB-HV scaffolds have shown suitable properties to sustain the co-culture of Kc and Fb, which constitutes a solid base for further work to demonstrate its potential for skin replacement.

13.P21
Potential of multiscale fibrous scaffolds for skin tissue engineering

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Mimicking structure, hierarchy and biological functions of native extracellular matrix (ECM) have been one of the major goals of tissue engineering. However, most of scaffold materials for skin tissue engineering highlights on resembling biochemical composition of native tissue. Here we present a rather innovative hierarchical nano/microfibrous chitosan/collagen scaffold, for skin tissue engineering. Scaffolds were developed by sequential electrospinning and freeze-drying. SEM microscopy showed formation of nano/microfibrous layers (~75 nm and ~10 μm fiber diameters). Physico-chemical properties of scaffolds (eg., porosity, tensile strength, swelling behavior, biodegradability) were tested. Scaffolds showed a distinct zone of inhibition against *E.coli*. Scaffolds were evaluated *in vitro* using 3T3 fibroblasts and HaCaT keratinocytes, for assessing matrices' cytocompatibility and cellular response. Presence of type-I collagen in scaffolds encouraged better cell attachment and improved cellular viability. In addition, scaffolds were tested in *ex vivo* human skin equivalent (HSE) model, as a preliminary alternative to *in vivo* animal testing. Results showed migration of keratinocytes along scaffold's surface, causing re-epithelisation of wound-a prerequisite healing and regeneration. Taken together, we observe that by closely mimicking structural/functional attributes of skin, here-in proposed chitosan/collagen scaffolds show great potential for skin tissue engineering application.

13.P22
Healing potential of O2- hydrogels on a full-thickness excision cutaneous wound in diabetic mice

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Foot ulcers and wound healing are problematic for patients with diabetes. At present, many therapies are directed at accelerating wound healing, such as applying electric currents, living skin equivalents, and pharmacological manipulation of factors that modulate the wound healing process. Oxygen (O_2) is a very important mediator associated with wound treatment, and its availability can limit healing rate. We tested the potential effects of O_2-hydrogel therapy on wound healing and regeneration. O_2-hydrogel treatment resulted in accelerated wound closure and formation of granulation tissue in the wound area compared with those in water-hydrogel treated mice. These data suggest that O_2 hydrogels may ultimately provide a novel therapy for accelerating wound healing in patients with diabetes.

13.P23
Comparison of blue and red low level light by LED on healing processes in a skin flap model in rats

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Most studies of low level light therapy have been performed in red or infrared range. We showed the significant impact of blue light to release nitric oxide from nitrosyl complexes. Here we aimed to compare the effects of red and blue light from LED on wound healing in a skin flap model. A skin flap supported by either left or right inferior epigastric neurovascular bundle was illuminated post-OP and on five consecutive days for 10 min with light-emitting diodes (LED) at either 470 nm or 630 nm with 50 mW/cm^2. On day 7 size of necrotic area, flap perfusion, histologic and immunohistochemical parameters were analysed. In both light treated groups wound healing was enhanced, necrotic areas were significantly smaller and flap shrinkage less pronounced compared to controls. Immunohistochemical analyses revealed profound effects of light on neoangiogenesis. In both light treated groups blood vessel count in the perimuscular layer of the skin was twice higher. In the subepidermal layer blue light doubled and red light tripled the amount of blood vessels. Consistently, tissue perfusion was twice higher in both light treated groups as determined by Laser Doppler Imaging. Our data suggest that red and blue light can enhance

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wound healing processes. By improving angiogenesis and tissue perfusion, light treatment can help to attenuate pathophysiological complications like ischemia-induced necrosis. LLLT would provide an easily applicable and cost-effective treatment for skin wounds.

13.P24
Effects of low level light therapy by LED on healing processes in porcine skin

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Treatment of donor sites after meshed skin graft harvesting is important to prevent infections and attenuate pain and regenerate the skin for further harvesting. Low level light therapy can be an efficient means to promote wound healing. Here we aimed to compare the effects of red and blue light from LED on wound healing in donor sites in a pig model.05 in Eight 0.5 mm thick epidermal wounds measuring 5 cm × 5 cm were made paravertebrally on the back of pigs. Wounds were illuminated post-OP and on day 2 for 10 min with light-emitting diodes (LED) at either 470 nm or 630 nm with 10 mW/cm^2. On day 5 planimetric, histologic and immunohistochemical parameters were analyzed. In both light treated groups wound healing was enhanced. Red light reduced open wound area by 49%, blue light by 62% ($P < 0.01$ vs. untreated control). Gene expression analysis showed down-regulated VEGF-A and VEGF-R2 reaching statistical significance in the blue light group, suggesting that wound healing is already completed. Furthermore, Coll I mRNA was decreased in both light groups, indicating less scar formation. Our data suggest that LLLT with red and blue light can enhance wound healing processes and may reduce scar formation. LLLT would provide an easily applicable and cost-effective treatment for surface wounds. Lorenz Böhler Fonds 12/08.

13.P25
Selection of new targets to modulate inflammation in diabetic foot ulcers

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Macrophages are key cells in the resolution of inflammation as they can secrete pro-inflammatory and wound healing cytokines. In the case of diabetic foot ulcers, macrophages are locked in their inflammatory phenotype. In this study, it was hypothesized that hyperglycemia is responsible for chronic inflammation in diabetic patients. The objective was to detect relevant genes with an altered expression in high glucose conditions. THP-1 cells were cultivated in normo or hyperglycemia for 60 days. Then, cells were differentiated into macrophage like cells with PMA and activated with LPS. Total RNA was extracted and analysed by hybridisation to microarrays. Differences in gene expression between normal and high glucose cells were determined. The microarray revealed that 546 genes were statistically up or down regulated in hyperglycemia. Among these genes, seven pro-inflammatory cytokines were up regulated in hyperglycemia. Several effectors of the Wnt5A signalling pathway were also up regulated. Regulation of GM-CSF and Wnt5A was verified and these molecules are potentially new targets to modulate inflammation. When Wnt5A links its receptor, the downstream effect is the increase of intracellular [Ca^{2+}]. The final effect is the production of pro-inflammatory cytokines. Cytokine GM-CSF is known to maintain the pro-inflammatory phenotype in macrophages. Hence, silencing of Wnt5A and GM-CSF using siRNA technology could lead to the modulation of inflammation in diabetic patients.

13.P26
Reducing lysil hydroxylase 2 expression in a fibrotic environment by introducing repressive epigenetic marks in the PLOD2 promoter

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Lysyl hydroxylase 2 (LH2), encoded by PLOD2, initiates pyridinoline (pyr) cross-linking of collagen. These hard-to-degrade cross-links are increased in fibrotic collagen, resulting in poorly digestible collagen. To date, no therapeutics has been developed that reduce pyr cross-linking. Therefore we aim to down regulate LH2 expression by targeted transcriptional repression of important transcription factor (TF) binding sites. To identify TFs and their binding to the PLOD2 promoter, we performed luciferase assays, low molecular weight compound (LMWC) inhibition of TFs, and chromatin immunoprecipitations (ChIPs). Influence of DNA methylation on LH2 expression was assessed by bisulfite sequencing and *in vitro* methylation of promoter-luciferase constructs. Currently we are engineering zinc-finger proteins to target transcriptional repressors to the PLOD2 promoter. The region -500 ATG showed the highest reporter activity after TGFβ1 stimulation. This region contains putative SP1 and Smad3 binding sites and LMWC inhibition of either TF reduced LH2 expression of stimulated cells. ChIP indicated binding of both TFs to the promoter. No differences in DNA methylation patterns were observed after stimulation. However, in vitro methylation of the PLOD2 promoter by M.SssI prevented reporter activity during stimulation. To conclude, our data indicates that introducing repressive epigenetic marks at indicated TF binding sites to be a potent tool in reducing fibrosis-related LH2 expression.

13.P27
Leptin promotes wound healing in the skin

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Leptin, a circulating hormone, has various physiological actions. Interestingly, skin wound healing was proven to be delayed in leptin-deficient ob/ob mice. However, little is known on mechanism of this phenomenon. In this study, we attempted to elucidate a role of leptin in wound healing of skin. First, chemical burn wounds were created on the back skin of male ICR mice. Wounds were covered with sustained-release absorbable hydrogel containing leptin or PBS. At day 4 and 8, wound repair was quantified by measuring the area of the epithelial defect. Also, the skin including the wound was extirpated for histological examination and immunohistochemical analysis for leptin receptor and CD31. Then, number of CD31-positive cells was counted. Moreover, gene and protein expressions of leptin receptor in human skin fibroblasts were analyzed by RT-PCR and Western blotting. As a result, the wound healing at day 8 was significantly enhanced in leptin-treated group. Immunohistochemistry revealed that leptin receptor was expressed in the healing skin. At day 8, more CD31-positive cells were observed in the subcutaneous area around ulcer in leptin-treated group. RT-PCR and Western blotting showed that human skin fibroblasts expressed leptin receptor. These findings suggest that leptin promotes wound healing in the skin by accelerating angiogenesis. The

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possibility that leptin may be useful as a new medicine to promote wound healing in the skin is also suggested.

13.P28
Antimicrobial and cytotoxic effects of silver nanoparticles in electrospun skin substitutes

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Silver nanoparticles (NPs) have emerged as good candidates to confer skin substitutes with protection capabilities against infections. Electrospun mats (EMs) are particularly adequate for such an application. We produced polyvinylpyrrolidone (PVP) EMs from solutions containing $AgNO_3$. EMs morphology was characterized by SEM. UV irradiation photo cross linked the polymer and induced the formation of NPs. Both processes, as well as the release of NPs from EMs to water, were analyzed by UV-Vis spectrophotometry. Weight loss of EMs when immersed in water was measured. NPs were characterized by TEM. Their activity against several microorganisms and toxicity to Human Ephithelial Kidney (HEK) cells were assessed, respectively, by disk diffusion/minimal inhibitory concentration and *in vitro* cell viability tests. Results indicate that as UV irradiation time increases the EMs weight loss decreases and absorbance at 290 nm increases. When either the ratio of PVP: $AgNO_3$ used or the UV irradiation time increases, the mean NPs size (all below 10 nm) and the dispersion of particles size increase. The NPs originate an absorbance peek (around 425 nm) which increases in the first 4 h of irradiation, then stabilizes, and shifts as the NPs mean size in a sample increases. The concentration of NPs released to water from an immersed EM increases in the first 2 h and then tends to stabilize.All EMs showed inhibitory effects on microorganisms and cytotoxicity to HEK due to the presence of NPs.

13.P29
Bilayered macroporous templates for skin tissue repair and regeneration

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Skin tissue engineering is a promising area of research where a number of tissue engineered medical products have been commercialized. Further research is required to be carried out to improve the quality of wound healing to mimic native tissue that can improve angiogenesis in scaffolds, reduce scar formation in sensitive areas and minimize antimicrobial resistance against wound pathogens. In our laboratory we have synthesized bilayered bioartificial skin grafts consisting of a regenerating layer and an antiseptic layer. The regeneration layer is made up of gelatin as a three-dimensional supermacroporous cryogel sheets for the dermis and two-dimensional thin films for the epidermis, respectively. The antiseptic layer consists of polyvinyl pyrrolidone cryogel sheet coupled with iodine for minimizing anti-microbial resistance against wound pathogens. Further in order to improve the angiogenic and regenerative properties of the scaffolds, gelatin microspheres of bioactive components such as mannose-6-phosphate, zinc oxide and fibrinogen, respectively have been incorporated into the dermal regeneration templates for specific applications. Cell culture studies in different types of skin cells such as NIH3T3, L929 and A431 have been studied and animal studies in rabbit model systems have shown promising results with the bilayered skin grafts.

13.P30
Gene expression profiling of negative-pressure-treated skin graft donor site wounds

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Negative-pressure wound therapy (NPWT) is widely used to improve skin wound healing. Although NPWT has been studied as a treatment for wound closure and healing, the molecular mechanisms explaining its therapeutic effects remain unclear. To investigate the effect of NPWT on gene expression, and to discover the genes most dominantly responding to this treatment during skin wound healing, we applied negative pressure on split-thickness skin graft donor sites from the first postoperative day (POD) to the seventh POD. Biopsies were collected from intact skin before graft harvesting, and on the seventh POD from the donor site wound. Each sample underwent a genome-wide microarray analysis. Gene expression profiles of NPWT-treated samples were compared with untreated control samples. In addition, we analysed wound exudates for volume, and for concentrations of leukocytes, erythrocytes, and hemoglobin. NPWT induced major changes in gene expression during healing. These changes ranged from 10-fold induction to 27-fold suppression. The genes most induced were associated with cell proliferation and inflammation, and the most down-regulated genes were linked to epidermal differentiation. Our results suggest that NPWT enhances specific inflammatory gene expression at the acute phase associated with epithelial migration and wound healing. However, its continued use may inhibit epithelial differentiation.

13.P31
The use of high-hydrostatic pressure treatment to decellularize normal skin

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Introduction: High-hydrostatic pressure (HHP) is a new decellularization treatment in order to produce acellular matrix. The aim of this study is to explore the possibility to produce acellular dermis (AD) from normal skin using HHP method.
Materials and Methods: Skin specimens were obtained from patients with informed consent. We compared four kinds of pressure to decellularize: 0 (control group), 100, 200, 100 MPa. Specimens were pressurized using a cold isostatic pressurization machine. We evaluated the structure of skin in H-E staining, and confirmed the structure of basement membrane of epidermis and capillaries using IHC staining for type IV Collagen and vWF. We counted viable cells using a WST-8 assay (Cell Count Reagent SF) before and after treating, 7, 14 days washing. The absorbance of the colored product, which represents quantitatively the degree of cell in skin, was read on a microplate reader at wavelength of 450 nm. Results: The epidermis was removed from skin after treating with pressure 200, 1000 MPa. The basement membranes and capillaries remain intact in all specimens that were treated with each pressure after treating, 7, 14 days. In the 200, 1000 Mpa groups, the numbers of viable cells (absorbance) were significantly smaller than those of other groups.
Conclusion: In this experiment, we could prepare AD from skin tissues using HHP. Next step, we will produce cultured skin using AD and cultured cells, and try to reconstruct skin defects using animal models.

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13.P32
Amniotic membrane attenuates TGFß signalling, induces c-jun expression and modifies the glycosilation profile of several proteins in HaCaT cells. Molecular lessons from the re-epithelialisation induced by AM in deep large-surface wounds

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Amniotic Membrane (AM) is a tissue of particular interest as a biological dressing due to its biological properties and immunologic characteristics. In patients with extensive wounds, AM was used as a wound dressing and induced a robust eptithelialisation and the formation of a well-structured skin. TGFß, initially released at the wound bed by platelets, has an important role in wound healing. TGFß induces migration of monocytes and fibroblasts into the wound bed. Additionally, TGFß induces a powerful cell cycle arrest in keratinocytes. All together, this prevents wound re-epithelialization before dermis is fully repaired. In big massive wounds, the high concentration of TGFß induced by inflammation may be the cause that prevents re-epithelialization. The application of AM may antagonise these TGFß effects. To test this hypothesis we used an epithelial model: HaCaT cells. We studied the effect of AM on the TGFß induced genetic response. HaCaT cells treated with or without AM were stimulated with TGFß. AM treatment triggered the activation of several signalling pathways, including MAP kinase pathway and modifies the genetic program that HaCaT cells exhibit in response to TGFß. In Mv1Lu cells, AM induced a migration response that was prevented by SP600125 (JNK inhibitor). Moreover, AM causes changes in the oligosaccharide branching of some proteins. Also, AM had several effects on TGFß-induced cell cycle arrest of HaCaT cells.

13.P33
Polysaccharide based hydrogels for the delivery of umbilical cord derived mesenchymal stem cells for cutaneous wound healing

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Introduction: Millions of patients world-wide suffer from acute and chronic wounds. Systemic and local injections of mesenchymal stem cells (MSCs) have proved effective for wound healing applications; however, alternative methods for the delivery of cells directly to the wounds are warranted. The incorporation of MSCs into a polysaccharide based carrier system would allow for their direct application to wounds.
Materials and Methods: Pullulan based hydrogels and porous scaffolds were fabricated by using sodium trimetaphosphate as a cross-linker under basic conditions. Gelatin was also included in some scaffolds to promote bioactivity. Porous scaffolds were fabricated by sodium chloride salt leaching and lyophilisation steps. MSCs were cultured from the Wharton's jelly of human umbilical cords and seeded on the materials. Live/dead staining allowed for the determination of cell distribution and viability within the cross-linked pullulan scaffolds.
Results: Pullulan hydrogels containing 10–25% gelatin were shown to improve MSC adhesion after 1 week of culture. Salt leaching and lyophilization steps resulted in porous sponge-like pullulan scaffolds which, when seeded with MSCs, showed relatively high cell viability after 1 day of culture.
Conclusions: The pullulan/gelatin based scaffolds promote MSC attachment and viability and thus, these constructs may act as carrier systems for the delivery of MSCs to wounds for potential cutaneous wound healing applications.

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14. A Novel Generation of Skin Substitutes to Clinically Treat Severe Skin Defects (in coop. EuroSkinGraft)

14.01
Keynote: A novel generation of skin substitutes to clinically treat severe skin defects

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This symposium is an attempt to present an overview of an FP7 HEALTH project, designated 'EuroSkinGraft' that aims at the production of novel, bio-engineered skin grafts, which are to be applied in Phase I and Phase II clinical trials. Two different autologous, bio-engineered skin grafts and an acellular, off the shelf skin substitution product, were designed to serve specific purposes in plastic skin surgery. All three products are intended to be applied in one surgical intervention. The speakers in this session will report: a surgeon's point of view concerning the project (second key note) the experiences made in previous, related clinical trials the science that preceded the development of the skin grafts on how a lab tries to analyze and evaluate the quality of the skin grafts prior and after transplantation a companies' point of view, which is involved in the projection how a Clinical Trial Center experiences regulatory and other aspects of the project. By presenting this global sight of the project, the organizers hope to initiate a stimulating and fruitful discussion about the approach in general and the undertaking of this type of clinical trial in Europe in particular.

14.02
Keynote: Skingineering for large full thickness skin defects

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Burn, plastic, and reconstructive surgeons are still facing considerable and sometimes unsurmountable problems when large or massive full thickness skin defects require definitive and functionally optimal autologous coverage. Today's options include repetitive split thickness grafting, cultured epidermis, and combinations thereof. If the patient survives, functional and cosmetical long term results are often far from satisfactory, in particular due to severe scarring. The growing organism of pediatric patients adds an additional challenge because scars do not proportionally grow with the patient. The goal of this presentation is to illustrate the above mentioned problems with typical clinical situations, to outline what the current therapeutic armamentarium can achieve, and, finally, to review the rationale for our attempts to bioengineer an autologous and clinically applicable full thickness skin analogue to overcome said shortcomings.

14.03
Cultured keratinocytes for burn wound treatment, preliminary results of a prospective randomized controlled intra-patient comparative study

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Standard treatment for large burns is transplantation with meshed split skin graft (SSG). A disadvantage of this treatment is that healing is accompanied by scar formation. Application of cultured autologous keratinocytes (CK) may enhance wound closure and improve scars. A prospective, multicenter randomized trial is currently being performed, in 50 adult patients with acute full thickness burns, an intra-patient comparison is made of the effect of treatment with CK with SSG compared to SSG alone. A biopsy is obtained from unaffected skin and cultured for 2–3 weeks. The expanded keratinocytes are seeded on a collagen carrier and transplanted onto the SSG. Primary outcome measures are wound closure and graft take (GT) after 7 days. Scars are evaluated at 3 and 12 months. Eighteen patients are included so far. There is a significant better wound healing in the areas treated with the CK compared to the standard treatment only, 67.1% (SD 27.2) compared to 61.1% (SD 27.4), $p = 0.01$) A positive trend is seen comparing mean GT, 89.1% (SD 15.0) and 85%,78% (SD 19.5). Three months post-operative, objective and subjective scar evaluations show a positive trend for the experimental area. The preliminary data show positive results for treatment of burns with CK, both in the early outcome parameters as well as in the follow-up at 3 months. In this presentation we will share our experience with coordinating and performing this multicenter study in clinical but also regulatory and logistic perspective.

14.04
The tissue engineering of human skin: Assembling an organ *in vitro*

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The engineering of skin substitutes and their application on human patients has become a reality. However, scientists and surgeons are still struggling to generate permanent skin substitutes. Constructing an autologous dermo-epidermal graft that rapidly vascularizes, optimally supports epidermal stratification and that can be conveniently handled by the surgeon, is now the ambitious goal. In the last decade, our extensive preclinical data on both small (rat) and large (pig) animal models have shown that cellular (consisting of keratinocytes and fibroblasts) dermo-epidermal skin substitutes based on collagen hydrogels optimally reconstitute full-thickness skin defects. Very recently, we have set up a European consortium (FP7 framework), the EuroSkinGraft, to perform both phase I and II multi-center clinical trials for our grafts. Apart from these achievements in the direct clinical application of skin

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substitutes, our research is focusing on the efficient pre-vascularization of skin grafts, on the characterization of epidermal stem cells and on the incorporation of melanocytes to reconstitute the 'color' and UV protection of human skin. In conclusion, our intense research on tissue engineering of skin has developed into clinically applicable products and their efficacy will now be tested and evaluated.

14.05
High density gene expression microarrays as a novel read-out tool in regenerative medicine

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The *in vivo* performance of tissue-engineered constructs is often based on generally accepted read-out parameters, like (immuno) histology. In this study, we probed an alternative strategy for *in vivo* evaluation, which is based on high-density gene expression microarrays and gene ontology analysis. This approach was applied for the identification of the biological processes occurring after implantation of an acellular collagen-based skin construct using a rat full-thickness wound model. A freely-available program (DAVID) was used to identify up/down regulated biological processes (GO-terms), and results were compared to wound healing/regeneration without a construct. Conventional (immuno) histology was used to validate the biological processes identified and it indicates that microarray analysis may provide a valuable, fast and unbiased tool to evaluate the *in vivo* performance of tissue-engineered constructs. However, challenges remain e.g., with regards to the development of specific GO-terms and annotation of the (rat) genome.

14.06
Development of a novel collagen-based dermal regeneration scaffold (Novomaix); control of pore architecture

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Introduction: The use of a dermal regeneration scaffold during transplantation of full thickness dermal wounds with autologous split skin graft is advocated to improve treatment outcome. The migration of different cell types into the dermal regeneration scaffold and the role of these cells in the regenerating tissue are directed by the composition and architecture of the artificial extracellular matrix. By fine-tuning the composition and pore architecture of the dermal scaffold, we anticipate to achieve improved wound healing and reduced scar formation during healing of full thickness wounds.
Materials and Methods: Porcine collagen Type-I, Type-III and insoluble fibrous elastin were used as starting materials for scaffold preparation. Porous scaffold architecture was obtained using a directional ice crystalization method followed by lyophilization.
Results: A collagen sponge scaffold manufacturing technology was developed to create orientated longitudinal pores as guiding structures for regenerating tissue during dermal regeneration. This method allows to achieve reproducible, uni-directional pore structures to obtain an optimal pore architecture. In addition, scaffolds had optimized handling properties compared to currently available dermal regeneration templates. (improved tensile strength, rapid rehydration).
Conclusion: A set of technologies was developed for manufacturing of dermal repair scaffolds with predictable and reproducible uni-directional pore structure.

14.07
Improving quality in non-commercial academic clinical research

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Investigator Initiated Trials (IITs) are clinical trials that are initiated by academic researchers and are aimed at acquiring scientific knowledge and evidence to improve patient care. As regulatory requirements for non-commercial IITs and industry sponsored trials are identical, the burden of complying with these is high for the investigators of IITs. In the last years inspections by Swissmedic (the Swiss federal regulatory authority) were focused on IITs. These inspections revealed several critical deficiencies in academic clinical research as to fulfil the requirements became very difficult and almost impossible to perform aside a clinical routine work in a hospital. For this reason clinical researchers in large academic and teaching hospitals need professional support to fulfil national, international and GCP requirements. The Clinical Trials Center (CTC) at the Center for Clinical Research, an institution of the University Hospital (and the University of Zurich) was founded to provide professional support, expert advice and services in the range of clinical research activities to achieve compliance with GCP and GMP guidelines, the Declaration of Helsinki and the national legal requirements. In Switzerland there is a close collaboration of Universities, University Hospitals and the Swiss National Science Foundation with the aim to improve academic clinical research. The services are subsidized by the University and provided to the researchers on a non-profit basis.

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15. Cardiac Regeneration

15.01
Keynote: Engineered heart tissue for myocardial regeneration

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This talk reviews some recent and ongoing work towards re-establishing the structure and function of injured myocardium through coordinated use of human stem cells, advanced scaffold and bioreactor systems. Human cardiomyocytes derived from embryonic stem cells by staged application of molecular regulatory factors were conditioned by electromechanical signals to enhance their maturation. Physical conditioning resulted in upregulation of multiple cardiovascular genes, synchronization of cell contractions and greater mechanical force generated by cultured cells. To incorporate signals provided by extracellular matrix, both native and synthetic scaffolds were developed, with the structure and mechanical properties similar to native cardiac matrix. To enable cultivation of a thick and functional cardiac patch, we also developed a bioreactor system providing interstitial flow through the cell-seeded scaffold (to maintain cell viability) and the application of electrical stimulation (to enhance cell function). Various types of cardiac patches were evaluated in small animal models. These studies suggest some interesting directions for the application of molecular, structural and electromechanical signals to regulate the differentiation and assembly of cardiac cell populations into a functional cardiac patch.

15.02
Keynote: Engineered heart tissue to model human disease

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We have developed means to generate 3D force-producing engineered heart tissues (EHT) from neonatal rat heart cells, human embryonic stem cell and induced pluripotent stem (iPS) cell-derived cardiac myocytes. The talk gives an overview on recent developments that are directed towards improving the system as an *in vitro* assay. (i) A semi-automated 24-well-EHT assay allows medium throughput screening of pro-arrhythmic and cardiotoxic drugs. (ii) Disease modeling. Human iPS cells have opened the possibility to model human diseases in the dish. To assess the validity of the EHT assay for this purpose we generated EHTs from myosin binding protein C (cMyBP-C) knockout (KO) and knockin mice (KI) and wild-type control EHTs (WT). KO and, to a lesser degree, KI EHT showed hypercontractility, increased sensitivity to external calcium, decreased response to the calcium sensitizer EMD 57033 and reduced sensitivity to verapamil. These results reproduce abnormalities observed earlier in cMyBP-C KO and KI mice and point to novel aspects of a hypertrophic cardiomyopathy phenotype. (iii) We have developed a new *in vitro* hypertrophy model by subjecting mature EHTs for 5 days to enhanced afterload. This intervention induces cardiac myocyte hypertrophy with molecular and functional hallmarks of pathological hypertrophy. Current efforts are directed towards improvement of current cardiac myocyte differentiation protocols from hiPS cells, a critical bottleneck for human disease modelling

15.03
Creation of cell sheet-based bioengineered heart tissue using ES/iPS cells-derived cells

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The bioengineered functional heart tissue is expected to function for repairing the broad injured heart. We have developed the cell sheet-based bioengineered vascularized heart tissue, however the function of stem cell-derived heart tissue remain elusive. Recently we have established the culture system with the suitable conditions for expansion and cardiac/vascular endothelial differentiation of mouse ES cells and human iPS cells using three-dimensional bioreactor. After the differentiation and purification steps using mouse ES cells and human iPS cells that express some drug resistant genes under the control of alpha MHC promoter, a large amount of cardiomyocytes were collected. The co-culture of ES/iPS cells-derived cardiomyocytes with the appropriate number of primary cultured fibroblasts on the temperature-responsive culture dishes enabled to form the cardiac cell sheets. Consistent with the findings that cardiomyocytes in cell sheets beat spontaneously and synchronously, Connexin 43 was expressed at the edge of the adjacent cardiomyocytes and the action potential propagation was observed between cell sheets. Furthermore when ES-derived CD31 positive endothelial cells were co-cultured with ES-derived cardiomyocytes and fibroblasts, robust vascular network formation was observed in the whole cell sheet area. These findings indicate that the layered cardiac cell sheets using stem cell-derived cardiomyocytes with vascular network might function as a regenerated heart tissue.

15.04
Ischemic preconditioning for cardiac tissue engineering with stem cells

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Ischemic preconditioning (IPC) is a powerful mechanism that protects the heart from damage by releasing mediators that promote cell survival. We have now used IPC to promote the growth and development of cardiac tissue from stem cells *in vivo*. We employed an *in vivo* vascularized tissue engineering strategy in rats, where polyacrylate chambers were placed around the femoral vessels. IPC was induced by three cycles of 5 min femoral artery occlusion interspersed with 5 min reperfusion. Chambers subjected to IPC generated bigger tissue constructs at 7 and 28 days post-implantation of 'empty' chambers, the IPC constructs showing significantly greater volumes of fibrin matrix, granulation tissue and vasculature. To investigate whether IPC was cytoprotective, neonatal rat cardiomyocytes (CMs) were implanted into chambers for 3 days: IPC significantly reduced CM apoptosis. Rat cardiac constructs harvested 4 weeks post-implantation contracted spontaneously and cardiac muscle volume was larger after IPC. We are now generating human cardiac tissue from human induced-pluripotent stem cells (hiPS), which are pretreated with trichostatin-A to produce functional CMs. Implanting spontaneously differentiated hiPS in the chambers produced teratomas, so it is clearly important to purify hiPS-derived CMs before growing them into constructs. In summary, IPC

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enhances tissue growth and protects implanted cells, and we will take advantage of this approach in future engineering of cardiac tissue.

15.05
Design of synthetic patch for congenital heart defect repair using self-assembled polycaprolactone and gelatin-chitosan hydrogel

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A 3-D scaffold comprised of self-assembled polycaprolactone (PCL) sandwiched in a gelatin-chitosan (GC) hydrogel was developed as a biodegradable patch for surgical reconstruction of congenital heart defects (CHD). These multilayered scaffolds have suturability and high tensile strength provided by the PCL core, along with cardiomyocyte binding sites, and control of the degradation rate provided by the GC hydrogel. Blended or pure MW PCL scaffolds were formed by self-assembly in an aqueous environment, then assessed for pore structure, elastic modulus, tensile strength and degradation time. SEM analysis showed that scaffolds containing lower MW PCL have larger pore sizes. These samples have ~1.8 MPa of elastic modulus. The degradation rate increased in scaffolds containing 10 kDa PCL in physiologic conditions. PCL scaffolds were coated with a GC mixture to facilitate cell attachment and form 3-D tissues. These coated scaffolds showed no significant alteration in tensile stress, strain and tensile modulus. However the compressive modulus of the composite tissue was in a range more similar to native tissue (~15 kPa for 1 : 1 = G : C). A 3-D hive like porous structure was formed with a mean pore diameter ~80 μm, allowing for invasion of neonatal rat ventricular myocytes (NRVM). NRVM were viable for 14 days, and formed spontaneously beating engineered tissues. In summary, we were able to form a multilayered scaffold containing NRVM with sufficient tensile strength for use in CHD repair.

15.P01
Perfusion of vascularized engineered heart tissue

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A critical factor for the potential clinical use of engineered heart tissues (EHT) for cardiac repair remains their limited size. Here we report on our efforts to integrate perfusable vessels in EHT to overcome this bottleneck. Adapting our previously published protocol, we generated EHT with a size of 22 × 15 × 1.5 mm spanning between six silicone posts hold by a silicone rack with hollow central posts. Thin calcium-alginate fibers were generated by extrusion technique and positioned into the outlet of the hollow posts. A reconstitution mix of neonatal rat heart cells, fibrinogen and thrombin was pipetted around the lower part of the silicone posts including the alginate fiber. For dissolving of the alginate fiber without impairing the EHT function incubation in 0.78 mM citrate solution or 0.78 μg/ml alginate lyase for 22 h was found to be optimal. This method gave rise to a tubular lumen (~100 μm) throughout the length of the EHT that could be connected to a perfusion system via the hollow silicone tubes. By varying flow rate, time point, duration and medium constitution of the perfusion we achieved viable beating EHT with increased cardiac myocyte density and distribution. Histological analysis suggested at least partial endothelial coverage of the lumen. This is the first report on a novel approach to achieve perfusion of living, actively contracting and force-generating 3D engineered tissues that is, in principle, applicable for all types of tissue engineering.

15.P02
The functional evaluation of ES cell-derived cardiac cell sheet with different types of fibroblasts

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The evidences that fibroblasts are widely used for the fabrication of cell sheets indicate the importance of the appropriate cellular network and the extracellular matrixes. However the suitable conditions for cardiac cell sheets remain elusive. In this study, we evaluated the function of cardiac cell sheets fabricated in co-culture with mouse ES-derived cardiomyocytes and a variety of fibroblasts by assessing sheet forming ability, action potential propagation and cardiomyocyte hypertrophy. Mouse ES cells-derived cardiomyocytes were co-cultured with three types of fibroblasts (mouse neonatal cardiac Fibroblasts (NCFs), mouse adult cardiac fibroblasts (ACFs) and mouse adult dermal fibroblasts (ADFs)) on FBS-coated UpCell for 5 days. After lowering temperature, it took around 75 min (NCFs), 30 min (ADFs), and 22 h (ACFs) to detach from dishes. When the extracellular action potential of each cell sheet was examined using multi-electrode system, synchronous contraction among cardiomyocytes was observed in the sheets with NCFs and ACFs, but not with ADFs. Finally the localization and the cell morphology of cardiomyocytes were elucidated with immunocytochemistry. Cardiomyocytes in cell sheets with NCFs and ACFs were elongated and evenly distributed, while those with ADFs showed the diminished size and localized. These findings suggest that cardiac fibroblasts might be supportive for cardiac function in cell sheet and the precise mechanisms of it remain to be determined.

15.P03
Effect of endothelial cell-cardiomyocyte 3D co-culture on the properties of an engineered cardiac tissue

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Endothelial cell (EC)-cardiomyocyte (CM) crosstalk through paracrine interactions is important in the control of myocardial development, growth and function such as contractility. Therefore, a functional engineered cardiac tissue should be able to accommodate ECs, not only for neo-vessel formation, but also for the proper function of the muscle cells. In this study we co-encapsulated ECs with CMs in photo-patternable hydrogels to assess the physiological effects of EC presence in a 3D engineered cardiac tissue under normal and pathological conditions. Our results showed that, EC presence did not hinder cardiac function under normal physiological conditions as proven by similar Connexin 43 and Troponin I expression and similar beating behavior throughout 15 days of culture. Moreover, EC presence improved CM survival significantly under oxidative stress conditions as shown by live/dead and MTS assays. Interestingly, our photopatternable system allowed us to distinguish between two different effects originating from ECs themselves, and from EC culture media. We found that at least one of the EC driven factors that govern this cardioprotective effect is Endothelin-1 (Et-1) and suggest that Et-1 has a positive cardioprotective effect locally. The engineered myocardium in this study, which we have control over the spatial distribution of its constituent cells, is important for cardiac regeneration and as a model tissue for investigating myocardial physiology.

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15.P04
Dynamics of engraftment of cardiac cell sheets *in vivo* and their angiogenic potential

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Myocardial tissue engineering has been pursed as a new possibility to repair the damaged myocardium. Previously, we have created cardiac cell sheets that have pulsatile function and endothelial cell network using the novel temperature responsive culture dishes, and demonstrated high viability of engrafted cells *in vivo* and the significant improvement of cardiac function. In this study, dynamics of vascular formation and engraftment of cell sheets were investigated after they were implanted. Cardiac cell sheets of Luciferase-transgenic neonatal rat or GFP-transgenic neonatal rat were implanted into dorsal subcutaneous tissues in nude rats. Real time PCR analysis revealed higher expression of HIF-1 in 6 h, VEGF and MMP-9 in 6 h and 1 day, and PDGF-BB and Ang1 during 3–28 days than other time points. The results correlated with the macroscopic view of the vascular formation inside the cell sheets: fast vascular formation in 6 h, functional vessel formation in 12 h, and their maturation in 7 days. Furthermore, the vessels formed initially possibly contributed to 1.5 year-survival of the cell sheets *in vivo* that shown by the imaging luciferase activities. The findings of the time course of mRNA expression that induce vascular formation and long-term survival of the cell sheets could be a driving force for *in vitro* tissue construction with appropriate cytokine control.

15.P05
The beneficial effects of delayed delivery on the efficiency of hydrogel therapy post myocardial infarction

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Remodelling following myocardial infarction (MI) can result in a progressive decline in left ventricular performance leading to heart failure. The delivery of hydrogels to infarcted hearts reduce remodelling through limiting increase in wall stress. The aim of this study was to directly compare the effect of injecting an enzymatically degradable polyethylene glycol (PEG) gel immediately or 7 days post-MI on pathological remodelling of the infarcted rat heart. Following permanent ligation of the left anterior descending artery in rats, PEG gel monomers were injected into the infarcted area and polymerized *in situ*. The gel was delivered either immediately or 1 week after infarct induction. Function was assessed by echocardiography and scar thickness quantified by histomorphometric analysis. The study was blinded and randomized. Degradation of fluorescently labeled gels was tracked over the implant period. Delay of treatment by 7 days improved fractional shortening at both 2 and 4 weeks post-MI and resulted in thicker scars relative to immediate treatment ($p < 0.05$). Gel distribution for immediate injection was intricately striated and temporary whilst 7-day delivery resulted in a cohesive mass that persisted for 4 weeks. We believe that the different morphologies were due to infarction-induced changes in the cardiac structure and influenced the degradability of the injectates. The results indicate timing of delivery is important and very early time points may be less beneficial.

15.P06
Relationship between exercise-induced blood lactate concentration and hematopoietic stem cell mobilization in heart disease patients

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Background: Hematopoietic stem cell (HSC) mobilization is not yet completely understood. Literature describes possible triggers such as the internal elevation of growth factors and chemokines, or the external influence of stress factors like hypoxia, and exercise. Exercise is known to be an evidence based part of cardiac rehabilitation. One effect of exercise could be an elevated concentration of HSCs in the peripheral blood. The aim of this study was to evaluate the influence of different blood lactate (La) concentrations on the number of HSCs in the blood of patients recovering from coronary incidences.
Methods: Seven patients (63.4 ± 7.0 years) performed 2–3 different randomized exercise test protocols ($n = 18$) out of four possibilities (ergometry, constant-load, short-interval or long-interval tests), producing different La-concentrations. Blood was drawn from the cubital vein before and instantly after each intervention. The number of HSCs in the peripheral blood was evaluated by means of flow cytometry ($CD34^+/CD45^+$). La-concentration was measured via ear-capillary method.
Results: There was a significant positive correlation ($r = 0.63$, $p < 0.01$) between the difference in HSCs in the peripheral blood and the maximal La-concentration accumulated during each test independent of the exercise mode.
Conclusions: An elevated La-concentration supports the increase of HSCs in the peripheral blood, possibly due to an enhanced release of progenitors from the bone marrow.

15.P07
Effects of macrophage polarization on mesenchymal stem cells in the context of myocardial regeneration

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The outcome of cardiac cell therapy will significantly depend on the inflammatory environment at the site of injury. Following myocardial infarction, tissue repair is mediated by pro-inflammatory (M1) and anti-inflammatory (M2) macrophages that are recruited during the early and late stages of cardiac ischemia. Repair cells delivered into the injured myocardium are inevitably subjected to this inflammatory milieu. The present study investigated how M1 or M2 environments affect the survival and function of mesenchymal stem cells (MSCs), which are the most frequently used cells in human clinical trials of heart repair. Human MSCs were cultured with M1 and M2 macrophages (both in direct and indirect culture) and the cytokines they secrete. For co-cultures, macrophages were polarized into M1 and M2 macrophages *in vitro*. The effect of polarization on the growth and function of MSCs was determined by measuring cell growth and differentiation

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potential. The M1 and M2 cytokines decreased and increased respectively the numbers of MSCs when compared to controls, and changed the differentiation potential of MSCs. There was a consistent increase in the number of MSCs during indirect and direct co-cultures with M2 macrophages. The data suggest that M2 macrophages augment survival and growth of mesenchymal stem cells while M1 macrophages inhibit the growth of MSCs. There seems to be a dynamic effect between macrophages and MSCs that may dictate the type of reparative response.

15.P08
Development of new bioactive implant to assist cardiac tissue regeneration (EU RECATABI Project)

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The main problem of many cardiovascular diseases is the presence of necrotic tissue. Current treatments consist in cellular therapy where stem cells are seeded into scaffolds and then grafted onto infarcted ventricles. Due to the adverse conditions of the damaged tissue it is difficult to maintain alive the implanted cells, therefore the main motivation of the EU granted project (RECATABI) is to develop a bioactive implant which will enhance cell survival. The bioactive implant under development consists of two components: an engineered micro-scale elastomeric biomaterial -which will match the requirements of the implanted tissue- and a soft nano-fiber self-assembling hydrogel (RAD16-I), which will provide adequate 3D-microenvironment. In this work we have evaluated the effect that RAD16-I would have on cardiac differentiation of subcutaneous adipose tissue-derived progenitor cells (subATDPCs). We found that when subATDPCs were cultured for 2 weeks in RAD16-I gels with cardiogenic media, the cells presented an aligned growth along the scaffold and preliminary qPCR results indicate up-regulation of cardiac markers at same culture times. We also analyze the effect of electrical stimulation detecting good cell viability and up-regulation of Troponin-I expression after 2 weeks of culture in control media. These results suggest that 3D environment provided by RAD16-I might be a good strategy to enhance the differentiation of suATDPCs into cardiac progenitor-like cells.

15.P09
Human cardiac progenitor cell sheets as a source of autologous contractile and vascular cells for cardiac repair

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Human adult heart harbors a population of resident progenitor cells that can be isolated by Sca-1 antibody and expanded in culture. These cells can differentiate into cardiomyocytes and vascular cells *in vitro* and contribute to cardiac regeneration *in vivo*. However, when directly injected as single cell suspension, the survival rate and retention is really poor, less than 1% of injected cells being detectable in the host tissue within few weeks. The present study aimed at investigating the possibility to produce scaffoldless, thick cardiac progenitor cell-derived cardiac patches by thermo-responsive technology. Human cardiac progenitors obtained from the auricles of patients were cultured as scaffoldless engineered tissues fabricated using temperature-responsive surfaces obtained by poly-N-isopropylacrylamide (PNIPAAm) surface immobilization. In the engineered tissue, progenitor cells established proper three-dimensional intercellular relationships and produced abundant extracellular matrix, while preserving their phenotype and plasticity. Cell phenotype and viability within the 3D construct were followed for 1 week, showing that no significant differentiation or apoptotic events occurred within the construct. After engineered tissues were leant on visceral pericardium, a number of cells migrated into the myocardium and in the vascular walls, where they integrated in the respective textures. The study demonstrates the suitability of such approach to deliver stem cells.

15.P10
Transplantation of bone marrow-derived human mesenchymal stem cell sheets attenuated left ventricular remodeling in porcine ischemic cardiomyopathy model

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Introduction: Clinical trials of bone marrow-derived autologous human mesenchymal stem cell (MSC) transplantation via needle injection have shown to be modest effects. Whereas, cell sheet technique have been proven to transplant abundant cells and enhance the efficacy of cell therapy. We hypothesized that transplantation of hMSC sheet may be feasible, safe and effective in treating ischemic cardiomyopathy (ICM).
Methods and Results: Human MSCs were acquired from bone marrow and they were positive for CD73, CD90 and CD105, and negative for CD11b and CD45.Ten hMSC sheets were created from total cell number of 1×108 h MSCs using thermoresponsive dishes. They were successfully transplanted over the infarct myocardium of porcine ICM model induced by placing ameroid constrictor on LAD without any procedure related complications (MSC group = 6: sheet transplantation with tacrolimus, Sham = 6). Premature ventricular contractions were rarely detected by Holter ECG in the MSC group. On echocardiography, cardiac performance of the MSC group was significantly better than that of Sham at 8 weeks after transplantation (EF; $51 \pm 5\%$ vs $35 \pm 5\%$, $p < 0.001$). On histological examination, LV remodeling was significantly attenuated compared with sham (cell size; 13 ± 1 μm vs 20 ± 2 μm, $p < 0.001$, fibrosis; $2.2 \pm 0.3\%$ vs $6.9 \pm 0.4\%$, $p < 0.0001$).
Conclusions: hMSC sheets improved cardiac function and attenuated LV remodeling in ischemic cardiomyopathy without major complications, indicating that this strategy would be applicable into clinical setting.

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15.P11
Biofunctionalization of CoCr surfaces with dimeric peptides and REDV elastin-like polymers to improve endothelization of cardiovascular implants

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To improve cardiovascular implant success, biomaterial surfaces are designated to modulate endothelial cells (EC) response in order to prevent atherosclerosis, restenosis and thrombosis diseases. We aimed to obtain a new family of biofunctionalized CoCr alloy surfaces by covalently-anchoring biofunctional molecules to enhance EC adhesion and growth. CoCr alloy (ASTM F90) discs were treated with plasma (PL) and etched with 5 M NaOH 2 h (NaOH) previous to silanization with 3-chloropropyltriethoxysilane (PL + CPTES and NaOH + CPTES) and functionalized with REDV elastin-like polymer and dimeric peptides (containing RGD, REDV, YIGSR). SEM, interferometry, contact angle, zeta potential, XPS and ToF-SIMS were used to determine physicochemical surface properties at each procedure step. Biofunctional molecules quantification and HUVEC cell studies were performed by means of QCM-D, FITC, and fluorescence microscopy. ToF-SIMS and XPS confirmed the immobilization of the biofunctional molecules on CoCr surfaces. NaOH + CPTES treated surfaces and the use of biomolecules enhanced EC adhesion after 4 h of incubation. This can be explained by the higher quantity of attached molecules, which suggests that the effect of nano-roughness and, the high electronegative surface charge are controlling the biomolecules adsorption and guiding cell response. Biofunctional molecules immobilization on CoCr surfaces represents an innovative technique to improve stents endothelization.

15.P12
The effects of electrical field stimulation on cardiomyocyte growth on collagen gel and 2D-coating

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Electrical field stimulation has been shown to improve cardiac cell differentiation, alignment and functional properties. In this study, neonatal rat cardiomyocytes were exposed to both long-term and short-term stimulation with the goal of investigating whether it is possible to achieve cell orientation and the maturation of cardiomyocytes with a novel, microelectrode array (MEA)-compatible electrical stimulation platform. Cells were cultured on gelatin-coated MEA chambers and on collagen gel.Cells were viable after electrical stimulation, but no orientation or other morphological changes were observed. However, the electrode wires in MEA dishes affected the cell orientation. Cardiomyocytes grew well on collagen gel but no differences between coating and collagen gel were seen in cells after stimulation. Cell contractions synchronized with pacing, but settled back to their original frequency in the absence of stimulation. The gene expression of Cx-43 and MYH-7 was stronger in stimulated cells than in controls. In summary, the surface topography was a stronger determinant of cardiomyocyte orientation than the applied electrical field stimulation, suggesting that the micro architecture of the biomaterials should be carefully designed for cell applications. However, electrical stimulation and its duration affected gene expression, so a stimulation system may prove useful to enhance the cardiac differentiation of stem cells.

15.P13
Secretome of apoptotic peripheral blood cells (APOSEC) attenuates microvascular obstruction in a porcine closed chest reperfused acute myocardial infarction model: Role of platelet aggregation and vasodilation

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Our group has recently found that paracrine factors secreted from apoptotic peripheral blood mononuclear cells (APSOEC) attenuate the size of acute myocardial infarction (AMI). The aim of this study was to determine the influence of APOSEC on microvascular obstruction (MVO) in a porcine AMI model. Treatment of AMI with cell culture supernatants derived from irradiated apoptotic peripheral blood mononuclear cells (APOSEC) resulted in a significantly improved microvascular perfusion (Myocardial blush grade: 1.3 ± 0.3 vs 2.5 ± 0.3; $p = 0.033$). Platelet activation markers (P-selectin, CD40L, PF-4, TSP-1) were reduced in plasma samples, suggesting an anti-aggregatory capacity of APOSEC. This finding was confirmed by *in vitro* tests showing significantly impaired aggregation of APOSEC treated platelets, paralleled by vasodilator-stimulated phosphoprotein-mediated inhibition. In addition, APOSEC evidenced a significant vasodilatory capacity on coronary arteries. HUVECs co-incubated with the compound substantially upregulated iNOS expression. Treatment of isolated coronary arterial segments with APOSEC resulted in a dilation of the vessels in a dose dependent manner (APOSEC from 5×10^5 cells: 23% dilation; 1×10^6: 26%; 5×10^6: 34%).Our data give first evidence that APOSEC reduces the extent of MVO during AMI. This explains the improved long-term outcome after APOSEC treatment in AMI as previously described.

15.P14
Evaluation of electrocardiographic analysis routines to characterize qualitative changes associated with mesenchymal stem cell (MSC) administration in a porcine model

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Stem cell therapies for potential treatment of myocardial ischemia are often evaluated in porcine models using various biomarkers diagnostic of ischemic status, as well as diverse methodologies for electrocardiogram (ECG) collection and analysis. This project was designed to assess the relative effectiveness of different sampling strategies with regard to providing definitive, quantitative data on ECG rhythm variants in the context of ongoing MSC therapy in a myocardial infarction (MI) model. Secondary objectives were to correlate key biomarkers (troponin, CK-MB) with ECG variants, particularly those of a malignant nature, as well as to determine the influence of volume of cell administration on study outcome measures. Twenty pigs ($n = 5$/group) underwent a myocardial ischemia protocol (Day 1) with subsequent (Day 4) injection ($n = 10$ sites/heart) of either 0.5 or 1.5 ml cell/carrier volumes per injection. ECGs were measured continuously and subsequently analyzed in discrete intervals using a range of sampling methods. Post-euthanasia heart tissue examinations included quantification of infarct

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volume and assessment of localized tissue response to injection procedures. Results demonstrate that contemporary minimalist strategies for ECG collection and analysis of arrhythmic potential are inadequate to define baseline arrhythmia and differentiate potential amelioration or exacerbation of ischemia-induced rhythm variants.

15.P15
Engineering of human 3D cardiac muscle patches based on a biological scaffold and specific bioreactor technology

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Remodeling processes of heart tissue after a myocardial infarct could cause cardiac insufficiency. Current innovative therapeutic concepts lack ideal autologous cell source, sufficient efficiency and missing long term success compared to already clinically proven cell transplants. We propose cardiosphere derived cells (CDCs) as possible cell source which can be generated from autologous biopsies. CDCs show characteristics of adult cardiac stem cells. Another human cell source can probably be umbilical cord stem cells (UCMSCs). They have the potential to differentiate into cardiomyocytes. Both cell types were isolated by enzymatic digest. The UCMSCs were differentiated with oxytocin or azacytedine. The CDCs and the differentiated UCMSCs were both seeded on a collagen-based matrix and cultured statically, under laminar or pulsatile flow in a bioreactor. After culturing for 7 days, immunohistochemical staining for cardiac markers were performed. All generated tissues express myocardial markers such as cActin, Connexin 43, and MYH7.To ensure the culture of cells in deeper regions of the scaffolds, the different seeding and cell culture conditions described above will be combined. Furthermore, cardiac differentiation will be verified immunohistochemically and by PCR. Patch clamp technology will be established for the functional characterization of the engineered tissues.

15.P16
Development of an electrical stimulation-based bioreactor to enhance cardiac commitment of human adult stem cells.

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Introduction: Recent studies have shown that an exogenous electric field (EF) can positively affect stem cell differentiation towards the cardiomyogenic lineage. However, the effects of pulsed EF involved in myogenic commitment are poorly understood. The aim of this work is to clarify the effects of mono and bi-phasic electrical current on human adult stem cells isolated from human adipose tissue and heart.
Methods: Device: The culture system is equipped with a chassis, including electrical wiring systems and housings for multiple removable culture chambers, made in PDMS in which stainless steel electrodes are embedded. The bioreactor is driven by a graphical-interface software. Biological experiments: human adult stem cells were seeded on collagen coated glass slides and 24 h later the EFs square monophasic or biphasic were applied for different periods (3, 8 and 24 h). Cells were analyzed for the expression of two cardiac commitment markers Connexin 43 (Cx-43) and Gata-4.
Results and Conclusion: When the two cell types were exposed to biphasic pulsed EF, Cx-43 was relocated at the cell membrane earlier and at higher levels. Bi-phasic stimulation induced also the up-regulation of the early cardiac transcription factor GATA-4. It is thus suggested that biphasic EF can be an effective stimulus to induce myogenic commitment for stem cells. Experiments which combine this stimulus with other cues, such as stiffness and topography of the culture substrate are in progress.

15.P17
Human cardiac biopsy-derived cells - a novel strategy for cell-based cardiac therapy

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Cell therapy represents a promising treatment option for cardiovascular diseases. We identified a novel cell type from human endomyocardial biopsies: cardiac-derived adherent proliferating cells (CAPs). The aim of our study was to investigate the effect of intravenous (i.v.) and intramyocardial (i.m.) administration of CAPs in Coxsackievirus B3 (CVB3)-induced myocarditis and angiotensin (Ang) II-induced heart failure. *In vitro*, CAPs decreased the CVB3-induced HL-1 apoptosis by 3.5-fold ($p < 0.05$) and the activation of splenic mononuclear cells by 2.9-fold ($p < 0.05$) in a nitric oxide- and interleukin 10-dependent manner. I.v. injection in CVB3-infected mice resulted in a decrease of cardiac apoptosis, CVB3 viral load, and cardiac mononuclear cell activity. These findings were associated with a 1.1-fold ($p < 0.05$) increase in LV pressure and a 1.3-fold ($p < 0.05$) and 1.3-fold ($p < 0.01$) improvement in dP/dtmax and dP/dtmin, respectively. With respect to fibrosis, *in vitro*, CAPs reduced the Ang II-induced proliferation, oxidative stress and collagen production in cardiac fibroblasts. Furthermore, i.m. injection of CAPs decreased the Ang II-induced accumulation of collagen I and III in Ang II-treated mice, which was paralleled by an improvement in LV systolic and diastolic function. In conclusion, CAPs represent a unique cardiac-derived cell type with promising features for the treatment of cardiovascular diseases.

15.P18
The role of electromechanical stimulation in engineered myocardium function

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Tissue engineering of myocardium represents a promising approach for the treatment of myocardial infarcts. A number of groups have attempted to recapitulate the physical stimulation (electrical and mechanical) present in the heart; however, these have been done in isolation, while combined electromechanical stimulation is present *in vivo*. Moreover, there has been no direct comparison of the two stimuli and their benefits. We hypothesized that alterations in the type of biophysical stimulation would significantly impact engineered myocardium function. Myocardial equivalents were created by entrapping neonatal rat cardiomyocytes (CM) in fibrin, and cultured under electrical, mechanical and combined electromechanical stimulation (synchronized pacing with release of stretch). After 2 weeks, constructs were harvested and analyzed via contraction force, DNA assay, histology and Western blots for important CM proteins. Stimulation resulted in increases in twitch force above static controls; however, no significant differences between stimulation types were noted. A less functional form of Connexin 43 (phosphorylated at Ser368) was upregulated in

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mechanical as compared to electrical stimulation, indicating better coupling with electrical pacing. Combined stimulation was similar to electrical stimulation in most aspects. Preliminary data indicate differences in function when altering the timing of the combined stimuli (synchronized vs offset), and future work will focus on this aspect.

15.P19
Engineering natural matrices: patterning cells derived from embryonic stem cells into implantable carriers that are designed to support cardiac tissue regeneration

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Biomaterials and methods for cell delivery in cardiac regeneration have been previously explored. These studies often ignore design factors critical for rebuilding tissue, by reducing cell retention and/or structural support. Key patch elements include biomaterial architecture, material strength, compliance, cell patterning, and incorporation of multiple cell types. We investigated the combinatorial use of decellularized tissues, moldable hydrogels, cell patterning, and cell-sheet engineering to develop an organized patch for treating myocardial infarctions based upon the hypothesis that alignment of cardiomyocytes will increase patch integration, cellular retention, and cardiac function. We show our previously developed wrinkled microchip, treated with thermoresponsive p-NIPAAM induces cellular alignment of transferrable cardiomyocytes sheets. Decellularized urinary bladder matrix (UBM) which supports cell survival is compliant and sutureable, does not allow maintenance of transferred cell-sheets. However, UBM filled with hyaluronan hydrogels (shown to be proangiogenic), created a surface capable of supporting cell sheets, allowing delivery of endothelial and cardiomyocyte cell types. Our data shows that an optimal design for generating a 3D cardiac tissue includes decellularized matrix seeded with endothelial cells in a HA hydrogel layered with aligned cardiac sheets fabricated using our 'wrinkled' microchips and the thermo-responsive polymer.

15.P20
A novel perfusion bioreactor for cardiomyogenesis

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Cardiovascular diseases are the leading cause of mortality in developed nations. Cellular therapies offer great potential, but face many limitations such as generation of sufficient cell numbers, purity and cardiac functionality. To achieve the production of clinically-relevant cell numbers, production of cardiac cells from pluripotent stem cells in controlled bioreactor cultures was attempted. We encapsulated murine embryonic stem cells (mESCs) in alginate-gelatin hydrogels and cultured in a novel perfusion novel bioreactor. Cardiomyogenesis was assessed in the 3D cultures under both fed-batch and perfusion modes. After 21 days, cell numbers in the perfusion bioreactor were similar to the fed-batch culture, generating 3.5×10^6 cells/ml, and were significantly higher than the static 3D cultures. The perfusion bioreactor also resulted in significantly higher cardiac gene expression (alpha-MHC and Troponin-T) and achieved structural organization of cardiac proteins and cardiac functionality. Furthermore, the perfusion bioreactor allows control of oxygen levels. Oxygen tension was then varied (4% and 20%) to determine the impact of oxygen on cardiomyogenesis. The usage of our bioprocess system facilitates a uniform supply of signals that enhances cardiomyogenesis compared to static controls, making it a feasible tool for future regenerative strategies for cardiomyogenesis.

15.P21
Injectable gellan/gelatin microspheres for the treatment of heart failure

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Injectable scaffolds are receiving a great interest as strategies to repair the infarcted myocardium. The aim of this work was the preparation and characterization of injectable microspheres for myocardial tissue engineering. Gellan/gelatin microspheres were prepared by a single water-in oil emulsion, using phosphatidylcholine as surfactant. The obtained particles underwent morphological, physicochemical and functional investigation. Loading with IGF-1 was then performed by adsorption. Loaded and unloaded particles, in combination with rat cardiac progenitor cells, were injected after cryoinjury on the rat heart. The morphological analysis showed a spherical shape and microporous surface. The infrared analysis confirmed the presence of interactions among the functional groups of the two biopolymers. Injectability through a narrow needle was verified. Preliminary *in vivo* tests, performed by three intramyocardial injections of cell loaded microspheres in a rat model of myocardial injury, documented that progenitor cells homed to the damaged myocardium and IGF-1 functionalization increased their engraftment. The results obtained showed adequate morphological, physicochemical, functional and biological properties for application as injectable scaffold for myocardial repair. This work was supported by the European Commission FP7 Programme, grant 214539.

15.P22
Mouse mesenchymal stem cells derived from induced pluripotent stem cells demonstrated myocardiogenesis potential

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This study is to derive mouse MSCs from iPSCs for myocardiogenesis potential study in experimental myocardial infarction models. Mesenchymal-like stem cells were isolated from mouse iPSCs by removing feeder cells and leukemia inhibitory factor (LIF). MSCs were enriched by conditioned medium with basic fibroblast growth factor (FGF2) and epidermal growth factor (EGF) supplements before phenotypical characterization by flow cytometry. Multipotent differentiation studies, including adipogenesis, osteogenesis and chondrogenesis have been carried out. Purified MSCs were further engaged to a recombinant cocktail formulated with transforming growth factor-beta 1, bone morphogenetic protein-4, activin A,retinoic acid,insulin-like growth factor-1,fibroblast growth factor-2, alpha-thrombin, and interleukin-6. Derived cardiopoietic mouse MSCs were transplanted into myocardial infracted murine model. The iPSC derived cells were negative for hematopoietic markers CD34 and CD133, pluripotency markers Oct4 and TRA-1-60, while being positive for mesenchymal markers, CD44, CD73 and CD90. These cells were induced into osteocytes and chondrocytes. High expression of homeobox transcription factor Nkx 2.5, ventricular myosin light chain MLC-2V, and sarcomeric alpha-actinin were observed in cardiopoietic mouse MSCs. Mouse iPSCs derived MSCs demonstrated myocardiogenesis potential induced by a recombinant cocktail of growth factors. They hold potential capacity to attenuate ischemic cardiomyopathy.

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15.P23
Controlling micro-structure to enhance de novo extracellular matrix deposition in elastomeric scaffolds for cardiac tissue regeneration

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Elastomeric scaffolds have been adopted for cardiac tissue regeneration in several scenarios including: myocardial wall, arteries and heart valves. Scaffold local deformations play a crucial role in, extracellular matrix (ECM) deposition, and tissue remodeling. To date, our understanding of how scaffold micro-architecture translates the organ level deformation into local deformation experienced by the cells remains inadequate. In this study we investigated on how strain levels and/or scaffold micro-structure impact on ECM formation. We hypothesized that (i) optimal levels of strain (ii) optimal micro - structure architectures can be identified to improve ECM production in quantity (collagen mass) and quality (level of structural organization). Vascular smooth muscle cells (VSMCs) seeded polyurethane scaffolds have been dynamically cultured using a uniaxial stretch bioreactor. Different levels of uniaxial strain ($n \geq 7$, 15, 30, and 50% at 1 Hz, 21 days) have been imposed, results (collagen mass) showed the existence of optimal strain levels (30%). Similarly, VSMCs seeded scaffolds with different micro-structure (groups having the same mechanical response but different number of fiber intersections/area) have been dynamically cultured for 21 days at 30% strain, 1 Hz, $n = 9$. Results showed the existence of optimal micro-structures, scaffolds with about 0.03 and 0.06 intersections/μm^2 produced 30.1 and 85.8 [ng/g] respectively (collagen mass).

15.P24
Dissection and reconstruction of the extracellular matrix - a cardiac regenerative niche

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Besides being tissue-specific, the extracellular matrix (ECM) is also developmental-stage specific and is actively involved in the tissue remodeling response to stress conditions, e.g. tissue injury. Thus, we hypothesize that the ECM plays a key role on determining whether the wound healing process develops along a regenerative response (fetus) or leads instead to scar formation (adult). To address this we are performing pioneering work by implementing *in vitro* model-systems of decellularized ECM from fetal and adult hearts. Herein we report on the fetal (E18) heart whole-organ decellularization, with well-preserved ECM structure and composition. Fragments of adult myocardium were decellularized using the same protocol to legitimate comparison of the bio-chemical, -physical and cardioinductive properties of the ontogenic distinct native ECM-systems. DAPI staining of decellularized matrices confirmed the absence of nuclei and immunofluorescence confirmed the lack of cardiomyocyte (CM) contractile components and specific cardiac-cell markers. Preservation of the collagen network that surrounded CM in the normal tissue, and correct collagen fiber assembly was demonstrated by Masson's trichrome stain and TEM, respectively. Aiming at exploring differential cell responses/dynamics while in contact with adult versus fetal ECM, rat neonatal CM were seeded onto the acellular matrices. A first sketch for the kind of cellular repopulation obtained throughout time will be presented.

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16. Heart Valve Generation

16.01
Keynote: The role of textiles in cardiovascular tissue engineering

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The scaffold plays a central role in the production of tissue engineered structures. Although the vision of tissue engineering is the complete autologous implant, the use of supporting materials enabling the formation of 3D structures is mostly necessary. Different kinds of scaffold materials are in use including biodegradable or non-biodegradable polymers, biological materials like fibrin, collagen, hyaloronic acid etc. and acellularized xeno- or homografts. Although cardiovascular tissue has made a significant step forward in the last decade, the tissue engineered constructs hold mostly insufficient mechanical properties for the arterial circulation. Therefore the textile reinforcement of tissue engineered structures allows the combination of the ideal properties of cell carrier scaffolds (e.g. hydrogels) with the defined mechanical properties of textile structures. The presentation will give an overview on the different kinds of textile scaffold materials and there use in reinforcing and organizing hydro-gel-based cardiovascular implants.

16.02
Autologous CD133$^+$ tissue-engineered pulmonary valved stent implantation: a percutaneous technique

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Introduction: The feasibility of percutaneously implanting autologous tissue engineered valved stents in the pulmonary artery was pursued to overcome the current limitation of degeneration and calcification of the bovine jugular vein valves.
Materials and methods: Porcine pulmonary heart valves and small intestinal submucosa were obtained from a slaughterhouse. To avoid stent strut related tissue damage small intestinal submucosa layer was put onto the inside of the pulmonary valved stent. The porcine valves were decellularized enzymatically. CD133$^+$ cells were isolated from bone marrow of juvenile sheep. After seeding the construct was placed in a dynamic bioreactor for 16 days. The autologous tissue engineered valved stent was implanted into the pulmonary artery with a deployment catheter. The constructs were explanted after 3 months.
Results: The angiography demonstrated orthotopic position of the stents ($n = 7$) at implantation and explantation. A confluent endothelial lining displayed typical cobblestone morphology. Immunocytochemistry demonstrated strong expression of α-smooth muscle actin in the leaflet. V Kossa staining demonstrated mild calcification on the annular side of the valve after 3 months. No signs of inflammation were detected by using anti-CD3, anti-CD20, anti-CD45 and anti-CD68.
Conclusions: In this study we demonstrated good function of the leaflets of CD133$^+$ tissue engineered valved stents after percutaneous implantation and 3-month follow-up.

16.03
Modification of human pericardium based on dynamic loading, a new approach for heart valve tissue engineering

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The challenge of heart valve tissue engineering is to create a living human biological heart valve prosthesis for clinical use. We prepared three cusp heart valve constructs from living autologous human pericardium harvested during cardiac surgery. These were then modified by conditioning in a dynamic culture system for up to 3 weeks and compared to unconditioned pericardial samples from the same patient and to the normal human aortic heart valve using immunohistochemical and biomechanical analysis. Dynamic loading was shown to influence extracellular matrix (ECM) composition and cellular differentiation. There was a three-fold increase in the total number of vimentin positive pericardial interstitial cells (PICs, quiescent phenotype) and a twofold increase in alpha-actin positive PICs (active, myofibroblast-like phenotype). Dynamic conditioning induced PICs proliferation as shown by the presence of the proliferation marker Ki-67 in conditioned samples. There was a substantial increase in collagen, elastin and glycosaminoglycan content as evidence of new ECM production. All results were statistically significant ($p \leq 0.001$). Our heart valve constructs were shown to have a normal transvalvular gradient (up to 6 mm Hg). The mechanical properties (secant elastic modulus) of human living pericardial tissue (13.1 ± 8.3 Mpa) are similar to native aortic heart valve tissue.
Acknowledgements: Supported by the Grant Agency of the Ministry of Health of the CR (project No.NT/11270).

16.04
Fibronectin coating supports early *in vivo* repopulation of decellularized aortic conduits in a rat transplantation model

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Proactive coating represents a promising approach to accelerate the *in vivo* neoendothelialization and medial repopulation of decellularized cardiovascular implants. Detergent-decellularized aortic rat conduits ($n = 24$) were coated with covalently Alexa488-labelled fibronectin (FN; 50 μg/ml, 24 h) and infrarenally implanted in Wistar rats (groupA; $n = 18$). Uncoated implants served as controls (groupB; $n = 18$). Preoperatively and at postoperative day 1 and week 1, 4 and 8, fluorescence-based detection of FN coating was conducted. Cellular

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repopulation was examined by histology and immunohistochemistry. RNA analysis after explantation was performed by quantitative realtime PCR. Unimpaired survival was 100%. Green FN fluorescence on both surfaces of the aortic conduits was bright at days 0 and 1 and decreased after 1 week, however, it was still present after 4 and 8 weeks. After 8 weeks, the intima-to-media (I/M) thickness was significantly in-creased in GroupA versus GroupB ($p < 0.01$), particularly due to a myofibroblast hyperplasia (αSMA^+). However, relevant medial repopulation originating from the adventitial zone was observed only in groupA ($p < 0.001$). In both groups vonKossa staining revealed sparse medial calcification and staining against inflammatory cell markers (CD3 & CD68) was negative. Biofunctional FN coating of cardiovascular implants proved feasible, persisted up to 8 weeks in the systemic circulation and induced a significantly increased medial recellularization.

16.05
Spatially selective cell embedding in fibrin based tissue engineered heart valves

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Introduction: Native heart valves (HV) are highly heterogeneous. In the wall, the cells stain positive for α smooth muscle actin (α-SMA) indicating a contractile phenotype, while hardly any α-SMA is detectable in the leaflet. On the contrary, the wall and the leaflets of tissue engineered heart valves (TEHVs) are normally seeded with the same cells. We aim at mimicking the spatial distribution of the cell phenotype of native HVs in a fibrin-based scaffold by means of a newly developed 2-step injection moulding process.
Materials and methods: HVs were produced by polymerizing ?brinogen with CaCl2, thrombin and cells. Non-contractile MFs from ovine umbilical cord arteries were embedded in the leaflet during the first moulding step. Contractile MFs from ovine carotid arteries were then used for the wall.
Results: An optimized chemical formulation of the fibrin gel enabled the two step-moulding process and the realization of a HV with the leaflets firmly connected to the wall. After a 3-week dynamic conditioning in a bioreactor, immunohistology confirmed distinct phenotypes in the different areas of the HV and consequent differences in the deposited extracellular matrix.
Conclusion: A biomimetic cell distribution is a step towards TEHVs with optimal heterogeneous properties, e.g. a mechanically stable wall and leaflets with no tissue contraction, a major limitation of TEHVs related to the cells' contractile activity.

16.06
Valvular tissue formation and remodeling under physiological loads

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A new *in situ* strategy for heart valve regeneration has been proposed, less time-consuming and with reduced infection risks as compared with the traditional *in vitro* approach. Previous studies demonstrated that *in vitro* tissue formation is enhanced by mechanical strains induced by pressure loads. What still needs to be investigated in order to allow for *in situ* tissue formation and remodeling is the behavior of freshly seeded cells in scaffolds under physiological conditions and the correlation between the applied deformation and matrix production. By applying an array of pressure differences on seeded scaffolds with varying mechanical properties, the deformation range that induces optimal tissue production can be defined. Experimentally obtained data serves as input for a computational model that enables the assessment of scaffold deformation. Polycaprolactone scaffolds were seeded with human venous vascular cells or adipose-derived mesenchymal stem cells. They were directly exposed to physiological transvalvular pulmonary pressure for 48 h in a pulse duplicator and afterward the expression of genes involved in extracellular matrix development was analyzed with qPCR. Expression of matrix genes was retained by both myofibroblasts and stem cells, but it was influenced by strain and mechanical properties of the scaffold. This study suggests that tuning the strain in *in situ* valvular tissue regeneration can control cell behavior in terms of matrix production.

16.P01
Development of textile scaffolds for tissue engineered heart valves

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Introduction: To overcome the limitations of conventional heart valves: rejection, calcification or thrombosis, a new heart valve is required.
Materials and methods: A nonwoven scaffold of polyurethane was developed by a patented fiber spraying process. With this method valve and aortic root were produced in order to develop heart valve substitutes for tissue engineering applications. To optimize the properties of the new valve scaffold, various process parameters were considered: the tensile strength of the aortic root and valve, pull-out strength and valve bending. The effects of these values were tested and evaluated by using scanning electronic microscopy methods.
Results: Fine fibers and denser nonwovens gave the best result for tensile strength. The valve showed the same strength as a human valve but higher elongation. Thick fibers are required to improve pull-out strength of the valves from the aortic root. The bending test showed that the porcine valves are more flexible than the polyurethane scaffolds. Perfusion tests demonstrated smooth opening and closing of the valves.
Conclusion: Modification of the parameters showed higher valve tensile strength and prevented separation of valves from aortic root. The valves remain flexible, open and close smoothly, having the same strength but higher elongation than homografts.

16.P02
Investigation of a new material for heart valve tissue engineering

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Limitations exist with current treatments for heart valve disease especially for paediatric patients, as no therapy is capable of growth or remodelling *in vivo*. Tissue engineering may be able to create a new therapy; however, there has been an inability to produce a scaffold with sufficient stiffness to resist the forces which result in non-coaptation of the valve leaflets. We proposed a scaffold which will have sufficient mechanical properties to resist the contractile forces of cells acting upon it; a fibrin gel reinforced with a collagen- glycosaminoglycan (CG) matrix. We have previously developed a mould for the con-

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struction of heart valve conduits. The scaffolds produced in a modified version of this mould, were evaluated on their pore size, porosity and homogeneity. Methods of adding the fibrin into the CG and the effect of crosslinking on the stiffness of the CG were assessed. A set of optimised freeze drying parameters were established under which a homogenous, repeatable scaffold was fabricated. Injection of the fibrin solution allowed for an even distribution of polymerised fibrin throughout the material. The proof of principle that fibrin can be successfully infiltrated into the CG material has been demonstrated and a stable ratio of fibrin to CG was determined in this study. Crosslinking was found to increase the stiffness of the material backbone which will improve the ability of the material to withstand the contractile forces of the cells on the material.

16.P03
Extracellular matrix structure of autologous human pericardium and significance for heart valve tissue engineering

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Collagen, elastin and glycosaminoglycans are responsible for the mechanical properties of the normal aortic heart valve and represent the major components of the heart valve extracellular matrix (ECM). Our objective was to compare the histological structure of autologous human pericardium to that of the human aortic heart valve. Normal aortic heart valves were obtained during heart transplantation and compared to autologous human pericardium before and after dynamic conditioning using histological assessment, immunohistochemical analysis and confocal microscopy to determine any correlation in structure. The ECM architecture and cellular organization of human pericardial tissue was shown to be very similar to that of the normal aortic heart valve. The pericardium possesses one layer whose densely packed collagen bundles closely resemble that of the lamina fibrosa of the native aortic heart valve by confocal microscopy. Elastin fibers are evenly and diffusely distributed throughout the entire thickness of the human pericardium. The distribution and cellular morphology of pericardial intersticial cells (PICs) closely resembles that of valve intersticial cells (VICs) of native aortic heart valve tissue. Dynamic conditioning of human pericardium positively influenced collagen, elastin and glycosaminoglycan production by PICs ($p < 0.001$).

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16.P04
The tube-in-tube: a tissue engineered autologous semilunar heart valve based on tubular leaflet design

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The function of the leaflets of the native semilunar heart valve (HV) can be performed by a simple tubular construct sutured along a circumferential line at the root and stitched at three single points at the sinotubular junction. Here we apply this concept and develop a tissue engineered autologous HV based on tubular leaflet design with the aim of obtaining a living valve with remodelling and growing capability, particularly important for the paediatric population. A fibrin based tubular structure embedding myofibroblasts from the human umbilical cord artery was realized by moulding. The structure was sutured inside a silicone rubber tube representing the root featuring the sinuses of Valsalva. A relatively uncomplicated suturing procedure resulted in a valve with rapid and smooth motion of the leaflets as revealed by high speed videos. The construct was dynamically cultivated for 3 weeks in a bioreactor. Immunohistological staining showed deposition of collagen which conferred good mechanical properties to the leaflets. The diameter of the inner tube was found to be a crucial parameter for valve performance in terms of height and area of coaptation, radial expansibility of the commissures and orifice area during systole. Alternatively, a complete HV based on the presented concept can be obtained by moulding also the scaffold for the root in case the complete valve needs to be replaced.

16.P05
Decellularisation of allogeneic aortic and pulmonary valves using low concentration SDS

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Over 200 000 aortic valves are replaced annually worldwide. Cryopreserved allografts are the 'gold standard' but suffer immunological responses resulting in reoperations. We have developed methods to remove the immunogenic cells from porcine aortic valves whilst preserving the biochemical and biomechanical properties (1, 2) and shown these to regenerate in the pulmonary position in sheep. This project aimed to translate this technology to human cardiac valves. Five aortic and three pulmonary cryopreserved valves were treated sequentially with: hypotonic tris buffer, 0.1% (w/v) SDS in hypotonic buffer plus protease inhibitors and then nucleases. The histology and DNA content of four different regions of the acellular valves was compared to untreated cryopreserved valves. In order to test the robustness of the process, studies were repeated on eight valves. Dilation tests were carried out on acellular and native valves (three aortic; three pulmonary). H&E staining of acellular tissues showed no evidence of cell nuclei or cell remnants. DNA assay confirmed 91–99.6% removal of DNA. Dilation tests showed no difference between treated and native valves. Allogeneic cardiac valves were successfully decellularised using low concentration SDS, whilst maintaining their histoarchitecture. Initial assessment of biomechanical properties indicated that treatment had no effect on compliance of the allogeneic valvular scaffolds.

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16.P06
Comparative analysis of material properties of fetal and adult porcine mitral heart valve leaflets

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The main function of the mitral heart valve is to enable unidirectional blood flow. The realization of heart valve function depends on adaptation of their material properties to progressive age-related changes in the cardiovascular hemodynamic during embryonic and postnatal development. The aim of this study to compare the material properties of fetal and adult porcine mitral heart valves and the associated structural and biochemical properties of their extracellular matrix during natural process of valvular tissue maturation. Using tensile tests it

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was demonstrated that the material properties of porcine mitral heart valves progressively increase with age. The collagen content of the adult heart valve as estimated by hydroxyproline assay increases three times as compared with fetal heart valves. Transmission electron microscopy demonstrated that the diameter of collagen fibrils increased in adult heart valves compared with fetal heart valves. Finally, the level of collagen cross-linking is lower in the fetal heart valve than the adult heart valve. In short, the reported age differences in the materialproperties of fetal and adult porcine heart valves were associated with increases in collagen content, the diameter of collagen fibrils and the level of collagen cross-linking. These data are essential to define the desirable level of tissue maturation in heart valve tissue engineering.

16.P07
Production and characterisation of low concentration SDS decellularised porcine pulmonary valvular conduits

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The aims of this study were to develop methods to produce an acellular porcine pulmonary valve conduit. A decellularisation process was developed incorporating trypsin digestion of the outside of the pulmonary artery and sequential treatment with: hypotonic Tris buffer (HTB; 10 mM Tris pH 8.0, 0.1% EDTA, 10KIU aprotinin), 0.1% SDS in HTB, DNase and RNase, and sterilisation with 0.1% peracetic acid. Histology showed the scaffolds were devoid of cells and retained gross histoarchitecture. DNA levels were reduced by >90% throughout the acellular tissue compared to fresh tissue. No functional genes were detected in the acellular tissue by PCR. Immunohistochemistry showed an absence of α-gal epitopes. *In vitro* biocompatibility studies showed the acellular leaflets were not cytotoxic to L929 cells. Fresh and acellular leaflets and arterial wall samples, were subject to uniaxial tensile tests, in circumferential and axial directions. No significant differences were observed in the tensile properties of fresh and acellular leaflet. With the exception of the elastin phase slope [significantly decreased in both directions], there were no significant differences in the tensile properties of the fresh and acellular pulmonary wall samples. Pulsatile flow testing indicated the acellular pulmonary roots had excellent hydrodynamic function and leaflet kinematics compared to fresh tissue. Overall, the acellular porcine pulmonary roots have excellent potential for clinical translation.

16.P08
Long-time conditioning of re-seeded, dezellularized homografts for aortic valve tissue engineering

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Introduction: Tissue engineered aortic valve prostheses are a promising approach for heart valve replacement. The aim of the study was to document the cell behaviour of seeded cells on decellularized homografts during a long-time low-flow conditioning period.
Material and methods: Fibroblasts (FB) and endothelial cells (EC) were isolated from human saphenous vein segments and were expanded in culture. After thawing, homografts ($n = 6$) were decellularized by detergents and were consecutively seeded with FB and EC using a special dynamic 3-D seeding device for 24 h. After initial FB- and EC adherence, cells were cultivated under static conditions for 6 days. Re-seeded homografts were exposed to a low pulsatile flow (750 ml/min for 9 days; 1100 ml/min for 3 days) in a self-made bioreactor for 12 days. Nativ, decellularized, seeded and perfused samples were analysed by scanning electron microscopy (SEM) and immunohistochemistry (IHC).
Results: IHC analysis revealed an intact cellular coating after perfusion. SEM demonstrated a confluent cell layer with EC-typical cobblestone relief after cell seeding and conditioning. Additionally, cell orientation in flow direction was detected after perfusion.
Conclusion: Long-time conditioning of re-seeded, dezellularized homografts permit cell adaption to flow conditions, offering a potential use of these tissue engineered heart valves in aortic valve replacement.

16.P09
Growth and differentiation of valve interstitial cells on aligned and randomly oriented nanofibres

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Polymeric materials have been extensively studied for their potential in tissue engineering of heart valves. We evaluated growth and differentiation of pig aortic valve interstitial cells (VICs) on aligned and randomly oriented electrospun nanofibres made of polylactide (PLA), polycaprolactone (PCL), polyamide 6/12 (PA), chitosan, and gelatine. In cell lysates, we measured concentrations of vinculin, talin, alpha-actin, vimentin, and ICAM-1 by the enzyme-linked immunosorbent assay. On aligned nanofibres, VICs grew in parallel with fibres. Nanofibres supported formation of alpha-actin cytoskeleton and focal adhesion plaques containing talin or vinculin. The highest concentration of both alpha-actin and vimentin was found in VICs cultured on aligned gelatine mats, which indicates a myofibroblast phenotype of VICs on this material. An increased concentration of vinculin was observed in VICs on both PA and chitosan mats. The lowest concentration of ICAM-1 was in cells cultured on gelatine mats, while higher concentrations were in cells on chitosan and PCL nanofibres. In the study, aligned nanofibres made of gelatine, chitosan and PA seem to be most favourable for VICs adhesion, growth and differentiation, and are promising for tissue engineered heart valves. Supported by the Grant Agency of the Ministry of Health of the Czech Republic (grant No. NT/11270 – 4), and the Grant Agency of the CR ('Centre of Excellence', grant No. P108/12/G108).

16.P10
Decellularization decelerates the calcification of aortic conduit implants in a standardized rat transplantation model

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The present study aimed at examining the calcifying degeneration of aortic conduit implants in dependency on the presence of donor cells in a rat transplantation model of accelerated cardiovascular calcification. Detergent-decellularized aortic conduits of Sprague-Dawley rats were infrarenally implanted (day 0) in Wistar rats suffering from an interventionally induced aortic insufficiency (AI) grade II–III (day-14) and fed with a procalcific diet, composed of high-dose vitamin D, cholesterol and calciumphosphate (groupA; $n = 6$). Cryopreserved implants served as controls (groupB; $n = 6$). Functional assessment was conducted by Doppler sonography at days-14, 0, 28 and 84. Graft explantation, histological and immunohistochemical analyses followed after 4 and 12 weeks. Functional competence of the implants and over-

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all survival of the rats were 100%. After 12 weeks, not only the native aorta, but also the implants were severely calcified, with significantly increased calcium burden in groupB ($p < 0.01$), accompanied by an invasion of Syndecan-3 positive cells. Moreover, in comparison to groupA, increased intimal hyperplasia with α-smooth muscle actin expressing cells, elevated intima-to-media ratio ($p < 0.001$) and an increased inflammatory activity was detected. In a standardized rat transplantation model, decellularization mitigated the pro-degenerative dietry effect on aortic conduit implants. Future work should elucidate the role of the observed chondroid cells in this context.

16.P11
Evaluation of the effectiveness of several antimicrobial disinfection protocols in tissue banking

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Introduction: The aim of our study was to monitor the efficiency of several decontamination protocols for cardiovascular and bone grafts with low-concentration antibiotic/fungicide cocktails at a temperature of 4 °C.

Materials and methods: Three different antimicrobial cocktails A, B and C were tested against eight bacterial and fungi test strains in duplicate with a concentration of 1×10^6 CFU in the first part of our study. Samples for the detection of microbiological growth were taken after 24 ± 2 h decontamination and the testing was performed with two different methods: aerobic and anaerobic bottle for testing in the BacT/Alert 3D for 7 days at 37 °C and soya-bean casein bouillon for 14 days at 20–25 °C and thioglycollate bouillon for 14 days at 30 °C. All samples which had no growth at 10^6 CFU after antibiotic treatment were considered as negative result. All microorganism with positive results at 10^6 CFU were retested in a stepwise concentration within the range of 10^3–10^6 CFU.

Results: Cocktail B and C showed the same results. All bacteria with a concentration of 10^6 CFU did not survive the antimicrobial treatment. The decontamination success with Cocktail A was similar except for pseudomonas aeruginosa which survived the antibiotic disinfection. All three cocktails had no impact on fungi.

Conclusion: Both cocktails B and C are qualified for routine bacterial decontamination of cardiovascular and bone grafts.

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17. Skeletal Muscle

17.01
Keynote: Determinants of skeletal muscle regeneration: results of preclinical and clinical studies

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Volumetric muscle loss is associated with aesthetic and functional morbidity, and surgical treatment options are limited. Skeletal muscle has more innate regenerative potential than most tissues in adult mammals, but the loss of greater than 20% of the mass of any single muscle group results in the formation of dense scar tissue. The use of appropriately prepared biologic scaffold materials composed of mammalian extracellular matrix (ECM) has been associated with robust skeletal muscle regeneration [1] and a predominant M1 to M2 macrophage phenotype switch; events which induce stem and progenitor cell accumulation *in situ* [2]. Preclinical models and the results of a human clinical trial will be presented. Results of these studies show the formation of functional skeletal muscle tissue induced by the use of ECM scaffold material and the associated recruitment of site appropriate stem and progenitor cells.

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17.02
Myogenic differentiation of mesenchymal stem cells by co-transplantation with myoblasts in the neurotised AV-Loop model

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Generation of axially vascularised muscle tissue aims at restoring damaged muscle tissue in clinical scale. Mesenchymal stem cells (MSC) could be used as alternative cell source to myoblasts due to their ability to be expanded to large cell numbers without losing their differentiation capacity. Previous *in vitro* experiments showed that co-cultures of primary myoblasts and MSC are able to undergo myogenic differentiation by stimulation with bFGF and dexamethason. In this study MSCs with and without myoblasts were transplanted in a novel rat AV-loop model (EPI-loop) after 2 weeks of prevascularisation. Neurotisation was induced through implantation of a motor nerve. Constructs (five specimens per group and time point) were harvested and analyzed 2 and 8 weeks after implantation by immunohistochemistry and quantitative polymerase chain reaction (PCR). Survival of stable transduced GFP-transduced MSC was assessed by fluorescence microscopy. After 2 weeks myotube formation and MEF2 und MHC expression were detected to a minor extend. RT-PCR showed an expression of myogenic markers in all specimens with strongest continuous expression after 8 weeks in the group with co-implantation of MSC and primary myoblasts. Thus neurotisation and bFGF and dexamethason may induce myogenic differentiation in MSC in an axially vascularized model. The next step towards generation of vascularized, innervated muscle tissue will be the use of electrospun nanofiber scaffolds.

17.03
Combined systemic and local delivery of stem cell recruitment for *in situ* tissue regeneration

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The concept of *in situ* tissue regeneration utilizes host responses to efficiently recruit neighboring stem and progenitor cells to a site of injury without the need for *in vitro* cell manipulation. For efficient tissue regeneration *in situ*, recruitment of sufficient numbers of stem cells should be achieved. In order to enhance infiltration of stem cells into implants, we developed a combination delivery system of 'systemic delivery' of substance P (SP) via intravenous injection and 'local release' of stromal-derived factor-1α (SDF-1α) from an implanted scaffold. In a subcutaneous implantation mouse model, flow cytometry and immunostaining results demonstrated that our combination delivery system significantly enhanced recruitment of mesenchymal stem cells, pericytes, and hematopoietic stem cells into the implants, as compared to no delivery and single local delivery groups. In a large traumatic defect model, the combination delivery system, incorporated with local delivery of insulin-like growth factor-1 (IGF-1), facilitated efficient recruitment of satellite cells and pericytes into the implants and more newly formed myofibers when compared to the no delivery groups. The incorporation of multiple regulatory signals into a scaffolding system and our combined delivery may be a promising approach for more efficient and effective muscle regeneration *in situ*.

17.04
Autologous progenitor cells in a hydrogel form a supernumerary and functional skeletal muscle *in vivo*

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Extensive loss of skeletal muscle tissue results in incurable mutilations and severe loss of function. *In vitro* generated artificial muscles undergo necrosis when transplanted *in vivo* before host angiogenesis may provide the amount of O_2 required for muscle fibre survival. Skeletal muscle tissue engineering has met with limited success, due to the complex tissue architecture and the presence of a dense microvascular network without which muscle fibers do not survive once implanted *in vivo*. Here we report a novel strategy exploiting the good survival and differentiation of mouse mesoangioblasts in a recently discovered biomaterial, PEG-Fibrinogen, and their ability, once engineered to express Placenta derived Growth Factor and embedded in this material, to attract host vessels and nerves while myotubes begin to form. Mesoangioblasts, embedded into PEG-Fibrinogen hydrogel, generate an additional muscle on the surface of the Tibialis Anterior. When PF embedded Mabs are implanted underneath the skin on the surface of the tibialis anterior (TA), the normal contractile activity of this muscle induces fiber orientation and maturation, resulting in an extra muscle that is morphologically and functionally very similar to the underlying TA. This strategy opens the possibility of *in vivo* autologous muscle creation for a large number of pathological conditions.

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17.05
Engineering muscle tissues on microstructured polyelectrolyte multilayer films

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The use of surface coating on biomaterials can render the original substratum with new functionalities which can improve chemical, physical, and mechanical properties as well as enhancing cellular cues such as attachment, proliferation, and differentiation. In this work, we combined biocompatible polydimethylsiloxane (PDMS) with a biomimetic polyelectrolyte multilayer (PEM) film made of poly(L-lysine) and hyaluronic acid (PLL/HA) for skeletal muscle tissue engineering. By microstructuring PDMS in grooves of different width (5, 10, 30 and 100 μm) and by modulating the stiffness of the (PLL/HA) films, we guided skeletal muscle cell differentiation into myotubes. We found optimal conditions for the formation of parallel-oriented myotubes and for their maturation. Importantly, the myoblasts were collectively pre-aligned to the grooves prior to their differentiation. Formation of myotubes was observed regardless of the size of the micropatterns and we found that their typical width was 10–12 μm. Their maturation, as characterized by striation of myosin heavy chain, was not affected by the topography except for the 5 μm wide micropatterns. We highlighted spatial constrains that led to an important nuclei deformation and further impairment of maturation within the 5 μm grooves. All together, our results show that PEM film combined with PDMS is a powerful tool for skeletal muscle engineering.

17.P01
Cell therapy for the treatment of compartment syndrome in rats

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Introduction: Compartment syndrome (CS) is a serious complication arising from a variety of extremity injuries and resultant swelling within the fascicles of the muscle tissue. The current standard of care for CS is fasciotomy, which relieves the intra-compartmental pressure but inflicts further tissue damage. We have developed a rat model of CS that closely mimics the sequelae of the human condition for the testing of cellular therapy for CS.
Methods: Neonatal blood pressure cuffs were placed on the hind limbs of nude rats. A pressure of 120–140 mm Hg was held for 3 h to induce CS in the anterior muscle compartment. Myoblasts (MPCs) derived from GFP^+ mice or vehicle alone (PBS) was injected in a 100 μl volume into proximal, center and distal regions of the TA at 4, 7 and 11 days after injury. The tibialis anterior (TA) muscle was removed14 and 28 days after injury and prepared for histological analyses.
Results: Immunohistochemistry revealed the presence of MPCs within the muscle tissue at 14 and 28 days after injury. GFP^+ MPCs differentiated into mature myofibers and integrated into the host tissue. A modest functional recovery was seen in muscles receiving cell injections.
Conclusions: The use of fluorescently labelled cells allows for the detection and localization of cells up to 28 days after injection. Injected MPCs are able to differentiate into mature myofibers. This data demonstrates the utility of MPC therapy for the treatment of CS.

17.P02:
Treatment of volumetric muscle loss injury with muscle-derived ECM and BMSCs promotes functional recovery in rat tibialis anterior muscle

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Introduction: Volumetric muscle loss (VML) is the traumatic or surgical loss of skeletal muscle that results in permanent functional impairment. VML therefore represents a challenging clinical problem for both military and civilian medicine. The purpose of this study is to improve functional recovery after VML with treatment of muscle extracellular matrix (ECM) and bone mesenchymal stem cells (BMSC).
Materials and methods: Male Lewis rats had a ~20% by mass, $\sim 1 \times 0.5 \times 0.5$ cm surgical defect created in the middle third of the tibialis anterior (TA) muscle. At the time of injury, VML was repaired with ECM in some (ECM) but not all rats (VML). In a subset of ECM-repaired rats, 1.0 million BMSCs were injected into the ECM 7 days post-injury (BMSC). TA muscle peak tetanic isometric force (Po) was measured via neural stimulation in injured and contralateral uninjured muscles at 2 or 4 months post-injury.
Results and conclusion: The contralateral control groups produced similar Po at the two (~11.6 N; $p = 0.791$) and 4 month time points (~12.6 N; $p = 0.572$). At both time points, VML resulted in a -30% Po deficit and ECM did not significantly improve these deficits (i.e., 2 and 4 months = -23 & -25%, respectively). BMSC did not improve functional recovery at 2 months (-22%), but this group was significantly improved (-18%; $p = 0.045$) at 4 months compared to VML. These results show that the treatment of VML with a biological scaffold combined with BMSCs can significantly improve muscle function.

17.P03
Tissue enginnered skeletal muscle

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A tissue-engineered skeletal muscle is a promising strategy for the reconstruction of skeletal muscle loss. It may also be applicable to treat the muscular dystrophy if the genetically modified cells were combined. However, the current major issue is a lack of angioarchitecture to maintain clinically applicable scale. In this study, a collagen scaffold having uniaxially oriented porous structure was developed for the large-scaled tissue-engineered skeletal muscle. The collagen scaffolds having diameter of 13 mm and length of 20 mm was prepared by a unidirectional freezing process of collagen solution. The C2C12 cells were seeded in the scaffold and cultured 1 week by using medium flow bioreactor. The scaffolds colonized with cells were implanted subcutaneously into the nude mouse. The explanted constructs were subjected to histological and contractile force evaluation. The scaffold was determined by SEM to have a uniaxial porous structure in communication with each other. The C2C12 cells were well seeded in the scaffold and dispersed throughout the entire structure. Sections of tissue-engineered skeletal muscle revealed myotubes had formed unidirectional orientation. After the implantation study, the construct was well maintained and showed further maturation of seeded cells. Furthermore, they showed much higher contractile force than them before implantation. This scaffold may be useful for giving the large-scaled tissue-engineered skeletal constructs.

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17.P04
Cellulose nanowhiskers and nechanotransduction as strategies for tissue engineering skeletal muscle from human mesenchymal stem cells

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There is a significant need for tissue engineering skeletal muscle for treatment of congenital deformities and trauma. We present a novel strategy for muscle tissue engineering using either Cellulose Nanowhiskers (CNWs) as nanoscale topographical cues, or cyclic strain to drive the myogenic differentiation of Mesenchymal Stem Cells (MSCs) in co-culture with a skeletal muscle cell line. By manipulating the mechanical and topographical aspects of the cell microenvironment, a pro-myogenic regime has been developed. CNWs, rod-like particles prepared from the marine invertebrate Ascidiella sp., are spin-coated onto a charged substrate. The resulting films are highly oriented, inducing contact guidance in myoblasts. Due to the nanoscale topography of the films (5–6 nm high features) terminal differentiation is upregulated and a high degree of myotube orientation results. An alternative approach uses cyclic strain to promote myogenesis via mechanotransduction. A FlexCell device is used to apply dynamic strain to cells using flexible culture surfaces. When an optimum regime is employed along with laminin coated surfaces, myogenesis may be promoted. Using such strategies, MSCs from bone marrow or adipose tissue are encouraged to fuse with nascent myotubes *in vitro*, introducing transcriptionally active nuclei of non-muscle origin. Such an approach has significant potential for tissue engineering skeletal muscle or for therapies for conditions such as Duchenne's Muscular Dystrophy.

17.P05
Age and gender limitation for the bioengineering of contractile muscle tissue for human muscle precursor cells (MPC)

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Introduction: MPCs are quiescent muscle cells capable of muscle fiber reconstruction. Therefore, autologous MPC transplantation is envisioned for the treatment of muscle diseases, many occurring in the aged population. However, density of MPCs and proliferation potential gradually decline with age. The goal of this research was to assess the limitations of age and gender on the ability to bioengineer contractile muscle with human MPCs.
Materials and methods: Human MPCs were harvested from the rectus abdominalis of 30 patients [15M, 15F] undergoing abdominal surgery [23–82 years]. Growth curves, muscle phenotype and function were analyzed by FACS and cytological assays. *In vivo* muscle formation and contractility was assessed by histology, WB and organ bath.
Results: We were able to confirm the myogenic phenotype, a great expansion potential and fiber formation for all ages and both gender. Male cultured cells had an increase differentiation ratio *in vitro* ($p = 0.03$). Female cells were more stable *in vitro*, grew faster ($p = 0.016$) and contracted better upon electrical stimulation ($p < 0.001$). To gain sufficient cells the biopsy size needed to be bigger in male and older patients ($p < 0.001$). MPCs of all ages were able to form muscle *in vivo*, but an age-dependent decline in contractile response could be detected.
Conclusion: Our results suggest that human MPCs for cellular therapies can be successfully isolated and grown from patients of all ages and gender.

17.P06
Macrophage and progenitor cell participation in a regenerative medicine approach to volumetric muscle loss

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Skeletal muscle has a robust capacity for regeneration following minor injury. However, this inherent constructive and functional remodeling response is limited following trauma that results in volumetric muscle loss (VML). Traumatic penetrating soft tissue injuries, tumor ablation, or large congenital muscle defects create a need for regenerative medicine strategies that can promote the de novo reconstruction of functional skeletal muscle tissue. Biologic scaffold materials composed of xenogeneic or allogeneic extracellular matrix (ECM) have been successfully used to repair or replace a variety of damaged tissues including skeletal muscle. The mechanisms responsible for this constructive remodeling response include progenitor cell recruitment and modulation of the host innate immune response among others. The present study examined the spatial and temporal pattern of macrophage accumulation, macrophage phenotype, and progenitor cell participation following surgical placement of an ECM scaffold within a mouse model of VML. Results showed a robust and heterogeneous population of macrophages at the surgical placement site, along with active multipotential progenitor cells. After 56 days, treatment with an ECM scaffold was associated with a site-specific and constructive remodelling outcome.

17.P07
Tissue-specific stem cell recruitment for *in situ* muscle tissue regeneration

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In this study we sought to utilize muscle-specific stem cells to regenerate muscle tissue through the use of a tissue scaffolding system. The objectives of this study were to evaluate a group of myogenic factors for muscle cell migration, proliferation, and differentiation *in vitro* and to investigate the possibility of using the tissue scaffolds to initiate cell mobilization and recruitment *in vivo*. Myogenic factor-containing scaffolds were implanted in the lower leg muscle of rats. Retrieved scaffolds showed progressive tissue ingrowth over time. By the fourth week after implantation, the scaffolds were completely infiltrated by host cells, including inflammatory cells and stromal mesenchymal-like cells. Interestingly, Pax7 was expressed within the implanted scaffolds at all time points. These findings indicate that host muscle satellite/progenitor cells are able to migrate into the implanted scaffolds. Furthermore, the myogenic factors effectively promoted myogenic cell migration, proliferation, and differentiation *in vitro* and the number of cells expressing Pax7 was increased within the implanted scaffolds that contained myogenic factors, suggesting that these factors can be used to mobilize muscle progenitor cells within an implant. We demonstrate that cells expressing muscle satellite/progenitor cell markers can be mobilized into an implanted tissue scaffold and that these cells are capable of differentiating into muscle cells.

17.P08
Effect of electrical stimulation on contractile properties of tissue-engineered skeletal muscle

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It is well known that cell behavior may be highly influenced by external stimuli. In this study, electrical stimulation was used as a tool to

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advance differentiation and enhance contractile properties of the tissue-engineered skeletal muscle. A porcine aorta was digested in an enzymatic solution to remove elastic fibers and cells inside. The acellular tissue was cut into circular forms and two of them were put apart on a silicone sheet as tendons. The C2C12 cells were embedded within cold type I collagen gel solution and the cell suspension was placed between two tendons. The construct was cultured for 2 days in DMEM containing fetal bovine serum and then the medium was shifted to DMEM containing horse serum to enhance differentiation of the cells to the myotubes. At the same time as medium shift, the constructs were placed between two platinum electrodes for continuous electrical stimulation for 7 days. The isometric twitch force of the tissue-engineered skeletal muscle increased by five times when the electrical stimulation of 5 V and 2 ms at 0.5 Hz was applied. The tetanus force increased by 5.5 times (223.8 μN vs 39.6 μN). The difference between the groups with and without stimulation was statistically significant ($p < 0.05$).These results suggest that the electrical stimulation may have a positive effect on the contractile properties of tissue-engineered skeletal muscle.

17.P09
Advanced differentiation of the C2C12 cell line and primary skeletal myoblasts by uniaxial cyclic strain

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Recent developments in skeletal muscle tissue engineering represent a promising approach for the treatment of musculoskeletal disorders. Although adult skeletal muscle cells sense and respond to mechanical forces, mechanical stimulation strategies to optimize myogenesis *in vitro* remain limited. We have recently shown that uniaxial cyclic tensile strain (CTS) drives the assembly and differentiation of the C2C12 myogenic cell line. Here, we aimed to validate the responses obtained with C2C12 using primary myoblasts. C2C12 and human skeletal muscle myoblasts (HSMM) were cultured on flexible-bottomed culture plates. Upon confluence, cells were subjected to uniaxial CTS during 48 h in reduced serum conditions. Differentiation profiles were assessed by morphological and biomolecular indicators. For both cell types, the uniaxial CTS protocol resulted in an array of cross-striated fibers aligned perpendicularly to the axis of strain. Furthermore, differentiation was enhanced by CTS, as evidenced by a short period of myogenin activation and significant increases in the myotube to myoblast ratio and percentage of myosin positive myotubes. Although the differentiation efficiency was similarly enhanced by CTS in both cell types, the number of HSMM-derived myotubes was significantly larger. The use of HSMMs in combination with CTS may represent a valuable approach to optimize engineering of functional skeletal muscle.

17.P10
Novel biomaterial strategies for repairing musculoskeletal defect

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One of the basic process after injury or fracture is bone repairing. This study is about our recent research in the field of bio-inspire materials and bone's tissue regeneration. Bone- engineering plainly is performed using biomaterials methods based on hydro gels or strontium containing bioceramics. Becauese of remarkable effects of Strontium ranelate on oral anti-osteoporosis drug via osteoblasts and osteoclasts it has a critical role in this way. Nowadays improvement of materials that is poly-γ-glutamic acid based can be use for tissue repair. Further thorough materials analyses of tissue engineered bone will be presented. Recent studies shown that many different cells such as mesenchymal or endothelial progenitor stem cells are used in bone regeneration application but in this way the main problem is the lack of suitable material can act as bone in both physical and structural point which can be joint to those cells in bone repairing. By investigating different analysis methods to micro-Raman spectra of mineralized nodules formed *in vitro*, we have found cell-source-dependent variations in relations between multiple bone-like mineral backgrounds. In this study comprehending the biological mechanism of bone formation *in vitro* that contribute to cell-source-specific materials differences may ease the improvement of clinically successful engineered bone.

17.P11
Phenotypic and functional muscle regeneration in a murine model of muscular dystrophy using a collagen matrix

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Therapeutic myogenesis holds great promise for treating many prevalent myopathies. This may be achieved by application of stem cells, growth factors or biomaterials. In previous work, we used an injectable collagen-based matrix, with properties similar to those of muscle extracellular matrix, to regenerate ischemic muscle. This study sought to characterize its regenerative potential in established murine models of: (i) degenerative Duchenne's muscular dystrophy (mdx mouse, $n = 3$); and (ii) atrophic Amyotrophic Lateral Sclerosis (MLC/SOD, $n = 3$). The EDL muscle is used to characterize disease progression. EDL muscles in both models received an injection of PBS or matrix (collagen I and chondroitin sulfate-C crosslinked with EDC/NHS). After 2 week, matrix-treated mdx mice were able to run 30% further ($p = 0.04$) and at speeds 19% greater ($p = 0.06$) than PBS controls; however, matrix treatment did not confer any improved mobility in MLC/SOD animals at 2 months ($p > 0.7$). Furthermore, matrix-treated mdx animals had greater levels of myogenin, desmin and Pax3 transcripts (by 2.0-, 1.6-, 4.0-fold; $p = 0.03, 0.07, 0.06$, respectively), while there were no differences in transcript levels in the MLC/SOD model. In both models, TNFα transcript levels did not differ among treatments ($p = 0.9$). Despite a lack of improvement or evidence for myogenesis in an atrophic model using a matrix therapy, the matrix presents itself as a candidate for phenotypic and functional regeneration of dystrophic muscle.

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18. Pancreas Replacement & Liver Regeneration

18.01
Keynote: Engineering an optimal implant for islet transplantation

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Clinical islet transplantation (CIT), the intraportal infusion of allogeneic pancreatic islets into a diabetic recipient, is a promising treatment for type 1 diabetes; however, the success of clinical islet transplantation is hindered by the location of the implant site, which is prone to mechanical stresses, inflammatory responses, and exposure to high drug and toxin loads, as well as the strong inflammatory and immunological response to the transplant in spite of systemic immunosuppression. To address these challenges, we are focused on three primary strategies: the development of scaffolds to house islets at alternative transplant sites; the fabrication of nano-scale encapsulation protocols for the immuno-camouflage of the transplant; and the production of bioactive biomaterials for the local delivery of oxygen and immunomodulatory drugs and/or cells. Three-dimensional scaffolds can serve to create a more favorable islet engraftment site, by ensuring optimal distribution of the transplanted cells, creating a desirable niche for the islets, and promoting vascularization. Nano-scale encapsulation can substantially decrease the need for systemic immunosuppression of the recipient, by preventing host recognition of surface antigens. Finally, localization of immunomodulatory agents to the site of the transplant can serve to concentrate protection to the site, while minimizing the side effects commonly observed with systemic delivery of these agents. Success in these strategies.

18.02
Therapeutic neo-islet engineering in subcutaneous site by islet sheet transplantation

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Recent clinical success in islet transplantation has encouraged further investigation into bioengineering islet tissues. The present study discusses our novel islet tissue engineering approach using a fabricated monolayered tissue sheet composed of dissociated single islet cells. Temperature-responsive culture dishes specific to islet cell culturing were prepared by covalently immobilizing a polymer, poly(N-isopropylacrylamide) to cuture dishes, followed by laminin-5 coating. Dissociated rat islet cells were plated onto the dishes. After reaching confluency, cells were harvested as a uniformly connected tissue sheet by lowering culture temperature. Electron microscopy revealed that numerous secretion granules and cell-to-cell connections were observed within the sheets. Upon transplanting islet cell sheets into the subcutaneous space of diabetic SCID mice, neo-islet tissues were successfully engineered. Therapeutic effectiveness of this islet bioengineering procedure was confirmed by the findings that hyperglycemia was successfully reverted to a steady euglycemic state. We also succeeded in creating islet sheets using cryopreserved islet cells. The present study succeeded in generating neo-islet tissues in diabetic individuals using monolayered islet sheets. Because of higher and persistent functionalities of the neo-islet tissues, our approach could contribute in advancing the regenerative medicine toward diabetes.

18.03
Coating Islets of Langerhans with cells improves sprout formation *in vitro*

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Background: Intrahepatic allogeneic islet transplantation in patients with type 1 diabetes has become an alternative for total pancreas transplantation. Long-term insulin independency is often not achieved due to severe islet loss shortly after transplantation. It has been suggested that co-culturing islets with endothelial cells and/or mesenchymal stromal cells (MSCs) enhances sprouting *in vitro*. We developed a new model of coating islets with cells to improve vessel formation and islet survival. We used various cell types and studied their effect on insulin responsiveness and sprouting.
Methods: Human islets from different donors were covered with MSCs, Endothelial-like MSCs (EL-MSCs) or HUVECs/MSCs in custom designed agarose microwells. At day 1 and 5, islets were challenged for insulin secretion. Sprouting of uncoated and coated islets was quantified at 24, 48, and 96 h on fibrin and Matrigel.
Results: Twelve hours after seeding, islets were uniformly covered with cells. All cell types significantly improved the formation of capillary like structures without decreasing islet functionality. Islets coated with EL-MSCs sprouted earlier and had more tubes per islet compared to MSC and HUVECs coatings. Currently we are performing *in vivo* studies to assess islet vascularization and revascularization time.
Conclusion: This study describes a reproducible method to improve islet sprouting *in vitro* without compromising islet function.

18.04
Novel oxygen generating biomaterial for enhancing cell survival under hypoxic conditions

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A significant challenge in implanting engineered tissues is sufficient oxygenation during engraftment. While various efforts have sought to accelerate implant vascularization, these methods still result in the insufficient implant oxygenation for extended periods. We have recently developed a novel oxygen generating biomaterial, based on the encapsulation of calcium peroxide within silicone, termed PDMS-CaO_2. PDMS-CaO_2 disks (0.08 cm^3) were found to generate oxygen over 1 month. We explored the potential of PDMS-CaO_2 disks to prevent hypoxia-induced cell death in beta cell lines and pancreatic islets. A PDMS-CaO_2 disk prevented hypoxia-induced cell death and maintained cell function and viability at levels equal to normoxic controls. Seeking to translate these findings *in vivo*, we developed a 3-D construct containing a PDMS-CaO_2 disk, loaded the implant with beta or islets and evaluated the capacity of our materials to prevent hypoxia-induced cell death *in vivo* and *in vitro*. We found the presence of a PDMS-CaO_2 disk within our 3-D constructs to significantly prevent hypoxia-induced loss in both beta cell and islet viability, with levels statistically equivalent to normoxic controls. Insulin responsiveness was also retained. Evaluation of effects *in vivo* is on-going. Overall, we have

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demonstrated the usefulness of our materials to mitigate hypoxia-induced cell death, which would be highly desirable for preserving the viability of the transplanted cells after transplant.

18.05
Micropatterned electrospun fibrous scaffolds for coculture of hepatocytes and fibroblasts

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Cocultivation of hepatocytes and fibroblasts plays an essential role in maintaining the hepatocyte phenotype, but the 'randomly distributed' coculture conditions lead to significant loss of liver-specific functions. Electrospun nanofibers have gained widespread interests for tissue engineered scaffolds because of their high specific surface area and highly porous structure. In this study, micropatterned electrospun mats are introduced using a patterned collector, including a series of parallel bands with width ranging from 200 to 400 μm, and hepatocytes and fibroblasts were seeded onto the spatially isolated bands. Blend electrospinning of poly(ethylene glycol)-poly(DL-lactide) (PELA) and galactosylated poly(DL-lactide) was used for primary hepatocyte attachment, while NIH3T3 fibroblasts were seeded on PELA fibers containing collagen I. Micropatterned hepatocytes and fibroblasts were visualized with fluorescent dye and found to locate predominantly within micropatterned bands. Compared with culture alone of hepatocytes and randomly distributed coculture of hepatocytes and fibroblasts, the micropatterned cocultures resulted in stabilized hepatocytes phenotype and aggregation for several days, and significantly higher urea synthesis and albumin secretion. The above results suggest the potential of micropatterned electrospun mats for tissue regeneration and therapeutic applications. This work was supported by National Natural Science Foundation of China (51073130).

18.P01
Encapsulation of protein microfiber networks supporting pancreatic islets

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Pancreatic islet tissue engineering is traditionally divided between macroscopic matrices, which attempt to restore the chemical and physical microenvironment, and microparticle-based immunoisolation, which eliminates the need for systemic immunosuppression. A method has been developed to produce and incorporate a network of crosslinked gelatin microfibers around a pancreatic islet within a barium alginate microcapsule. This technique is the first example of the immunoisolation of intact islets within a fibrous protein matrix. Microfibers 22.3 ± 0.4 μm in diameter were produced from a novel vortex-drawn extrusion system. Microfibers and islets were encapsulated within 294 ± 4 μm 1.6% barium alginate microparticles by electrostatic-mediated dropwise extrusion. The islets, which were extracted from Sprague–Dawley rats, were analysed over a 21-day preliminary *in vitro* study. Fluorescent viability staining indicated a significant increase in viability for the fiber-laden particles relative to fiber-free control particles at days 7, 14, and 21. The fiber-laden system also reduced the incidence of islet disruption from 31% to 8% at day 21, and showed evidence of islet-fiber adhesion. Further investigation into benefits of islet encapsulation within an extracellular matrix fiber network will be the subject of future studies. Additionally, the process has applications in the immunoisolation of other large aggregate systems.

18.P02
Pseudo-islets as an *in vitro* model to study biomaterials for transplantation of islets of langerhans

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Background: Although allogenenic islet transplantation offers a promising therapy for type 1 diabetes patients, more than 60% of the islets are lost shortly after transplantation. To improve islet survival, biomaterials that can serve as a supportive and protective islet carrier are being developed. However, fast screening of these biomaterials is hampered by the scarce availability of donor islets. To address this problem, we have developed a high-throughput platform for generation of pseudo-islets as a standardized model for pancreatic islets.
Results: Non-adherent agarose microwells (fabricated using negative molds of PDMS) enable the formation of stable INS-1E beta cell aggregates with a well-defined size that is linearly correlated to the seeding density and ranges from 50 to 200 micrometer. The pseudo-islets secrete insulin when challenged with a high glucose concentration, mimicking the response of islets of Langerhans. Insulin secretion of the pseudo islets was found to be dependent on aggregate size after 7 days of culture, suggesting that cell-cell signaling plays a key role. To further develop the model, we are currently generating pseudo-islets of multiple cell types and testing their function.
Conclusion: This study demonstrates a novel method for the controlled generation of stable cell aggregates as a model for islets of Langerhans. This model enables *in vitro* evaluation of new biomaterials that can improve islet transplantation for type 1 diabetes.

18.P03
Transplantation of co - aggregates of dissociated islet cells and sertoli cells to treat type 1 diabetes in mice.

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Transplantation therapy utilizing isolated, donor islets of Langerhans (islets) has been employed successfully to treat insulin - dependent diabetes mellitus (type 1 diabetes). However, patients who undergo islet transplantation must maintain an immunosuppresive drug regimen to prolong graft survival. The long term effects of immunosuppression involve complication, and improved approaches which avoid their use are desired. We examined co - transplantation of islets with sertoli cells which are known to have immunosuppressive ability. We describe a method for the preparation of cell aggregates of islet and sertoli cells, their *in vitro* function, and their potential to cure diabetes. Islets and sertoli cells were isolated from BALB/c mice. Aggregates of 1500 dissociated islet cells and 1500 sertoli cells were prepared via hanging drops. The sertoli cells occupied the core part while islet cells engulfed the sertoli core aggregate. The sertoli portion of co - aggregates continuously released activin, and islet cells could regulate insulin release in response to glucose concentration changes. Eight hundred co - aggregates were transplanted into each diabetic C57BL/6 mouse via the portal vein, and their blood glucose levels were monitored. Six of seven recipients demonstrated normoglycemia for more than 100 days.

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18.P04
Release of exendin-4 from multilayered alginate microcapsules protect islet cells after transplantation

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Multilayered alginate microcapsules coated with a semipermeable poly-L-lysine (PLL) membrane have been investigated for the encapsulation and transplantation of islets as a treatment for type 1 diabetes mellitus. The long lasting glucagon-like peptide-1 (GLP-1) receptor analogue, exendin4, has been shown to enhance cell mass in rodents through inhibition of cell apoptosis, stimulation of cell proliferation and islet neogenesis. Furthermore, exendin-4 reduces cytokine-induced apoptosis in purified rat islet cells. Here we generate the multilayered alginate microcapsules with an outer alginate layer that can be used for the delivery of exendin-4. The influence of alginate concentration and viscosity on outer layer, release kinetics, insulinotropic properties and protection against inflammatory cytokines in porcine neonatal pancreatic cell clusters (NPCCs) *in vitro* was investigated. The technique results in a stable outer layer of alginate with high viscosity 2% alginate displaying the most sustained release. The released exendin-4 promotes insulinotropic performance and also decreases apoptosis induced by sTNFα in porcine neonatal islet cells. The effect of exendin-4 from multilayered alginate microcapsules *in vivo* was also investigated. In conclusion, this technique could be used to generate encapsulation that delivers exendin-4 for successful islet transplantation.

18.P05
Hepatocyte embedded-functional gel-filled scaffold is effective for the construction of liver tissue-like structure

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Introduction: Liver is a central organ for metabolism in our body and is complicated structure. Therefore, Liver Tissue Engineering (LTE) reconstructing a functional liver tissue is one of the most important and difficult themes in Tissue Engineering field. In this paper, growth factor-immobilizable ECM was developed for the creation of effective cell transplantation technology and the effectiveness was evaluated by animal experiments.
Methods and results: Heparin-collagen conjugate and solubilized ECM of liver were developed as growth factor-immobilizable materials. VEGF and HGF were immobilized on these functional materials and the immobilized ratios were more than 90%. Adult rat hepatocytes on these functional materials well expressed various liver-specific functions *in vitro*. Adult rat hepatocytes or fetal liver cells-embedded functional gel-filled macroporous scaffold (CGS) was subcutaneously transplanted into rat. Hemoglobin content in transplanted samples and viability of transplanted hepatocytes were enhanced in the functional gel by combining 70% partial hepatectomy treatment. The effectiveness enhanced by spheroid transplantation. Furthermore, transplanted fetal liver cells form liver tissue-like structure with vascular network. Therefore, hepatocyte embedded-functional gel-filled scaffold is a potential method to create LTE.
Acknowledgments:
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18.P06
Multi-layered hepatocyte co-culture systems mimicking the liver microstructure

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Since hepatocytes are known to lose their functions within several days under monolayer culture conditions, novel culture systems, which allow hepatocyte to preserve their morphologies and functions for a long-term period, are required to be established. The present study was conducted for creating highly functional hepatocyte culture systems mimicking *in vivo* liver microstructures. Various types of multi-layered co-culture systems were produced as follows: (1) rat hepatocyte sheet (Hep), (2) Hep-Hep, (3) Hep-bovine carotid artery endothelial cell sheet (EC), (4) EC-Hep, (5) EC-Hep-EC and (6) Hep-EC-Hep. Multi-layered co-culture systems were cultured and assessed by functional and morphological analyses. Among co-culture systems tested, a triple-layered hepatocyte sheet sandwiched between EC sheets (EC-Hep-EC) was found to show the highest albumin and urea synthesis values. In regard to morphology at 7 days, hepatocytes cultured in a sandwich configuration between EC sheets (EC-Hep-EC) possessed cuboidal cell shape and intact bile canaliculi, similar to the morphology observed in early stage of monolayer hepatocytes culture. EC-Hep-EC group structurally resembled the liver microstructures was the most effective structure for preserving not only hepatic functions but also characteristic hepatic morphologies. These cell sheet based three-dimensional (3-D) co-culture system will be a useful tool for tissue engineering approaches and an assessment tool for drug screening.

18.P07
Liver derived decellularised matrices in bioreactors

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Hepatic tissue and its derivatives have a wide range of *in vitro* applications. Reproducing the 3D organisation of the liver and ensuring an effective mass transport of oxygen, nutrients and wastes is crucial in developing a robust analog *in vitro*. There is interest in using tissue derived extracellular matrix as a biological scaffold for hepatocytes and other liver cells since hepatocytes seeded on decellularized scaffolds have been shown to possess higher metabolic activity than monolayer cultures. This study was aimed at obtaining a 3D *in vitro* liver model in a Quasi-Vivo bioreactor using pig liver derived extracellular matrices (dECMs). Different decellularisation procedures were investigated in order to obtain dECMs from bulk untreated hepatic tissues. The absence of any cellular component within the dECMs was confirmed using H&E staining. The total protein content per gram of dECM was determined with the Bradford protein assay. Results showed that the proposed decellularisation procedures do not significantly alter the extracellular protein content respect to the one of fresh liver. Moreover, the morphology of dECMs, investigated using micro-tomography (micro-CT), confirmed a highly porous structure with interconnected pores ranging from few to hundreds of micrometers in diameter. Once characterised, dECMs were seeded with hepatocytes and cultured in dynamic conditions within our bioreactors, obtaining robust 3D liver models with high levels of protein expression.

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18.P08
Modified gelatin as encapsulation material for HepG2 cells: a study of viability and functionality

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Introduction: Hepatocyte culture is highly sensitive to the extracellular matrix for the maintenance of viability and differentiation. It is known that modification of a substrate with galactose is correlated with increased hepatocyte functions. Therefore we aim to investigate the feasibility of a chemically crosslinked gelatin hydrogel,containing galactose side groups, for the encapsulation of HepG2 cells.
Materials and methods: Cell viability was assessed by MTT assay and live/dead staining. Functionality was investigated using IHC stainings and real-time PCR.
Results: The MTT assay shows a significant reduction of the amount of cells. After 24 h, no further reduction is observed and the encapsulated cells remain viable at least during 7 days. This is confirmed by the live/dead staining and was the case in both the galactosylated and non-galactosylated hydrogel.Staining shows a maintenance of albumin and HNF4α synthesis and glycogen storage in both hydrogels. Quantification of expression of several hepatocyte specific genes shows higher expression of all investigated genes in the galactosylated hydrogel, compared to the non-galactosylated hydrogel and the control.
Conclusions: The viability and functionality tests indicate that the chemically crosslinked gelatin hydrogel is suitable for the encapsulation of HepG2 cells. Modification of the hydrogel with galactose improves the maintenance of hepatocyte functions and might be more suitable for the cultivation of hepatocytes.

18.P09
In vitro and *ex vivo* functional evaluation of a hollow fiber type bioartificial liver module immobilizing ES-derived hepatic cells

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We previously reported the hepatic differentiation of mouse ES and iPS cells using hollow fiber (HF)/organoid culture. In this study, we evaluated the performance of an ES cells-immobilized bioartificial liver module based on HF/organoid culture *in vitro* and *ex vivo*. Mouse ES cells were immobilized in a module with one-layer textile HF sheet. The HF module was cultured under the perfusion condition. To induce hepatic differentiation, differentiation-promoting agents were added to the culture medium (sodium butyrate, dexamethasone, oncostatin M, and insulin-transferrin-selenium). Male Wistar rats weighting 250g were used for the animal experiments. The liver failure model with 60% spontaneous recovery ratio. After induction of liver failure, the rats were connected with the ES-module with differentiating ES cells or control-module (without cells), and 1 hour extracorporeal circulation at 1 ml/min was initiated. We measured changes in the blood biochemistry level. The liver specific functions were detected by 2 weeks of culture. In animal experiments, all of the 3 rats applied the control-module died. However, the rat applied ES-module, although the number of application has only been one case, recovered and the blood biochemistry levels a week after become equivalent to the healthy level. Although it needs further researches, these results shows that our module have the potential for supporting liver functions and inducing liver regeneration of the liver failure rats.

18.P10
Bioengineering human livers with primary liver progenitor cells - in situ differentiation into biliary ducts and drug metabolizing hepatocytes

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Liver transplantation is presently the best therapy able to extend survival for end-stage liver disease. Nevertheless, the waiting list for liver transplantation is extensive and many patients will not survive long enough due to dramatic organ shortage. To address this unmet clinical need, our lab and others have recently developed whole organ scaffolds for liver bioengineering. The engineered organoids have shown putative hepatic markers and exhibit some hepatic functions. Nevertheless, they lack extensive biliary ducts and mature hepatocytes capable of drug metabolization.To address these issues, we have seeded large numbers of human fetal liver progenitor (hFLC) and endothelial cells (hEC) and allowed them to differentiate in situ in the liver bioscaffolds for 2 weeks.Immunohistochemistry showed progressive tissue organization with clusters of hepatocytes (albumin, α-fetoprotein, cytochrome P450 2A, 3A, Hep-1 positive). Several long ductular structures staining positive for biliary markers (CK19, EpCAM, and ASBT - bile salt transporter) were also present throughout the bioengineered liver. When exposed to diazepam and 7-ethoxycoumarin, the differentiated hepatocytes were able to metabolize these drugs into some of their metabolites, showing broad cytochrome P450 activity. These results represent a clear proof of principle that immature hFLCs, used for liver bioengineering, can lead to fully functional liver tissues for transplantation.

18.P11
Development of a multilayered fibroblast/hepatocyte sheet for regenerative medicine

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Single layer hepatocyte sheets have already been developed as an effective method for hepatocyte transplantation (Ohashi et al., Nat. Med. 2007). In this study, the effect of our newly-developed multilayered fibroblast/hepatocyte sheet was evaluated and the results were compared to those obtained with single layer hepatocyte sheets *in vivo*.TIG-118 cells (human skin-derived fibroblasts) were inoculated onto the UpCell (temperature-responsive PIPAAm culture dish, CellSeed Inc.) at a density of 2.3×10^4 cells/cm^2, and formed a confluent monolayer within 2 days of beginning the culture. HepaRG cells (human hepatoma-derived cells) were inoculated onto the TIG-118 cell layer at a density of 1.39×10^5 cells/cm^2 (TIG+HepaRG). As a control, HepaRG cells were cultured on the UpCell at the same density (HepaRG). The HepaRG and TIG+HepaRG cell sheets were harvested and transplanted independently onto the subcutaneous sites of NOD/scid mice after 4 day of culture. To evaluate the hepatocyte function, the human albumin concentrations in the mouse serum were assayed in each group.The mean human albumin concentrations of mice transplanted with the TIG+HepaRG cell sheets were significantly higher than those of mice transplanted with the HepaRG cell sheets (528 ± 192 vs 84 ± 78 ng/ml/10^6 transplant-cells ($p < 0.01$), respectively).In conclusion, the hepatocyte function of the newly-developed multilayered fibroblast/hepatocyte sheets was better than that of single layer hepatocyte sheets.

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18.P12
Construction of liver tissue *in vivo* with preparative hepatic irradiation and growth stimuli: lower invasive techniques and progenitor cells

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Introduction: We have succeeded in selective proliferation of transplanted hepatocytes by providing a growth stimulus concomitant with hepatic irradiation (HIR) that can suppress proliferation of host hepatocytes. We have made experiments focusing on I) lower invasive techniques and II) progenitor cells. Methods and Results; I) 1)partial HIR. Dipeptidyl-peptidase IV (DPPIV)-F344 rats received partial HIR (only 30% of whole liver) and portal vein branch ligation (PVBL). Hepatocytes from DPPIV+rats were transplanted at 1×10^7 intrasplenically after HIR+PVBL. 2) HGF supply. C57Bl/6 mice received HIR, followed by injection of a adenovirus vector expressing HGF. Hepatocytes from the liver of Rosa mice expressing beta-gal were transplanted at 1×10^6. With both preparations, 16 weeks later, the donor cells constituted more than 70% of the hepatocytes of irradiated lobe, and they expressed connexin 32, PCK1 and glycogen storage. II) After HIR+PVBL with DPPIV-rat, (i) small hepatocytes from DPPIV+rat were transplanted at 2×10^6. After 3 months, more than 70% of host hepatocytes were replaced. (ii) adipose-derived stem cells from DPPIV+rat that were CD90/34$^+$, CD45/31- were transplanted. Although donor cells were detected, no proliferation was observed. Conclusion; HIR can be given to a part of liver and is a low-invasive and effective means for transplanted hepatocytes to proliferate. Even smaller number of small hepatocytes can construct liver tissue with their prevailing proliferative ability.

18.P13
Adult human liver derived stem cells differentiate into hepatocytes in in partial hepatectomy-induced liver regeneration

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The human liver stem cells (HLSCs) are recently identified new type of stem cells. It has previously been shown that HLSCs expressed mesenchymal markers among which vimentin, CD73, CD90, CD105 but not hematopoietic stem cell marker. The HLSCs may be a candidate seed cell for liver regeneration. To research the survival and migration *in vivo* of HLSCs and to explore the expression of hepatocyte-like function. Thirty SD rats (weighing 120–150 g) were randomly divided into 3 groups (groups A, B, and C, $n = 10$). The rats of groups A and B were made the 2/3 partial hepatectomy model, and the rats of group C underwent open abdominal operation without hepatectomy. The HLSCs were transplanted into the intraportal in groups B and C with a cell density of 1×10^7/ml and a volume of 0.2 ml; the same volume of normal saline was injected in group A. After 4 and 8 weeks, transplanted HLSCs were detected in the rat liver by immunohistochemical staining in serial sections. Sections from rat liver were stained the anti-human albumin, HepPar and AAT antibody revealing specific detection of the respective antigens in human liver, only. And human albumin secretion was detected in rat serum of groups B and C by ELISA assay. In conclusion, the ability of isolated HLSCs to proliferate and differentiate into hepatocyte-like cells *in vivo* leads to propose them as an attractive expandable cell source for stem cell therapy in human liver diseases.

18.P14
In vitro recapitulation of the hepatic metabolism using *in vitro* liver model from murine ES/iPS cells

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Introduction: Polygonal and multipolar hepatocytes in the liver are surrounded by sinusoids, by the bile canaliculus, and by adjacent hepatocytes. It is only in the context of hepatic tissue architecture that hepatocytes can express their specific and multiple functions. Hepatocyte polarity exerts a major influence on the cell's physiology. Recently, we established a unique system of *in vitro* liver model derived from murine ES/iPS cells, IVL.
Method: The IVL, consisting of not only hepatocytes, but also endothelial networks, together with cardiac mesoderm differentiation, was induced after the embryoid body formation.
Results: To confirm the functionality of the cultures, first, dichlorofluorescein diacetate (CDFDA) was added into the IVL. Green fluorescent CDF accumulated at the boundary of the cells in the IVL, but not in primary hepatocyte culture. Second, we tried to enhance urea cycle by addition of L-ornithine in the IVL or liver perfusion system. Urea production increased and ammonia decreased in a dose-dependent manner with respect to the amount of L-ornithine both in the IVL and the liver perfusion system, but not in primary hepatocyte culture.
Conclusion: Here, we demonstrated that architectural and functional properties in the IVL was quite similar to the liver perfusion system, but different from the culture of primary hepatocytes. The IVL has great promise to be useful for drug metabolism and pharmacokinetics in liver as an alternative to animal experiments.

18.P15
Transplantation of CD34$^+$ human umbilical cord blood derived stem cells in rabbits' fibrotic livers

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Introduction: The prognosis of patients with liver fibrosis is poor as its therapeutic treatment remains inadequate. Liver transplantation is currently the only curative approach, an alternative method of treatment to whole organ transplantation is essential.The aim of this work was to investigate whether human umbilical cord blood (HUCB)CD34$^+$ stem cells could improve liver fibrosis in an experimental model.Methods: Liver fibrosis was induced in V-Line New Zealand male rabbits using allyl alcohol.CD34$^+$ magnetically fortified stem cells derived from HUCB were transplanted undifferentiated into the liver through portal vein infusion after being assessed for purity and viability using the flowcytometric immunophenotyping technique. Experimental animals were not subjected to immunesuppression.2 months after transplantation, they were sacrificed for assessment in comparison to positive control group. Assessment was done through liver function tests and histopathological analysis, images were analyzed using image analysis system.
Results: results were statistically analyzed and showed significant improvement of liver functions and significant regression of fibrotic areas in stem cell treated group in comparison to the control group. All rabbits survived healthy through the study period without immune suppression.
Conclusion: The purified viable HUCB CD34$^+$ stem cells are able to survive and engraft in fibrotic liver. Toleration of these cells across HLA barrier.

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19. Urogenital Tract

19.01
Keynote: Amniotic derived cells for cell based therapies

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Human amniotic fluid cells, which have been used as a diagnostic tool for the prenatal diagnosis of fetal genetic anomalies, can also harbour a therapeutic potential for human diseases, as different populations of fetal-derived stem cells have been isolated from amniotic fluid. Mesenchymal stem cells were the first to be described, which possess the higher proliferation and differentiation plasticity of adult stem cells and are able to differentiate towards mesodermal lineages. Amniotic fluid stem (AFS) cells have also more recently been isolated from humans and rodents. They are characterized by the expression of the surface antigen c-kit (CD117), the type III tyrosine kinase receptor of the stem cell factor. AFS cells represent a novel class of broadly multipotent stem cells with intermediate characteristics between embryonic and adult stem cells, as they are able to differentiate into lineages representative of all three germ layers but do not form tumours when injected *in vivo*. Finally, c-Kit(+)Lin(-) cells derived from amniotic fluid displayed a multilineage hematopoietic potential. These characteristics, together with the absence of ethical issues concerning their employment, suggest that stem cells present in the amniotic fluid might be promising candidates for gene and stem cell therapy of several human disorders. It is possible that, because of they are easy to derive during gestation, AFS cells could have a specific role for the treatment of prenatally diseases.

19.02
Keynote: Human endometrial mesenchymal stem cells and novel meshes as an autologous cell-based therapy for pelvic organ prolapse (POP)

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POP is the herniation of bladder, bowel &/or uterus into the vagina causing incontinence and sexual dysfunction. 19% of women require treatment by reconstructive surgery and re-operation for surgical failure is common. Meshes provide structural support but fail to repair tissues. We propose to use cell-based therapy for POP using autologous mesenchymal stem cells, isolated from the highly regenerative endometrium (eMSC) delivered in novel meshes. EMSC were isolated from endometrial biopsies by enzymatic dissociation and flow cytometry ($CD140b^+CD146^+$) or magnetic bead ($W5C5^+$) cell sorting. Xcelligence and MTS viability assays showed that optimized culture of eMSC compliant with GMP was with fibronectin matrix and in-house DMEM/FGF2/EGF serum-free and Lonza TP-SF® media in 5% O_2 in both 2D and 3D. We fabricated 3 novel warp-knitted meshes, polyetheretherketone (PEEK), polyamide (PA) and PA+gelatin (PA+G) composite to deliver eMSC into tissues. EMSC attached and proliferated on all meshes *in vitro*, but not commercial polypropylene. Biomechanical assessment of the meshes (minus cells) in a rat abdominal hernia repair model showed increasing stiffness over time in load elongation curves;greatest for PA+G by 90 days. Collagen deposition mirrored stiffness, macrophage infiltration and degree of foreign body reaction and was similar for all meshes, indicating similar biocompatibility. EMSC seeded onto PA+G mesh may provide a novel autologous cell based therapy for pelvic organ prolapse.

19.03
Keynote: Pre-establishment of a vascular network for urinary sphincter engineering

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The use of autologous muscle precursor cells (MPC) is a promising strategy in reversing sphincter muscle damage. Until now, the construction of large volumes of functional muscle tissue by MPC transplantation is limited by insufficient vascular supply. The transient expression of VEGF165 in transplanted MPCs was not sufficient in supporting sustained muscle growth. Therefore, we employed fibrin gels which allow cell-demanded release of covalently bound TG-VEGF121 (TGV). The stability and biological activity of TGV over 3 weeks was demonstrated *in vitro* by ELISA, HUVEC proliferation assay and activation of the VEGF signalling pathways (pVEGF-R, ERK1/2). *In vivo* data revealed robust induction of new vessels by fibrin-bound TGV over a period of 12 days, whereas non-bound VEGF121 failed to support vessel formation (histology/IHC/vessel density). Further, we demonstrated that remaining TGV liberated from explanted fibrin gels (day 12) retained its biological activity. Taken together, these data demonstrate that fibrin constructs containing covalently-bound TGV can form a robust neo-vascular network due to its prolonged delivery in a bioactive form. Within a translational context, this slow release material can be injected directly into a damaged sphincter before cell therapy. Well-vascularized tissue could support functional muscle tissue development after cell injection. This method could impact many organ systems and help to overcome current limitations in organ engineering.

19.04
Cell therapy for the treatment of neurogenic urinary incontinence (UI) in female nonhuman primates

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Treatments for women with urinary incontinence (UI)are not always adequate and alternatives are needed. Our approach was to test the efficacy of autologous skeletal muscle precursor cell (skMPC) therapy on sphincter regeneration in a primate model of neurogenic UI. Female monkeys were divided into 3 groups: control; sphincter injury (bilateral pudendal nerve transection); or sphincter injury + sphincter injection of 5-million skMPC. *In vivo* urodynamic and nerve stimulation studies were done prior to, and at 1 and 3 months post-injury, followed by histological assessment of sphincter structure and cell content. The injured group had 40% reduction in urethral sphincteric pressure, 80% reduc-

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tion of sphincteric response to pudendal nerve stimulation and 52% loss of muscle and neuronal content. skMPC treatment restored pre-injury urethral pressures, nerve responses and muscle and motor end-plate content by 3 months post injection. Female primates share with women similar anatomy and physiology of the genitourinary tract. As such, this study provides direct translational evidence that cell skMPC therapy may help regenerate both nerve and muscle loss associated with this form of UI.

19.05
Repopulation of primary renal cells for whole organ engineering

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Shortage of donor organs has been a continued problem for patients with end stage renal disease. A regenerative medicine approach-using patient's own cells seeded onto a renal scaffold could be an alternative method to donor transplantation. This study describes the preliminary results using porcine primary renal cells seeded onto acellular renal scaffolds for the engineering of a whole kidney. Primary cultures of porcine renal cells were expanded, characterized and seeded into multiple regions of acellular whole kidney scaffolds at a concentration of 5×10^6/ml (total of 200×10^6 cells) using a needle. The cell seeded renal scaffolds were placed in a perfusion bioreactor at 37oC, 5% CO2, with 10 ml/min flow rate. H&E, immunohistochemistry and TUNEL staining were performed after 3, 7, 14, 21 and 28 days. Cell seeded renal scaffolds showed excellent cell attachment and early cell organization. TUNEL staining demonstrated that cells were viable up to 28 days. Maintenance of the renal cell phenotypes was confirmed by staining with antibodies specific to Aquaporin-1, Aquaporin-2, Aquaporin-4, EPO and Ezrin. In conclusion, acellular porcine renal scaffolds seeded with primary porcine renal cells showed evidence of cell attachment and early cell organization. Cells are viable for up to 28 days and maintain their major renal cell phenotypes. These results provide preliminary data for the generation of a transplantable renal graft as a potential treatment for end-stage renal failure.

19.06
Autologous mesenchymal stem cells seeded on a nanofibrous PLLA matrix for urethral reconstruction

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Introduction: We evaluated whether bone marrow mesenchymal stem cells (BMSC) seeded on 3D nano-fibrous poly-L-lactic acid (PLLA) matrix could be used as a novel tissue engineering-based therapy for the treatment of urethral strictures.
Materials and methods: Human BMSC and the human bladder smooth muscle cells (SMC) used as controls. The cells were seeded onto PLLA matrices and cultured for 3 days. Four types of cell-matrices were produced: myogenically-differentiated BMSC-PLLA, BMSC-PLLA, SMC-PLLA and cell-free PLLA. These constructs were implanted subcutaneously in athymic mice. The implants were harvested 4 weeks later and were assessed in terms of blood vessel density and endothelial cell markers and immunocytochemistry was used to visualize SMC markers and human nuclei.
Results: BMSC expressed the SMC markers desmin and myosin 14 days after they were induced to differentiate *in vitro*. The PLLA scaffold provided a 3D structure maintained myogenic differentiation of the induced BMSC, and promoted tissue remodeling with rich capillary formation *in vivo* compared to control. Numbers of cells expressing SMC makers, endothelial cell markers, human nuclei, and vessel density significantly increased in induced BMSC-PLLA grafts.
Conclusions: Our results demonstrate that myogenically-differentiated BMSC seeded on a nano-fibrous PLLA scaffold could be used for cell-based tissue engineering for urethral reconstruction or other urological tissue repair.

19.P01
Bio-engineered human kidney for the study of nephrotoxicity and kidney disease

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Introduction: Current work in toxicology and kidney disease relies upon animal models and cells cultured in monolayer. Neither method is capable of recapitulating the biology or response of the human kidney *in vivo*. This is significant for the determination of drug toxicity and the study of kidney disease as the data drawn from these model systems makes it difficult to draw relevant conclusions. We have bio-engineered human kidney tissue to test drug nephrotoxicity and mimic the biology of kidney diseases such as ADPKD.
Methods: Human kidney cells were embedded in a matrix of collagen and Matrigel, allowed to form structures, subjected to drugs and analyzed for kidney injury and cell death. For modeling ADPKD, the cells were infected with a tet-responsive shRNA against pkd1 and gene silencing was initiated after tissue development. Tissues were analyzed for changes in morphology, function, gene expression and protein localization.
Results: Bio-engineered human kidney tissues were able to mimic *in vivo* structure and function. Additionally, the tissues were more sensitive to drugs than 2D monolayers of cells.
Conclusions: Our human kidney model mimics the human kidney in its response to drugs, its structure and its function. This model will provide relevant data for drug development both in terms of drug toxicity and kidney disease research while reducing the number of animals used in research.

19.P02
Hypertonicity maintains a differentiated renal epithelial monolayer: a promising approach for bioartificial kidney

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The development of a successful bioartificial kidney (BAK) faces some challenges such as overgrowth and dedifferentiation of epithelia on synthetic membranes. It is known that hypertonicity suppresses the proliferation of renal epithelia and modulates the synthesis of the extracellular matrix proteins, therefore one could envisage that hypertonicity may overcome these challenges. Human epithelial cells were treated with 300 (regular medium), 400 and 500 mOsm media (made by adding NaCl) for 8, 24, 48 and 72 h. We found that 400 and 500 mOsm suppressed or tended to suppress the mitochondrial activity after all time points, suggesting the suppression of the overgrowth of cells. Morphological evaluation revealed that 400 and 500 mOsm media maintained an intact epithelial monolayer, while 300 mOsm medium treatment resulted in a disrupted layer of cells, where some cells looked like mesenchymal cells. 400 and 500 mOsm treatments for 72 h significantly induced the mRNA expression of E-cadherin, EpCAM and ZO-1, while it induced slightly, but significantly N-cadherin and had no effect on α-SMA and K-cadherin. 500 mOsm media significantly induced the mRNA expression of Col1A1 after 72 h, while there was no effect on the mRNA expression of Col4A1 and fibronectin. Our

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results are promising for BAK, since they suggest that hypertonicity inhibits the overgrowth of the cells and maintains an intact differentiated monolayer, which are some of the major obstacles in the development of BAK.

19.P03
Regenerating kidney tissue with a neo-kidney augment implant containing selected renal cells and natural biomaterial

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Chronic kidney disease (CKD) is a global public health concern involving progressive loss in renal function. New treatments to restore renal function thereby delaying or eliminating dialysis and transplant are needed. We have identified a population of selected renal cells (SRC) that positively affect several aspects of the CKD condition. Biomaterial addition to SRC provides cell stability, enhanced shelf life and targeted delivery. This study reports on the development of a Neo-Kidney Augment (NKA) product containing SRC and natural biomaterials that, upon implantation into rat or canine kidney, catalyzes kidney tissue regeneration. SRC are obtained from a kidney biopsy and density gradient separation of cells. NKA product prototypes use SRC and gelatin-based hydrogel biomaterials. SRC have been shown to provide a significant regenerative stimulus in the rodent models of CKD, delaying disease progression and reduced disease-related mortality. A canine nephrectomy model was used for evaluation in the large animal model of CKD. Treatment with SRC results in a statistically significant increase in uromodulin (THP) and a decrease of vitamin D binding protein in the urine, indicative of restoration of tubular cell function. Histological evaluation in the dogs revealed that NKA product prototypes were well tolerated. These observations provide evidence that selected renal cells and natural biomaterial may be effective for neo-kidney tissue regeneration in chronic kidney disease.

19.P04
The feasibility of using autologous renal cells from diseased kidneys for the treatment of renal failure

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Cell-based therapies have been proposed as a means to augment and restore renal function in patients with chronic kidney disease (CKD). However, it is uncertain whether cells obtained from diseased kidneys exhibit similar cellular characteristics as normal kidney (NK) cells. In this study we examined whether primary renal cells obtained from CKD patients retain normal phenotypic and functional characteristics for cell therapy in patients with renal failure. Donor kidneys (3NK and 3CKD) were obtained from Carolina Donor Services, NC. Primary renal cells were isolated and culture expanded. Proximal, distal, and podocyte cells were isolated and characterized. Analyses of specific cellular functions were measured and compared between the cells. Normal and CKD isolated cells showed similar phenotypic and functional characteristics. The percentage of proximal and distal tubular and podocyte cell populations from both groups showed no statistical difference. SEM images of proximal tubular cells showed the presence of normal microvilli in both cell groups. There was no significant difference in oxidative stress or functional analyses performed. This study shows that renal cells obtained from diseased kidneys possess similar characteristics as normal kidney cells. This study confirms the possibility of using autologous renal cells as a reliable cell source for the treatment of renal failure.

19.P05
Using whole organ decellularisation to create recellularised kidney constructs

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Renal transplantation is the optimal form of renal replacement therapy but is critically restricted by the limited pool of donated organs. The technique of whole organ 'perfusion' decellularisation produces extracellular matrix (ECM) bio-scaffolds which can be recellularised to create a potentially functional organ construct. Wistar rat whole kidneys were decellularised with 1% w/v SDS. Characterisation of decellularisation included histology (H+E), immunohistochemistry(IHC) for ECM components, vascular corrosion resin casting with Batson's no. 17 kit, and ECM growth factor quantification. Recellularisation with i) rat primary renal cells and ii)rat bone-derived mesenchymal stem cells were performed within a bioreactor for 7 days. Recellularised construct viability was shown with live/dead staining and Alamar blue assay and characterisation by histology, IHC for renal cell differentiation, and electron microscopy. Rat kidneys were successfully decellularised using the perfusion method to create whole kidney ECM bio-scaffolds. Characterisation of the bio-scaffold demonstrates good decellularisation, preservation of ECM components/growth factors, renal-specific architecture and preservation of the vasculature. Recellularisation studies show penetration and distribution of viable cells throughout the bioscaffold architecture within 'renal-like' structures, suggesting an appropriate response to structural and bio-inductive cues within the bio-scaffold.

19.P06
Production and characterization of human renal and ileal ECM scaffols: the ideal platform for renal and intestinal bioengineering investigations

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Backround: It is important to identify new sources of transplantable organs because of the critical shortage of donor organs. Tissue engineering holds the potential to address this issue through the decellularization/recellularization of whole organs. We are currently implementing this technology to produce extracellular matrix scaffolds (ECMs) from human kidneys and small intestine.
Methods: Segments of normal human ileum (NHI) and human kidneys (HK) discarded from transplantation in reason of severe glomerulosclerosis were decellularized with detergent-based solutions. After rinsing with PBS to remove detergents, the so-obtained ECMs were processed for histology and vascular imaging as previously described (Orlando et al. ANN SURG, in press)(Totonelli et al BIOMATERIALS 2012).
Results: acellular intestinal ECMs were consistently obtained from NHI. As well, acellular renal ECMs were successfully produced from HK regardless of the degree of glomerulosclerosis of the discarded kidneys. ECMs from both organs retained innate architecture and molecular components. H&E and DAPI confirmed acellularity. Imaging revealed an intact vascular tree with no leak.
Conclusions: Our investigations show that both NHI and glomerulosclerotic HK discarded from the organ donor network, can be successfully decellularized to produce acellular ECMs. ECMs maintain their

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3D architecture and vasculature and so may represent the ideal platform for kidney and intestine bioengineering studies.

19.P07
Muscle precursor cells for the treatment of fecal incontinence

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The surgical repair of external anal sphincter is still limited today. We hypothesize that autologous Muscle Precursor Cells (MPC) injected into the damaged anal external sphincter muscle are able to form new functional muscle tissue.In this research rodent MPCs were harvested and characterized by FACS, immunostaining and fiber formation assays. We established a fecal insufficiency model, which induces a constant sphincter damage and allows for detailed functional assessment of the external and internal anal sphincter. Morphological and functional outcome after MPC injection was compared up to 6 weeks. Collagen-only injections served as controls. We demonstrated that rodent MPCs were reproducibly harvested, cultured and expanded *in vitro*. MPC-injection for anal sphincter reconstruction resulted in improved sphincter contraction in response to electrical stimulation ($p = 0.016$). Significant improvement of sphincter contraction at 4 weeks (18.3 vs 8.7 cmH_2O; $p = 0.003$) and at 6 weeks after MPC injection (27.6 vs 11.1 cmH_2O; $p < 0.001$) was demonstrated. No difference between the groups was found for the resting pressures. Formation of new tissue with myofibers was demonstrated by immunostaining.We conclude that cell therapy using cultured MPCs might be a promising option for the treatment of anal sphincter insufficiency in near future.

19.P08
Re-endothelialization of acellular kidney scaffolds for whole organ engineering

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The lack of transplantable allografts for renal failure has prompted the development of engineered whole kidneys. Decellularized solid organs such as the kidney hold great promise due to the maintenance of structural integrity while removing resident cellular components. However, acelluar kidney vasculature needs an endothelial cell lining to avoid blood clotting when transplanted into a living recipient. In order to maintain blood circulation and induce renal functions, re-endothelialization of acellular scaffold is necessary. In this study, we describe an endothelial cell seeding method that permits effective endothelial cell coating on the vascular walls of the decellularized porcine renal scaffolds. Histological results showed that a cell seeding technique, consisting of combined static and ramping perfusion of the renal vessels facilitated the development of a well-organized and viable endothelium throughout the renal scaffold. Furthermore, the cell-seeded (MS1) scaffolds appeared to be functional as evidenced by the maintenance of stable parenchymal pressures during blood perfusion and significant inhibition of platelet adhesion on cell-seeded vascular surfaces, as compared to the unseeded scaffolds. These results are a promising indicator that the formation of a fully endothelialized vascular tree could prevent blood clotting and deliver oxygen and nutrients to a tissue-engineered kidney *in vivo*.

19.P09
Uretheral muscle regeneration using injectable gelatin-based hydrogel with heparin nanogel

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Injectable hydrogels have been received the tremendous attention due to the minimal invasiveness and maximal effectiveness for delivery of therapeutic agents and regeneration of damaged tissues. In this study, *in situ* cross-linkable gelatin hydrogels incorporated with heparin nanogels were prepared via enzyme-mediated reaction using horseradish peroxidase for urethral muscle regeneration. For this study, gelatin-poly(ethylene glycol)-tyramine (GPT) and heparin-Pluronic (HP) were synthesized by PNC and EDC/NHS chemistry. The HP nanogels (HPNP) were prepared by the direct dissolution method and the HPNP loaded GPT hydrogels (GPT/HPNP) were fabricated by the enzymatic reaction. The bFGF was released from the hydrogels for 30 days in a controlled manner due to the heparin binding affinity. To assess the smooth muscle regeneration, *in vivo* animal study was carried out, resulting that the most effective muscle tissue regeneration was shown in the case of the GPT/HPNP containing bFGF as compared to other hydrogels and controls. The obtained results demonstrated that the injectable GPT/HPNP hydrogels have a great potential for the urethral sphincter muscle regeneration.
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19.P10
A bedside collagen-PLGA nanofibrous construct for autologous transplantation of minced bladder mucosal

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Introduction: Bladder regeneration using minced bladder mucosa is an alternative to costly and time-consuming conventional *in vitro* culturing of urothelial cells. In this method, the uroepithelium expands *in vivo* and the patient body appears as an incubator. With our preliminary successes, designing an appropriate scaffold that supports *in vivo* cell expansion and surgical handling in a clinical setting was our aim. This study, investigates cell expansion in a hybrid construct of collagen/poly (lactic-co-glycolide)(PLGA).
Materials and methods: An electrospun PLGA mat was placed on a semi-gel collagen inside a mold and covered with a second collagen layer. After gel formation, minced particles of pig bladder mucosa were seeded on the hybrid construct and then processed by plastic compression (PC). The scaffolds were incubated for 2, 4 and 6 weeks *in vitro* for further studies.
Results: Tensile tests show an increase in tensile strength of 0.6 ± 0.1 MPa in PC collagen to 3.6 ± 1.1 MPa in hybrid construct. Morphological studies, histological staining and SEM show that the construct has kept its integrity during the time and proliferated urothelial cells have reached confluence after 4 weeks and a multi-layered urothelium after 6 weeks.
Conclusion: We have designed a mechanically robust scaffold that permits surgical handling and tissue expansion *in vivo*. The construct is easy-to-use for clinical application in an ordinary surgical operating theater for bladder regeneration.

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19.P11
Adipose-derived stem cells (ADSCs) and muscle precursor cells (MPCs) for the treatment of bladder voiding dysfunction

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Bladder outflow obstruction is common in the elderly and can result in bladder voiding dysfunction (BVD). The goal of this research was to evaluate the use of adult stem cells for the treatment of BVD in a rat model. Adipose-derived stem cells (ADSCs) from the inguinal region and Muscle Precursor Cells (MPCs) from the soleus muscle of adult male Lewis rats were harvested, expanded in culture and characterized. Bladder outflow obstruction was induced by tying a suture around the urethra in male Lewis rats (6–8 weeks old). After 6 weeks the development of a hypocontractile bladder was confirmed by urodynamic studies, organ bath and molecular expression. Injection of ADSCs or MPCs into the bladder wall and synchronous deligation was performed 6 weeks after the obstruction. 4 and 8 weeks after cell injection, morphological and functional changes were assessed. Obstructed rats had a significant larger bladder weight, lower maximum bladder pressure and lower contractility than age-matched rats. Labelled ADSCs and MPCs were detected after 4 weeks in the bladder wall expressing smooth muscle-specific markers. Organ bath analysis and urodynamic studies showed an improved contraction and higher maximal bladder pressure 4 and 8 weeks after cell injection. The same trend was seen on RT-PCR and Western-Blotting where gene expression and protein translation of important contractile proteins after stem cell therapy were upregulated.

19.P12
The effect of porosity on tissue ingrowth and vascularization in electrospun hybrid scaffolds for bladder regeneration

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Introduction: Scaffold porosity governs cellular infiltration, tissue ingrowth and early revascularization which promotes oxygenation of the graft. We investigated the role of porosity of hybrid scaffolds consisting of bladder acellular matrix (BAM) and electrospun poly(lactide-co-glycolide) (PLGA) mimicking morphological characteristics of the bladder wall. Scaffolds with different porosity seeded with smooth muscle cells (SMCs) were evaluated in a cystoplasty model.
Materials and methods: Single-spun scaffolds (SSS) were compared with a scaffold with increased porosity (CSS) obtained by co-spinning of PLGA and Polyethylene glycol. Scaffolds were characterized by scanning electron-microscopy. *Ex vivo* proliferation assays and histological examinations were performed. 16 rats received partial cystectomy followed by augmentation cystoplasty with seeded SSS or CSS. Morphological and histological studies were performed after 2 and 4 weeks.
Results: The porosity of the micro-fiber scaffold ($4.4 \pm 0.7\ \mu m$) was $75.8 \pm 1.2\%$ (SSS) and $80.7 \pm 1.8\%$ (CSS). *Ex vivo* evaluation showed an increased cell proliferation on CSS. *In vivo* histology revealed a bladder wall-like structure with urothelial lining, and SMC infiltration. The micro vessel density was significantly increased in CSS after 2 and 4 weeks ($p = 0.04$, $p < 0.001$ respectively).
Conclusion: We were able to demonstrate that increased scaffold porosity significantly enhances cell infiltration and revascularization in bladder TE.

19.P13
Compressed fibrin-collagen scaffolds for enhancing bladder tissue regeneration: a bioreactor cell culture system

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Bladder tissue engineering plays a pioneering role among many branches of tissue engineering. Surgical repair of the urinary tract is required when the tissue is damaged by malignancies, trauma, or congenital pediatric disorders (Atala, 2011). The reconstructive procedures used in clinic have several drawbacks such as secondary malignancies, intestinal adhesions, and chronic infections. Therefore the latest developments in bladder tissue engineering have been aiming to design novel cellular scaffolds and culture techniques for urinary tract repair. The use of bioreactors in tissue engineering is regarded as a further step in comparison with conventional tissue engineering techniques. In this this study, compressed collagen-fibrin scaffolds were assessed under dynamic culture conditions inside a novel bioreactor system imitating the dynamic environment of the bladder. Mechanical properties, degradation, morphology, and *in vitro* biocompatibilities via smooth muscle and urothelial cells of these engineered gels have been investigated. Currently biological performance of the compressed gels is being evaluated by AlamarBlue assay, Live/Dead staining, and immunohistochemistry.
Acknowledgements: This study is supported by the European Commission under the 6th framework programme (EuroSTEC).
Reference:
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19.P14
Single cell analysis of porcine and human urothelial cells

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An estimate of 400 million people worldwide suffers from bladder diseases. The epithelium of the urinary tract, the urothelium, is an epithelial with many unanswered questions, concerning tissue homeostasis, injury and disease. In the case of congenital anomalies and acquired diseases when replacement surgery is necessary, the current treatment plans are not optimal. Tissue-engineered constructs will be a clinical option. The aim with the tissue-engineered constructs is that it should function throughout a patient's lifetime. Elucidating, the specific cells that are important for functional tissue-engineered constructs for the bladder will have a clinical impact. We addressed with single cell analysis, the capacity of single urothelial cells to proliferate and differentiate, to observe if there was an *in vitro* cell hierarchy that could be traced back to what we could observe *in vivo*. RT-PCR was used to investigate the single urothelial cells gene expression. We observed different growth capacity and ability to form colonies of clonal cell lines originating from human and porcine urothelium. Differences in gene expression could be observed between the clonal urothelial cell lines. Currently, these clonal urothelial cell lines differentiation capacity is being investigated with implantation sub-kidney capsular in nude mice. This study will bring us closer to an understanding of how the urothelium self-renew and differentiate *in vitro* and *in vivo*.

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19.P15
Human amniotic membrane scaffolds promote urothelial cell differentiation: a potential application for the urinary tract reconstruction

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A challenge in tissue engineering (TE) is to establish a fully functional tissue that could be used to replace damaged or defective tissues or organs. Amniotic membrane (AM) has unique biological and mechanical properties that promote cell growth and thus presents an excellent scaffold for TE.Our aim was to determine urothelial cell (UC) differentiation on three different AM scaffolds. Normal porcine UCs were seeded on cryopreserved human intact AM, denuded AM or AM stroma, and cultivated for 3 weeks. UCs differentiation was determined by electron microscopy and immunofluorescence of urothelial differentiation-related markers.On all three AM scaffolds the normal urothelium composed of basal, intermediate and superficial cells expressing uroplakins was established. The highest differentiation stage of UCs was demonstrated on AM stroma scaffold. Superficial UCs cultured on AM stroma were large, with apical plasma membrane shaped in the microridges, discoidal vesicles in the apical cytoplasm and the highest fluorescence intensity of uroplakins. Our findings indicate that AM stroma scaffold enables the development of tissue-engineered urothelium with molecular and ultrastructural properties comparable to native urothelium. Since such urothelium enables the effective permeability barrier with immediate protection of the underlying stroma, we consider the highly differentiated urothelium on the AM stroma scaffold suitable for use in the urinary tract reconstruction.

19.P16
Injectable pDNA-loaded bulking agent for the treatment of urinary incontinence

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The injection of bulking agents has been commonly used as the first line therapy for the treatment of urinary incontinence due to its low cost and the minimally invasive procedure compared to surgical techniques. In spite of the short-term success achieved by this therapy, the procedures are hindered by the need of multiple injections caused by the resorption or particle migration over time, indicating that the cure rate relies on the passive bulking effect by the volume injected. In this study, we prepared plasma DNA (pDNA; encoding for bFGF) complex-loaded polycaprolactone (PCL) particle/poly(DL-lactic-co- glycolic acid) (PLGA) mixture as an injectable bioactive bulking agent which can provide bulking effect and stimulate the defect tissues around urethra for the effective treatment of urinary incontinence. The pDNA complex was continuously released from the PCL/PLGA mixture over 3 months. The released pDNA complex was effectively transfected into the cells around urethra and synthesized the bFGF from the cells. From the animal study using a rat model, it was observed that the pDNA complex-loaded PCL/PLGA mixture has effective cure behaviors for urinary incontinence as follows: the narrowed urethral lumen, the regeneration of smooth muscle around the urethra, and the effective urethral contractions. The pDNA complex-loaded PCL/PLGA mixture may be a good candidate as an injectable bioactive bulking agent for the treatment of urinary incontinence.

19.P17
Combination of human adipose-derived stem cells and cross-oriented bilayered scaffold for urethra reconstruction

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An organ or a tissue in body is commonly composed of several layers and components. However, traditional tissue engineering fabricated with conventional scaffold and seed-cells provides solutions by dressing or stuffing, which means to repair physically, not regenerate functionally. Clinically, urethra diseases including congenital malformation, trauma, tumor, etc. cause urethral defects. Doctors are often stumped for the lack of smooth muscle cells (SMCs) which belong to stable cells and hardly regenerate. Urethra tissue engineering has shown great potential for urethral reconstruction. The native urethra is characterized for inner longitudinal and outer circular layers of SMCs in anatomy, which is confirmed to be the foundation for storage and emiction of urine. Therefore, we manipulated electrospinning technique to prepare oriented nanofibers and then manufactured a bilayered and cross-arranged scaffold by vertical overlying. On the other hand, the SMCs were induced by human adipose-derived stem cells (ASCs) and seeded on the scaffolds to construct muscle tubes. The biocompatibility of ASCs-derived SMCs and nanofibers including proliferation, attachment and spreading were investigated. In addition, the cell-scaffold construct was functionally detected by organ bath and the contraction and relaxation could be controlled by atropine and carbachol. Our findings paved a new way for functional urethra tissue engineering in the design from biomimetic construction to biological function.

19.P18
A novel tissue engineering construct using human endometrial mesenchymal stem cells and polyamide mesh for pelvic organ prolapse (POP) repair

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POP is defined as the descent of one or more of the pelvic structures into the vagina and includes uterine prolapse, vaginal vault prolapse, anterior or posterior vaginal wall prolapse. The treatment of POP includes conservative surgical treatment and/or implantation of synthetic/biological mesh. However, the long-term outcome of synthetic mesh surgery is unsatisfactory due to surgical failure and post-surgical complications. Our aim was to improve the *in vivo* biocompatibility of a novel synthetic Polyamide gelatine coated mesh with a novel source of mesenchymal stem cells (MSC) using a tissue engineering approach. Human endometrial MSC (eMSC) were isolated from hysterectomy tissue by FACS, seeded onto scaffolds (25 × 10 mm, 500.000 cells/mesh) and subcutaneously implanted dorsally in immunocompromised rats for 7, 30, 60 and 90 days (n = 8/gp). Flow cytometry was used to detect eMSC after explantation. Immunohistochemical assessment of foreign body reaction and tissue integration was performed using collagen antibodies (I, III, VI), CD31, CD45, CD68, and alpha-smooth-muscle-actin antibodies. The implanted materials were well tolerated, no mesh erosions or shrinkage occured at any timepoints. The meshes with cells attracted fewer macrophages and promoted vessel formation. This tissue engineering application might be an alternative option for future treatment of POP.

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19.P19
Biomechanical properties of implanted mesh: a rat study for pelvic organ prolapse repair

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Pelvic organ prolapse (POP) is a major hidden burden affecting millions of women worldwide. POP is the herniation of the bladder, bowel and/or uterus into the vagina. Polypropylene (PP) mesh is widely used to support the pelvic organs, but has inferior biomechanical properties to vaginal tissue. In this study, we have fabricated mesh from a range of polymers, and using a rat hernia model compared implanted mesh biomechanical properties to native tissue properties. Polyetheretherketone (PEEK), polyamide (PA) and gelatin coated PA (PA+G) knitted mesh were implanted into the abdominal cavity of rats. A commercial PP mesh (Polyform™, Boston Scientific) was also implanted for comparison. After 7, 30, 60 and 90 days mesh were explanted and assessed for their biomechanical properties. Similar levels of stiffness were determined for the non-implanted mesh and day 7 explants in the toe region (up to 20% strain) of the load-elongation curves. Increased stiffness was determined for the day 30 explants in this region. Within the linear region of the curves, the day 30 explants were stiffer, with lower strain at mesh rupture, than the day 7 explants. Throughout implantation the PA+G mesh was generally the strongest of all meshes, including the PP mesh, with most similar biomechanical properties to adjacent rat tissue. These results indicate that implanted PA+G mesh possessed similar levels of stiffness and breaking load/strain to native rat tissue, and may be a candidate in POP repair.

19.P20
Novel biomaterials for pelvic organ prolapse (POP) repair

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POP is the descent of one or more of the pelvic structures into the vagina and includes uterine, vaginal vault as well as anterior and posterior vaginal wall prolapse. Treatment of POP includes surgical treatment and/or implantation of synthetic mesh. However, the long-term outcome of synthetic mesh surgery is unsatisfactory due to surgical failure and post-surgical complications. We have fabricated 3 novel warp-knitted synthetic scaffolds, Polyetheretherketone (PEEK), Polyamide (PA) and gelatine coated PA (PA+G). Our aim was to compare the *in vivo* biocompatibility of these meshes with a commercial Polypropylene (PP) mesh. A SD rat abdominal hernia model was used to implant PA, PEEK, PA+G, PP meshes (25×35 mm, $n = 24$/gp). After 7, 30, 60, 90 days tissues ($n = 6$/mesh) were explanted for immunohistochemical assessment of foreign body reaction and tissue integration, using CD31, CD45, CD68, alpha-SMA antibodies. CD68+ macrophages showed a trend toward decreased numbers in PP compared to PA, PA+G and PEEK. The smooth muscle content at 60 days was significantly higher in PA and PA+G meshes compared to PP ($p > 0.05$), but there were no significant differences at 90 days. The collagen content was similar for all meshes at 90 days($p > 0.05$). PA, PA+G and PEEK appear to have similar biocompatibility properties compared to PP, inducing a similar foreign body reaction response. These novel meshes may provide an alternative option for future treatment of POP.

19.P21
Development of ovarian follicle-like structures

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The ovary contains cells that secrete hormones, which maintain female sexual characteristics as well as egg production. Loss of ovarian tissue function due to various endocrine and fertility disorders requires chronic estrogen and progesterone administration. While conventional hormone replacement therapy is able to maintain female sexual characteristics, this therapy is unable to restore ovarian tissue function, which includes the production of eggs. This is critical for premenopausal women who desire to conceive. Creation of ovarian tissue that produces eggs would provide numerous benefits to women of all ages. Toward this goal, this study aimed to develop an ovarian cell cultivation system that could lead to the production of eggs. We investigated whether adult ovarian cells could produce oocytes and form follicle-like structures. Ovarian cells from 3-week old female rats were isolated and cultured with glial cell-derived neurotrophic factor and leukemia inhibitory factor. Colonies were placed under 3D collagen gel. Cell growth, gene expression and hormone production were assessed. As culturing continued, oocytes protruded from the clusters of ovarian cells and formed compact colonies resembling follicle-like structures. These cells expressed germ cell markers and zona pellucida, and exhibited steroidogenic capacity. We conclude that adult ovarian cells obtained from rats are capable of maturing into oocytes and form ovarian follicle-like structures.

19.P22
Transplantation of an alginate-matrigel matrix containing isolated ovarian stromal cells: first step to develop an artificial ovary

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For women diagnosed with leukemia, transplantation of cryopreserved ovarian tissue after disease remission is not advisable due to the risk of reintroduction of malignant cells. To restore their fertility, a biodegradable artificial ovary that allows isolated follicles and stromal cells (SCs) to survive and grow needs to be developed. The aim of this study is therefore to test the survival and proliferation of isolated SCs encapsulated in an alginate-matrigel matrix. For this, 4 NMRI mice were ovariectomized and SCs were isolated from their ovaries. Groups of 50 000 SCs were embedded in an alginate-matrigel matrix for fixation (fresh controls), 1 week of *in vitro* culture (IVC) or grafting. SC proliferation (Ki67), apoptosis (TUNEL), scaffold degradation and vessel formation (CD34) were analyzed. After IVC or grafting, the beads degraded, losing their original round form. Infiltrating blood capillaries were observed in the grafted beads. CD34(+) cells were found around and inside the matrix. Proportions of 2.3%, 18.3% and 15.5% of cells were Ki67(+) in fresh, IVC and grafted beads respectively. The results from control were statistically lower than the other 2 groups. The percentage of TUNEL(+) cells was 0%, 1.5% and 6.9% in fresh, IVC and grafted groups respectively. The results were statistically different between groups. This study showed that our matrix is a promising choice to graft SCs. The next step will be incorporating isolated follicles and ensuring their survival.

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19.P23
Reconstruction of endometrial tissue using cell sheet technology

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Introduction: Endometrial tissue plays a critical role in embryo implantation and development. An endometrial abnormality is related to female infertility. Hence, the regeneration of endometrium allowing fertilized ovum to implant might be valuable in the field of fertility treatment. The present study aimed to reconstruct endometrial tissue using cell sheet technology.
Materials and methods: Uteri resected from female C57BL/6 mice aged 4 to 5 weeks were treated with collagenase. Isolated cells were cultured for 3 days using a temperature-responsive cell culture dish. Confluent cells detached themselves as a contiguous cell sheet by reducing temperature. The cell sheet slightly shrinking was re-cultured for constructing a thick tissue, which was then harvested 3 days later and stained for immunohistochemistry.
Results: After re-culture, the shrunk cell sheet was reconstructed to be a multilayered endometrium-like tissue, which was found to consist of cytokeratin 18 and vimentin positive cells showing the characteristics of endometrial epithelial and stromal cells, respectively. Moreover, estrogen receptor-positive cells and progesterone receptor-positive cells were also observed in the tissue.
Conclusion: The endometrial cell sheet was constituted of essential cells in endometrial regeneration and contained cells expressing endometrium-specific hormone receptors. The endometrial cell sheet was speculated to be valuable in the regeneration of functional endometrium.

19.P24
Urine derived stem cells with high telomerase activity: an optimized cell source for regenerative medicine in urology

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Introduction: The purpose of this study was to evaluate whether telomerase activity can be detected in urine derived stem cells (USC) derived from healthy, middle-aged donors, and to determine whether USC that express telomerase (USC-TA+) possess greater proliferative capacity than USC that do not express telomerase (USC-TA-).
Methods: One hundred and fourteen USC clones were isolated from the urine of 10 healthy donors (ages ranging from 20–50 years) and cultured. The telomerase activities of these USC and human bone marrow stromal cells (BMSC) were measured at p2, 7, and 11 using a TRAP-ELISA assay.
Results: About 70% USC clones expressed telomerase activity. USC-TA+ clones demonstrated more population doublings (PD) and shorter doubling times (DT) than USC-TA- clones regardless of the age of the donor. The average PD was 67.50 ± 5.1 at p17 in USC-TA+ compared to 37.6 ± 2.9 at p8 that in USC-TA-. The average DT was 26.9 ± 2.0 h in USC-TA+ compared to 35.9 ± 1.4 h in USC-TA-. The karyotype of all USCs was normal and did not appear to depend on TA level. Both USC-TA+ and USC-TA- expressed mesenchymal stem cell markers. Conclusions: USC that expressed telomerase activity possessed higher proliferative capacities and could be maintained for a higher number of passages. This information further enhances our characterization of USC-TA+ and supports the potential clinical utility of these cells for cell based therapy in urology.

19.P25
Xeno-free culturing of human muscle precursor cells (MPC) for clinical application

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Autologous cell therapies are envisioned as a promising therapy for many diseases including urinary incontinence, where sphincter muscle damage leads to urine leakage and significantly reduces life quality. Recent animal models demonstrated the validity of the method showing regeneration of sphincter muscle tissue after injection of MPCs. Currently, theses cells are expanded in xenogenic media containing fetal bovine serum (FBS). However, before MPCs can be applied clinically it is mandatory to reduce the potential immunogenic reaction and infection risk by removing any xenogenic contaminants. In this research human MPCs were expanded in xeno-free medium using pooled human platelet lysates (pHPL) or pooled human Serum (HS). We assessed the expansion potential, the differentiation by FACS and the fibre formation *in vitro*. Further, we assed the *in vivo* muscle tissue formation after injection and contractility by organ bath. Cells grown in standard FBS medium served as controls. Our results clearly demonstrate that HS is no substitute for FBS with the MPC showing signs of senescence and decreasing in growth. Using pHPL we were able to expand the MPCs while maintaining the myo-phenotype as demonstrated by FACS and IHC for MyoD, desmin and MHC. Expanded MPCs gave rise to contractile muscle tissue *in vivo*, comparable to tissues grown using cells expanded in FBS. In conclusion, our results show that pHPL is a suitable substitute for FBS and may be used for clinical application.

19.P26
Biological characterization of human urine-derived stem cells from the upper urinary tract for potential use in bladder regeneration in patients with end-stage bladder disease

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Introduction: Because the upper urinary tract is usually normal in patients with end stage bladder diseases or bladder cancers, we hypothesized that stem cells obtained from the upper urinary tract (uUSC) may be an alternative cell source for tissue engineering applications designed to treat these conditions.
Methods: Urine samples were collected from the patients with normal upper urinary tracts during renal pyeloplasty. Cells were isolated from this urine and extensively expanded *in vitro*.
Results: The maximum population doubling of uUSC was 56.7 (average 44.04 ± 8.59) and the cells were grown up to p14. The uUSC also expressed mesenchymal stem cell surface markers. These cells could differentiate into smooth muscle-like cells expressing smooth muscle-specific gene transcripts and proteins. In a collagen lattice assay, these myogenic-differentiated uUSC displayed contractile function that was similar to those in native smooth muscle cells. Urothelial-differentiated uUSC expressed urothelial-specific genes, proteins and functional tight junction markers. Moreover, karyotype analysis on several passages of uUSC clones showed normal chromosomal content and structure.
Conclusions: uUSC possess expansion and urothelial and myogenic differentiation capabilities, and can potentially be used as an alternative cell source in bladder tissue engineering for patients with end stage bladder diseases who require cystoplasty.

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20. Trachea/Oesophagus/Chest - Repair and Replacement

20.01
Determining the in vitro and in vivo immune response towards decellularized porcine tracheal allografts for airway transplantation

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I-Malignancy, subglottic stenosis and traumatic injury to the trachea require surgical resection. The advances in airway allotransplantation focused on tracheal decellularization techniques which allow for removal of the immunogenic components. Despite the multiple advances in this field, the local and systemic immune response to these decellularized tracheae has not been explored. M-Tracheae were harvested from Yorkshire pigs ($n = 6$) and decellularized using three different decellularization protocols. Tracheae were evaluated histologically and by immunostaining for MHC1 and MHC2 markers. CFSE labelling in a Mixed Lymphocyte Reaction (MLR)assay was used to assess $CD4^+$ and $CD8^+$ T cell proliferation following incubation with tracheal pieces. Native and decellularized allografts were heterotopically transplanted and segments were biopsied each week for 3 weeks for histology, immunohistochemistry, PCR and flow cytometry looking at infiltrating cells. R-Following decellularization, cells within the glands continue to stain for anti-MHC1 and anti-MHC2. MLR using tracheal pieces as antigenic stimulants showed an increase in the proliferation index of $CD4^+$ T cells using Protocol B. In vivo heterotopic transplantation revealed a delay in leukocyte infiltration following decellularization and an increase in $CD4^+$ $CD25^+$ $Foxp3^+$ T cells. The infiltrating macrophages were of an M2 phenotype. C-Decellularization delays leukocyte infiltration and results in subtle changes in the type of cells recruited.

20.02
The efficiency of stratified epithelial cell sheet fabrication by the addition of IL-1 receptor antagonist

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Cell sheet-based epithelial regenerative medicine has been applied to the skin, cornea, and oesophagus. However, some problems as rapid provision of regenerative medicine products, coculture with feeder cells, and additional cholera toxin, are still remaining. For the coming full-fledged regenerative medicine, to build refined culture systems have become an urgent matter. Oral mucosal epithelial cells or human epidermal keratinocytes were cultured on temperature responsive cell culture inserts. Gene expression was analyzed by Taqman Gene Expression Assays. Secreted IL-1 receptor antagonist (IL-1ra) was detected by ELISA. Rat oral mucosal epithelial cells showed substantial proliferation in the medium containing canine oral mucosal epithelial cell culture supernatant. IL-1ra gene was up-regulated in the growth moment of cultured canine oral mucosal epithelial cells. In addition, released IL-1ra was detected in canine oral mucosal epithelial cell culture supernatant. In the absence of 3T3 feeder cells and cholera toxin, rat oral mucosal epithelial cells or human epidermal keratinocytes showed remarkable proliferation by the addition of IL-1ra, and also robust cell sheets were harvested. IL-1ra showed growth promotional effect on stratified squamous epithelial cells. IL-1ra is expected to fulfill more proper epithelial culture condition and contribute to the further improvement of regenerative medicine for clinical applications.

20.03
Isolation of $CD34^+$ epithelial stem cells and decellularized oesophageal scaffolds holds potential for the production of a tissue-engineered oesophagus

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Abstract not available.

20.04
Esophageal reconstruction in six patients: a regenerative medicine approach

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Barrett's esophagus with high grade dysplasia and esophageal adenocarcinoma have increased in incidence and now represent the sixth leading cause of cancer death. The standard of care for these conditions is esophagectomy. In spite of significant reductions in mortality reported at experienced centers, esophagectomy is associated with morbidity rates approaching 50%. Therefore, there is a strong impetus to move therapy towards esophageal preservation in patients with neoplastic lesions limited to the intramucosal region. Herein, we report the use of biologic scaffolds composed of extracellular matrix (ECM) for esophageal reconstruction. In pre-clinical models, critical size, full circumferential defects were repaired with minimal stricture formation if autologous muscle tissue was placed in direct apposition to the ECM. Recently, the use of ECM was extended to a pre-clinical model of endoscopic mucosal resection (EMR) such as that currently conducted for Barrett's disease. Complete reconstruction of the mucosal layer was achieved. Based upon these findings, six patients with stage 1 esophageal adenocarcinoma were treated with aggressive EMR and the associated placement of ECM material. Three patients subsequently received fundal plication surgery to cure reflux. The successful results of this clinical translation with 3 year follow up will be reported.

20.05
Keynote: Tissue engineering – from bench to bedside and back to bench

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End-stage organ failure is one of the major challenges for medicine and this can worsen due to demographic changes. Despite enormous efforts in medical and basic research, allogenic organ transplantation is proba-

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bly the only curable treatment option for these patients. However, the growing shortage of donor organs and the need for lifelong immunosuppression with its associated morbidity are some requirements for novel therapeutic option. Tissue engineering (TE) is becoming a very promising alternative. Early clinical application has demonstrated the feasibility of using TE to replace damaged tissue and organs with rather simple architecture. Preclinical findings also suggest that even higher complex organs can be successfully engineered and transferred to the clinic in the near future. Basic components of the tissue engineering concept include i) a scaffold ii) cells iii) a bioreactor and iv) pharmaceutical intervention/bioactive molecules. Recently, the first clinical trachea transplantation was performed with seeded autologous cells on a synthetic-based nanocomposite. Although the feasibility of a concept with great potential was demonstrated, further efforts must be done to elucidate underlying pathways and mechanisms. Only continuous evaluations and re-investigations of the already clinically applied methodology can provide stronger evidence to transfer the concept into clinical routine. The proof of concept has been done – so let us now improve the concept!

20.P01
A tissue-engineered fibroblast sheet as a pleural substitute for chest surgery

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We previously have reported that tissue-engineered fibroblast sheets readily close visceral pleural defects, followed by the deposition of extracellular matrix-enriched tissue on the lung surface with an excellent biocompatibility. Tissue-engineered fibroblast sheet is a good pleural substitute. The present study examined human fibroblast sheets. Skin specimens (leaf shape, 10 × 5 mm) were excised from the lateral chest for preparing an access port of patients who were performed with video-assisted thoracoscopic surgery under general anesthesia in compliance with the ethical guidelines of Tokyo Women's Medical University. Oral and written informed consents were obtained from individual patients. The obtained confluent primary cells were passaged and cultured for additional 1 week before cell sheet harvest. When culture temperature was decreased to 20 °C, all the cells detached themselves as a single contiguous cell sheet within 1 h. Harvested fibroblast sheets were transplanted directly to the defects of a visceral pleural injury model in athymic rats without the need for sutures or additional adhesive agents. After 4 weeks, rethoracotomy was performed. Transplanted pleural substitute was significantly thicker with ECM which own produced by itself. By the analysis of fluorescence in situ hybridization, human fibroblasts had remained in the cell sheet and hardly invaded into the host lung. Human fibroblast sheets would be useful as a pleural substitute for chest surgery.

20.P02
Optimizing seeding conditions for respiratory epithelium on fibrin hydrogel

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Fibrin gel has proven a valuable scaffold for tissue engineering. We have shown that proliferation and differentiation of respiratory epithelial cells on fibrin gel compares to culture on collagen-coated, microporous membranes. To achieve confluence quickly, we optimized the seeding density of the cells to fibrin gel and the time cells need to adhere onto fibrin gel. Cells from porcine tracheae were suspended in 500 μl medium and seeded on 1.9 cm^2 wells coated with 500 μl fibrin gel at densities of 2, 4, 8, 16 and 32 × 104 cells per cm^2. To assess the unanchored cells after seeding, the concentration of cells in the removed medium was measured using a Casy cell counter. Live microscopy images were taken every day to evaluate a cell layer confluence. To optimize adhesion time of the cells, cells in concentration of 8 × 10 ^ 4 were seeded and medium was removed after 1, 2, 4, 8, 12 and 24 h. Unanchored cells were measured as described above. Cells seeded with density of 8–16 × 10 ^ 4 cells per cm^2 reached confluence at first. Lower densities of seeded cells take unproportionally longer to cultivate to a confluent cell layer. Cell concentration in the removed medium did not lower after 12 h of attachment time. Seeding cells at a density of 8–16 × 10 ^ 4 cells per cm^2 is most effective in quickly reaching a confluent cell layer of respiratory epithelium. Cell attachment mainly takes place in the first 12 h of seeding.

20.P03
Development of a novel 3D bellows scaffold and application of monolayered mesenchymal stem cells for tracheal reconstruction

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Artificial tracheal grafts should have not only enough compressive strength to maintain an open tracheal lumen, but also sufficient flexibility for stable mechanical behavior, similar to the native trachea at the implant site. In this study, we developed a new 3D artificial tracheal graft using a bellows design for considering its in vivo mechanical behavior using indirect solid freeform fabrication technology. Due to tracheal critical functions, such as airflow and mucociliary clearance, it is very important to regenerate mucociliary and airtight epithelium rapidly. Therefore, we applied cell sheet technology at the inner wall of bellows graft using temperature-responsive culture dish. Monolayered mesenchymal stem cells (MSCs) have multipotent and self-propagating properties into transplanted tissue because cell-to-cell adhesion proteins are maintained. The MSCs sheet-coated bellows graft was implanted into cervical tracheal defect of rabbit for 8 weeks. Post-implantation evaluation was performed by bronchoscope and histological analysis. In vivo result, the bellows graft was completely incorporated into regenerated connective tissue and no obstruction at the implanted site was observed for up to 8 weeks after implantation. And engrafted MSC sheet is well induced to form morphologically and functionally normalized tracheal epithelium. The data suggested that the developed bellows tracheal graft and monolayered MSCs may be a promising alternative for tracheal reconstruction.

20.P04
Reconstruction of circumferential tracheal defect by pedicled tissue-engineered trachea in rabbits

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In this study, we tested the feasibility of autologous tissue-engineered trachea (TET) for functional repair of circumferential defects of the trachea in rabbits. Auricular chondrocytes, cultured and expanded in vitro,

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were seeded onto polyglycolic acid (PGA) cylindrical scaffolds to form cell-polymer constructs. After 14 days of in vitro culture, the constructs were implanted into the sternohyoid muscle or subcutaneous tissue for 6 weeks to form TET. In experimental group (n = 10), TET replaced a six tracheal rings of the cervical tracheas with the sternohyoid muscle pedicle as carrier. In control group (n = 10), construct was implanted into subcutaneous tissue and TET was used as free graft without the muscle pedicle. After trachea reconstruction, the animals in experimental group could breathe spontaneously without assistance. The engineered cartilage remained the cartilaginous characteristics and was surfaced with a layer of squamous epithelium after 1–2 weeks, which transformed to pseudostratified ciliated columnar epithelium at 6~8 weeks. In control group, the epithelium growth rate was 1~2 weeks later, and part of engineered cartilages were proved to turn into fibrous tissue. At 6 months after reconstruction, there were six animals survived in experimental group while none survived in control group. There is statistical difference between the two groups. This study successfully repaired circumferential tracheal defect in rabbit with autologous TET.

20.P05
Transplantation of fabricated autologous epidermal cell sheet for prevention of esophageal stricture after circumferential ESD in a swine model

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ESD is an accepted treatment for early esophageal carcinoma and high grade dysphasia. However, resection of a large size mucosal area, such as circumferential ESD, induces severe stricture formation. Our aim was to investigate the feasibility of autologous skin epidermal cell sheets for prevention of stricture after ESD in a swine model. Primary epidermal cells were isolated from the lower abdominal skin of miniature pigs (n = 8), cultured for 18 days at 37 °C on temperature-responsive culture inserts. Transplantable cell sheets were harvested from the inserts by reducing temperature to 20 °C. Eight pigs were subjected to general anesthesia and circumferential esophageal ESD. In four pigs, an epidermal cell sheet, using fabricated autologous epidermal cells, was endoscopically transplanted to the central ESD site. The remaining four pigs were subjected to circumferential ESD only. Necropsy with histologic assessment was performed at 1 and 2 weeks. All control pigs after 2 weeks had endoscopic findings of severe esophageal constriction. In the control and epidermal cell sheet groups, the mean degree of weight gain were -10.3% and 0.3% and the mean degree of mucosal constriction were 88% and 56%, respectively. In Epidermal cell sheet group, histological assessment showed early re-epitelialization and mild fibrosis in muscularis. Fabricated autologous skin epidermal cell sheets can be utilized for prevention of esophageal severe constriction after circumferential ESD in swine model.

20.P06
Transplantation of autologous human oral mucosal epithelial cell sheets for esophageal endoscopic resection

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Endoscopic resection (ER) is a minimally invasive technique for the treatment of early-stage esophageal neoplasms. However larger-sized esophageal ER can frequently cause stricture due to ulceration after ER. We reported an innovative treatment using autologous mucosal epithelial cell sheets in a canine model to prevent an esophageal stricture after ER. In this study, we used a novel treatment for esophageal ER using cell sheet technologyin clinical setting. Epithelial cells were isolated from patients' own oral mucosa tissue and were cultured for 16 days in temperature-responsive culture inserts. Individual cell sheets were transplanted to the ulceration immediately after esophageal ER. All the patients underwent endoscopy every week until conformation of complete epithelialization of affected area.We performed procedures on 10 cases, all of them male. Esophageal ER was safety performed in all cases. The circumferential range of the ulceration was from half to fully circumferential. The number of cell sheets ranged from one to eight. Patients experienced no dysphasia or stricture following the procedure (apart from one case). The median value of time until complete healing was about 3.5 weeks. Oral mucosal cell sheets can potentially be used to prevent postoperative esophageal stricture after ER, thereby improving post-operative quality of life of patients.

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21. Ocular Area

21.01
Keynote: Corneal tissue engineering for fundamental and clinical applications.

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The cornea is one of the most transplanted tissues, accounting for more than 50 000 grafts each year in North America. As the population ages, the number of patients needing corneas for corneal dysfunction is expected to rise. In addition, the increasing severity of the eye banking exclusion criteria for donor tissue will reduce accessibility to corneal transplant and increase waiting times. Recent progress in tissue engineering has made it possible to consider new solutions to the problems of corneal tissue shortage. Autologous cells are more suitable for permanent tissue replacement since they are not rejected by the patient's immune system. The approach used in our laboratory is to use the cell's regenerative capacity for the engineering of an autologous tissue. We have used this approach to engineer the corneal epithelium, corneal stroma and the corneal endothelium. Stromal keratocytes can form thick sheets of extracellular matrix in vitro that can be used as carriers for the engineering of the corneal endothelium. Tissue-engineered corneal endothelia are functional in vivo, even when reconstructed using dystrophic endothelial cells. Potential applications for these models are numerous, and include in vitro pathophysiological and pharmacological studies, as well as clinical applications to treat corneal diseases. Support: CIHR, FRQS ThéCell Network, FRQS Research in Vision Network, Fondation du CHA.

21.02
A tissue-engineered plastic compressed collagen substrate suitable for human corneal endothelial layer transplantation

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Fuch's endothelial dystrophy affects many people over the age of 40 (5% of the >40 US population). Treatment involves replacing the failed endothelium with donor tissue. Currently one donor only treats one recipient. Human corneal endothelial cells (hCECs) can be expanded in culture but require a suitable substrate and carrier for transplantation. We describe, for the first time, the use of plastic compressed collagen (PCC) as a novel substrate for hCEC culture and as a potential carrier for transplantation. PCC constructs were produced using neutralised rat-tail type 1 collagen. hCECs, isolated from the Descemet's membrane of human corneas ($n = 3$) were cultured on FNC coated dishes and then seeded onto constructs. Constructs were cultured for 4 days, then analysed with light microscopy, immunochemistry and scanning and transmission electron microscopy (EM). hCECs seeded onto constructs were seen to have a typical cobblestone appearance and the majority of hCECs strongly expressed ZO-1 at cell-cell junctions and Na^+/K^+ ATPaseα1 on the cell surface. EM revealed overlapping finger-like projections onto juxtaposed cells with tight junctions, apical microvilli and anchoring filaments into the collagen substrate, all features indicative of a functional endothelial layer. This novel method provides expanded hCECs with an ideal carrier, superior to any other biomaterial previously trialled for this purpose and importantly allows one donor cornea to potentially treat multiple patients.

21.03
In vivo evaluation of a biomimetic human cornea stroma model generated with cornea stem cells and biomaterials

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Introduction: Fibrin-agarose biomaterials previously demonstrated to be adequate scaffolds for the development of bioengineered corneas. The aim of this work is to evaluate the in vivo behavior of a fibrin-agarose corneal stroma substitute in animal models.
Materials and methods: Human cornea stroma keratocytes were isolated from the human cornea limbus, and cultured in specific culture media. Then, an artificial cornea stroma was generated using fibrin - 0.1% agarose biomaterials with keratocytes immersed within. These tissues were evaluated ex vivo and in vivo by intracorneal implant in laboratory rabbits at both the clinical and the histological levels.
Results: The analysis showed that keratocytes were able to proliferate and spread within the scaffold from the 3rd day of culture. In vivo results showed an initial inflammatory process, with reabsorption of the implanted biomaterials around the second month of the implant. Transparency of the rabbit cornea was recovered after 5–6 months. Histologically, the corneas showed a process of fibers remodeling, with a high number of keratocytes spreading among the collagen lamellae.
Conclusion: Our results suggest that fibrin-agarose scaffolds are fully biocompatible and could be used for the generation of an artificial cornea scaffold for clinical use.Acknowledgements: Supported by FIS11/1582 from the Spanish Instituto de Salud Carlos III and P10-CTS6060 from Junta de Andalucia.

21.04
Keynote: Application of iPS cells for outer retinal reconstruction

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For the outer retinal damage, cell transplantation is expected as one of the future treatments. In that case we should transplant both photoreceptor cells and retinal pigment epithelial (RPE) cells in the end. Now we have the culture methods that induce directed differentiation of ES/iPS cells into those most needed retinal cells. As for photoreceptor transplantation, it is still necessary to adjust the transplantation condition to make the grafted cells form proper synapses with host neurons.As for RPE cells, we reported induction of RPE cells from primate ES cells (Kawasaki et al. PNAS 2002) and effective transplantation of them to RCS rats (Haruta et al. IOVS 2004). We are now preparing the

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protocol for the first in man clinical trial of human iPS cell-derived RPE (hiPS-RPE) transplantation. All the hiPS-RPE lines demonstrated a similar expression pattern of RPE signature genes. The hiPS-RPE sheets always have the mature function such as phagocytosis, growth factor secretion, cell polarity and barrier formation. We have never observed tumor formation from these cells in multiple tumorigenecity tests. With those cells we will be able to restore visual function to some extent but it does not mean we can regenerate the retina to be normal. For the successful development of regenerative medicine, we should consider precisely what we will be able to do with iPS cells.

21.05
Keynote: RPE Transplantation-Clinical Experience and New Concepts

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The Ludwig Boltzmann Institute for Retinology and Biomicroscopic Lasersurgery, Austria

No abstract available.

21.P01
Rapid fabrication of corneal epithelial cell sheets by oxygen controlled methods

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Introduction: Autologous epithelial cell sheets for treatment of ocular surface diseases are fabricated for several weeks. If the manufacturing period is shortened, the production will be more cost-effective. Recently, it was shown that hypoxia enhances the proliferation of various stem cells (1). On the other hand, oxygen concentration affects the differentiation of corneal limbal epithelial cells (2). In this study, we investigated whether the epithelial cell sheets are fabricated rapidly by oxygen control.
Materials and methods: Primary rabbit limbal epithelial cells were cultured in cell culture inserts. Oxygen conditions are as follows: (1) O2 20%, (2) O_2 2% and 3) O_2 2–20%. In condition 3, cells were cultured until reaching confluence in hypoxia. After confluent, the cells were cultured in O_2 20%.
Results: In condition 2 and 3, cells enhance the proliferation and reach confluence earlier within 2 days comparing with condition 1. In contrast, when O_2 concentration was changed to 20% from hypoxia after confluent, multilayered corneal epithelial cell sheets were fabricated (condition 3) as well as condition 1.
Conclusions: We developed a novel method for rapid fabrication of the epithelial cell sheets. Our results indicate that the novel method is effective for rapid fabrication of the cell sheets.
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21.P02
Development of tissue engineered stem cell niches for corneal repair

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Corneal blindness occurs as a result of limbal epithelial cells (LEC) deficiency due to causes such as chemical burns, Aniridia or radiation. LEC are located in the limbus at the Palisades of Vogt in specific microenvironments or stem cell niches. In some cases of corneal disease limbus and niches are destroyed. In this situation cells from the conjuctiva migrate to the cornea producing scar tissue which reduces vision. Our aim is to develop an experimental model of the limbus in which to study LEC activity. Specifically, we are designing a corneal outer ring with micropockets to simulate LEC microenvironments. We hypothesise that the micropockets will aid in tissue regeneration by providing protection for LEC.PEG-diacrylate outer rings were fabricated using photopolymerisation microstereolithography. The polymer was mixed with the photoinitiator (Camphorquinone) at 1% w:w and then irradiated with a blue laser (473 nm) at times ranging from 30 to 120 sec. Rabbit limbal fibroblasts were seeded at 1.75×105 cells/ml, incubated for 7 days and observed using fluorescence and scanning electron microscopy. We demonstrated that cells can be attached to the scaffolds and located inside the artificial niches. We also explored different methods for biofunctionalizing the basic PEG-structure e.g. using biotinylated fibronectin and we are developing a new biodegradable ring alternative.This work provides a technique for producing artificial niches for studying LEC behaviour in vitro.

21.P03
Generation of a biomimetic human cornea model using mesenchymal stem cells

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Introduction: Human Wharton Jelly Stem Cells (HWJSC) are one of the major sources of non-embryonic stem cells with high proliferation and differentiation capabilities. The aim of this study is to evaluate the ex vivo differentiation potential of HWJSC into corneal epithelium.
Methods: We first generated primary cell cultures of corneal stromal keratocytes and HWJSC from small tissue biopsies. Then, we generated a cornea stroma substitute made of cultured keratocytes immersed in fibrin-agarose gels. Finally, HWJSC were seeded on the surface of the stroma substitute using air–liquid culture technique to induce the epithelial layer differentiation. Histological and Immunofluorescence studies were performed after 7, 21, and 28 days of ex vivo culture.
Results: Histological analysis demonstrated that HWJSC were able to form a single epithelial-like cell layer at 7 days of submerged culture and two to four layers after 14 days ex vivo. The maximum level of stratification was identified after 10 days of air–liquid culture technique. Immunofluorescence analysis revealed a progressive expression cytokeratin 3/12, especially after 3 weeks of culture.
Conclusion: Our results show that HWJSC are potentially able to differentiate into corneal epithelial cells, suggesting that this could be a promising cell source for cornea regeneration.
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21.P04
Regulation of genotype and phenotype of corneal stromal cells

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Control and maintenance of keratocyte phenotype is vital to developing in vitro tissue engineered strategies for corneal repair. In this study the influence of topographical and chemical cues on mechanical, phenotypical and genotypical behaviour of adult human derived corneal stromal (AHDCS) cells in three dimensional (3D) constructs are examined. Topographical cues are provided via multiple aligned electrospun nanofiber meshes and chemical cues are examined using different media supplements. A non-destructive indentation technique and optical coherence tomography are used to determine the elastic modulus and dimensional changes, respectively. qPCR analysis revealed that the shift between keratocyte and fibroblast marker expression could be adjusted by both chemical and topographical factors. The results demonstrate

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that changing the surrounding niche from 2D (TCP) to 3D (collagen hydrogel) conditions in serum-containing media increased keratocyte marker gene expression and decreased fibroblast marker expression which was further enhanced by removal of serum, media supplements and the presence of orientated nanofibers. There was a correlation between elastic modulus, contractile characteristics and gene expression. The combination of non-destructive monitoring techniques and analysis of gene expression provide important feedback for optimizing culture condition, which has not previously been shown in 3D corneal models.

21.P05
Low-intensity ultrasound stimulation to enhance the recellularization of corneal stroma

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Ultrasound-based techniques have been widely applied for medical diagnosis. In the past years, some studies have demonstrated that ultrasound may have some effects on cells and tissue, and could thus be applied for treatment as well. Recent results evidence the beneficial aspects of applying ultrasound stimulation to biological fields, among others, to improve the cell viability, increase the blood flow, and improve tissue healing and regeneration, as well as to enhance adipose cell proliferation, metabolic activity, and differentiation of cells. In this work the effects of ultrasonic stimulation on the proliferation of corneal tissue culture are presented. A parametric study is carried out to understand the influence of several characteristics of the ultrasonic source, such as the frequency, the energy, the time period or the wave form. A bioreactor is developped to enhance the cell proliferation process, with the aim of standardizing the recellularization process of the cornea. Xenografts have been obtained both ex vivo and in vivo to determine its potential usefulness. A low-intensity ultrasonic source is applied to stimulate the cell proliferation. The pressure field has been carefully described by making use of an inmersion scanner. Statistical growth curves and proliferation ratio are obtained for different configuration of ultrasonic stimulation. The authors would like to thank the MICINN and SAS through Project grants DPI2010-17065 and PI-0308.

21.P06
Generation of a complete anterior lamellar human cornea using decellularized stromas

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Introduction: In the present study, we generated a bioengineered human cornea based on an acellular porcine corneas recellularized with human stromal and epithelial corneal cells.
Material and methods: Pig corneas were decellurarized using 1.5 M NaCl for 12 h. Once decellularized, the xenografts were washed in PBS and the excess of liquid was removed in a specific chamber with sterile 3 MM paper towels for 30 min. NaCl-decellularized corneas were recellularized with human keratocyte cell cultures. Finally, a human limbal rim explant was placed on top of the corneal construct to promote epithelial cell growing. Histological analysis of decellularized and recellularized corneas was performed.
Results: The NaCl-decellularization method properly removed the cells from the stroma, and the integrity of the collagen bundles was preserved. After the recellularization process, human keratocytes spread and grew into the acellular porcine corneas. Human limbal rim promoted the epithelial corneal cell growing, obtaining a well-developed stratified epithelium.
Conclusion: This method allowed us to generate partial human artificial corneas based on porcine xenografts, which could have potential usefulness for clinical and experimental uses.Supported by grants FIS PI11-2680 from Instituto de Salud Carlos III and SAS PI462/2010 from Junta de Andalucia, Spain.

21.P07
Comparison of the relative effects of culture conditions and cytokines on subcultured corneal keratocyte phenotype

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Corneal disease is the second-leading cause of eye disease globally with roughly 8 million people who are blind and about 284 million who are visually impaired. The current treatment for corneal disease is corneal tissue transplant and the demand for tissue exceeds supply, making a tissue engineered (TE) cornea highly desirable. In order for a TE cornea to be useful, it must be transparent which requires downregulation of light scattering alpha-smooth muscle actin (αSMA) and upregulation of the native corneal marker, aldehyde dehydrogenase 1A1 (ALDH1A1). In this study we expose the tissue to a culture environment that represents a close corneal replica to determine which signals are needed for recovery of corneal phenotype. Specifically, we use a 3D collagen matrix, cornea-specific proteoglycans decorin and biglycan, and IL-1 receptor antagonist (IL-1ra) to recover the native corneal phenotype. Both decorin and IL-1ra have been shown to reduce haze in vivo. We have shown that the 3D matrix is capable of strongly downregulating αSMA, even in the presence of the pro-inflammatory cytokine, TGF-β, and causing partial recovery of ALDH1A1. The expression of ALDH1A1 in 3D cultures also shows a time-dependent trend. We have also shown that the 3D culture environment provides a relatively stronger signal to the cells than collagen alone, TGF-β or IL-1ra in terms of α-SMA expression levels. Finally, we have determined that IL-1ra has no practical effect on subculture cell populations.

21.P08
Optimized cultivation of human corneal endothelial cells for transplantation

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Corneal transplantation is a common transplant procedure to improve visual acuity by replacing the opaque or distorted host tissue by clear healthy donor tissue. However, its clinical utility is limited due to a lack of high quality donor corneas. Bioengineered neo-corneas, created using an expandable population of human donor-derived corneal endothelial cells (HCECs), could address this shortage. Thus, the objectives of this study were to establish HCEC sourcing with various isolation methods, including enzymatic digestion, culture medium components, and ECM proteins. HCECs were isolated from discarded corneas with various aged donors after surgery. In order to improve the cell attachment, tissue culture plates were coated with adhesive proteins before cell plating. The cultured HCECs were characterized by immunofluorescence for ZO^{-1} and Na^{+}/K^{+}-ATPase. HCECs were successfully isolated from 32% (86/269) of donor corneas. HCECs were expanded to the numbers needed to re-endothelialize neo-cornel constructs, while

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expressing markers typical to HCECs. The donor age and isolation method significantly affected the HCECs culture results. Under all conditions tested, ECM protein coating promoted cell-cell interactions and proliferation of HCEC. The results indicate that the corneal endothelium can be regenerated by seeding the cultured HCECs on a proper carrier material. This study demonstrated that the bioengineered neocorneas could be a new potential source for transplantation.

21.P09
Bioengineering gelatin hydrogel for corneal endothelial cell repair

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Introduction: Corneal endothelium can be cultured in vitro, and together with appropriate scaffolding material, is transplanted inside of patient's eye to repair the dysfunctional endothelium. Gelatin is a hydrolysed product from collagen, and has been extensively used in medical field. The purpose of this study is to develop a bioengineering gelatin hydrogel for corneal endothelial cell repair.
Methods: Gelatin hydrogel was obtained by cross-linking with1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) / N-hydroxysuccinimide (NHS). Human corneal endothelial cells (hCECs) were seeded on the gelatin hydrogel. New Zealand rabbit was used for the implantation of gelatin hydrogel covered with/without hCEC.
Results: A flexible and transparent hydrogel was obtained after cross-linking gelatin film with EDC/NHS. hCECs were seeded directly on gelatin hydrogel, and tight confluent cell layer was formed on hydrogel surface after several days culture. The growth rate of hCECs on gelatin hydrogel was similar to that on the tissue culture plate. ZO^{-1} and K-Na-ATPase markers were expressed by hCEC seeded on gelatin hydrogel. Round shaped gelatin hydrogel with a 7 mm diameter was easily implanted inside of rabbit eyes with only 2–3 mm incision, and no obvious inflammation and rejection response were found.
Conclusion: The Gelatin hydrogels, seeded with hCECs can be used to create corneas for transplantation and overcome the shortage of donated corneas.

21.P10
Bioactive peptide amphiphile nanofibers for cornea regeneration

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Corneal opacification is the most frequent cause of blindness. Although keratoprosthesis and keratoplasty are the most common methods of treatment, there are some limitations in their use. Peptide amphiphile (PA) molecules offer a promising new approach for regenerative medicine studies. These molecules are biocompatible and biodegradable and can be engineered to include bioactive sequences. The bioactive groups are used for mimicking the active epitopes in the natural extracellular matrix. In addition, the nanofibers formed by self assembly of PA molecules form a network that resembles the morphological properties of the natural matrix. Here, we utilized various PA molecules in order to determine their effects on human keratocytes in in vitro conditions. The PAs were designed to contain laminin or/and fibronectin-derived epitopes. The biocompatability and bioactivity of the PAs were analyzed via cell viability and proliferation assays with Alamar Blue and BrdU assays, respectively. The adhesion and morphological properties of keratocytes on PA surface was characterized by phalloidin/To-Pro-3 staining. Our results showed that PAs enhance the viability of these cells. Moreover, PA molecules have ability to augment cell proliferation. Keratocytes can also spread on these PA networks similarly to collagen matrices. In conclusion, we established that PA nanofibers can be used as an efficient scaffold for keratocytes and thus offer a new platform for corneal tissue engineering.

21.P11
Fabrication of novel artificial corneal substitute using silk protein derived materials

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Corneal transplantation is most promising way for recovering from corneal blindness. However, donor shortage in all over the world is serious problem. Therefore, development of an artificial cornea is one of the solutions to overcome this complicated situation. Silk protein derived materials such as fibroin have been tested as an artificial cornea because of its high biocompatibility. However, perfectly reliable material has not been established yet. We focused on novel silk protein derived material such as sericin gel films (SF) and hornet cast films (HF) as a novel artificial cornea. These films have high transparency and enough mechanical strength for clinical use. Therefore, we evaluate SF and HF under various sterilized conditions. Using autoclave treated HFs, we could establish bio-safety artificial cornea. However, low permeability of film materials affected the homeostasis of host cornea adversely. To solve this problem, we tried to fabricate electrospun fiber based materials from silk derived protein. From aqueous fibroin solution, we could prepare nanofiber mat. Our biological tests revealed that corneal cells could attach not only the surface of fiber mat but also the inside of fiber mat with keeping its phenotype. This result suggests that the materials might be applicable for artificial cornea. Unfortunately, current fibrous mats have insufficient transparency. Now we are spending our efforts to increase transparency of silk proteins-based fibrous substrates.

21.P12
Biological cornea glue - in vitro evaluation

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Introduction: An efficient biological method of corneal tissue repair that replaces surgical sutures is still unmet. Transglutaminase-2 (TG2) stabilizes connective tissue by covalent isopeptide crosslinks. We hypothesize that TG2 based tissue glue will covalently bond the corneal tissue layers to replace the need of sutures for wound closure.
Materials and methods: Human cornea was tested for the presence of TG2 and FXIIIa substrate sites using histochemical assay and imaged with fluorescent microscopy, electron microscopy and second harmonic generation imaging. The ability of endogenous and exogenous TG2 in crosslinking collagen was studied on fibroblast cultured matrix using enzyme inhibitors and commercial TG2 followed by gel electrophoresis. The enzymatic adhesion in corneal tissue was studied in cadaveric porcine corneal flap wounds by delivering concentrated enzyme to the tissue interface with or without substrate additives and then mechanical testing using instron tester.
Results: Human cornea showed abundant substrate sites for TG2 and a good colocalization of its substrate sites on the matrix proteins. Both endogenous and exogenous TG2 produced collagen crosslinking in the matrix. In mechanical testing, the breaking shear stress of the corneal flap wounds closed after the delivery of the active enzyme with the substrate additives was greater than the strength of fibrin glue.

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Conclusion: Human cornea, rich in TG2 substrate sites is an ideal target for TG2 based repair.

21.P13
Corneal stromal substitute by aligned multilayer of compressed native collagen: measuring and improving corneal transparency

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Transparency is a major requirement for human cornea. Part of this is due to corneal stromal architecture, with its well-defined, orientated collagen lamellae. Here we describe a novel method to construct corneal stroma based on multilayering of collagen scaffold, with improved corneal transparency. For this it is important to define and measure the necessary optical performance, and test the hypothesis that increasing collagen layer density increases blurring. Multilayered collagen scaffolds were made using plastic compressed collagen layers of different volumes. We also developed a novel measure of image blurring due to increasing number of layers by analyzing the increase of grey-edge intensity around sharp black image lines, through corneal constructs. This blurring coefficient of optical function was correlated with layer number and total collagen content, with routine histology.Analysis of transmission and blurring coefficient clearly demonstrated that for the same total collagen content, a larger number of thinner layers significantly improved optical performance. H&E sections showed a well-developed lamella collagen structure where collagen layers were well integrated, one above the other. Compressed collagen multilayers not only produced structurally promising corneal constructs but also showed that increasing numbers of thinner collagen layers improves optical performance. Multilayering of collagen is emerging as a good process fabricating corneal stroma.

21.P14
Evaluation of light transmittance in a model of decellularized tissue for use in corneal regeneration

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Introduction: In this work, we have descellularized mice intestinal wall tissues as scaffolds for cornea engineering, and we determined the transparency levels of these decellularized tissues for use in cornea regeneration.
Methods: Mice small intestine fragments washed in PBS and subjected to one of the following decellularization agents: (1) immersion in 0.1 M SDS solution during 48 h, (2) ultraviolet irradiation (UV) for 10', (3) ultrasound exposition (US) for 10'. Optical analysis was carried out by transmittance quantification in order to determine the tissue transparency.
Results: Although the spectral behavior of all decellularized tissues was similar to the control pig cornea (higher transmittance for longer wavelengths), transmittance ranged between 33 and 77% of the incoming light. The UV treatment proved to be the most effective in preserving tissue transparency, followed by SDS and, finally, US.
Conclusion: The optical study of decellularized tissues suggests that UV exposition of the tissues could preserve more efficiently the extracellular matrix structure than US and SDS methods. We therefore suggest that the UV method should be preferentially used for cornea applications.
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21.P15
Characterization of the effect of visible electromagnetic signals on corneal fibroblasts

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Corneal opacity is a major cause of visual impairment. Although corneal transplants are very successful, LASIK surgery limits the number of viable donors. Tissue engineering has the potential to provide transparent corneas with the structural integrity to withstand transplant. We are investigating the use of visible light signals to increase the transparency of cultured rabbit corneal fibroblasts (RCFs) by influencing intracellular protein expression. Transparency requires corneal crystallins (CC) expression and low α-SMA expression, but standard cell culture induces a decrease in CC expression and an increase in α-SMA in RCFs. Since CCs are involved in the oxidative stress response, a controlled light environment may decrease α-SMA and increase CC levels. We developed a photoreactor that emits light at controlled wavelengths and intensities. RCFs seeded at a density of 10 000 cells/cm^2 in a 12.5 cm^2 culture flask were incubated in the dark, under normal light conditions, or while exposed to red (660 nm), green (570 nm) or blue (470 nm) light at discrete intensities in the range of 5–81 mW/cm^2. Cells were lysed after 8–14 days and α-SMA was found with Western Blot. Compared to normal conditions, red and blue light reduced α-SMA the most, with high intensities decreasing cell proliferation, indicating that light is an important signal in regulating protein expression. Further work will be done to confirm these effects and to examine the effects of other wavelengths.

21.P16
Towards developing a biodegradable cell carrier with nanofeatured surface topography for RPE cell transplantation

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A supportive substrate is crucial for retinal pigment epithelium(RPE) transplantation. To investigate the influence of substrate topography on RPE behavior, various fibrillar and smooth poly L-lactide/ϵ-caprolactone(PLCL) substrates were prepared by electrospinning and solvent casting, respectively. Surface topography was analyzed by SEM. Fetal human RPE(fhRPE) were seeded on substrates at 10E4 or 2 × 10E5 cells/cm^2. Cell morphology was observed by phase contrast microscopy. Growth curves were counted semiautomated with ImageJ. Acellular fibrillar and smooth membranes were implanted into rabbit subretinal space. SD-OCT images were obtained after 4, 7 and 14 days, followed by histology. It was found the average diameters of PLCL fibers were 200, 550 and 1100 nm. RPE attached on all substrates at comparable densities (5451 ± 1247 cells/cm^2), but differed in footprint with decreased spreading on rougher topography. The highest proliferation rate was determined on 200 nm fibers. Decreasing growth rates were observed with increasing fiber diameters. RPE seeded at high density contracted or detached from all groups at 3–14 days. Intraoperative handling of implantation was safe. Preliminary SD-OCT results showed increased retinal reflectivity and subretinal fluid above both implant types, suggesting inflammation. In conclusion, the

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200 nm fiber diameter topography appears to induce superior proliferation in fhRPE. Experiments now focus on PLCL modification to reduce the inflammatory responses in vivo.

21.P17
Retinal pigment epithelial cell viability and functionality on electrospun PLLA biomaterial scaffolds

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Introduction: The aim of this study was to characterise the interaction of human retinal pigment epithelial (RPE) cells with electrospun poly-L-lactide (PLLA) biomaterial scaffolds for retinal tissue engineering.
Methods and materials: Physico-chemical analysis of polymer scaffolds was performed using scanning electron microscopy, X-ray photoelectron spectroscopy and fourier transform infrared spectroscopy. Biological analysis of RPE cells was performed using biochemical assays, immunocytochemistry, flow cytometry and real time qPCR.
Results: SEM analysis revealed a sub-topography on the PLLA electrospun fibres and confirmed fibre diameter of between 1.49–2.17 μm. XPS showed surface chemical composition of the PLLA electrospun scaffolds was not altered during the electrospinning process. Biological analysis showed improved RPE cell viability and proliferation on the PLLA electrospun scaffolds over 21 days. RPE cells exhibited continued gene and protein expression of RPE65, TJP1 and RLBP1 over 21 days cell culture.
Conclusions: This study demonstrates the potential application for electrospun polymer scaffolds as a vehicle for clinical RPE cell delivery. The electrospun PLLA supported RPE cell attachment, proliferation and differentiation and maintained good cell morphology. From this study it is clear that electrospun biomaterial scaffolds could be used to mimic retinal tissues for the potential treatment of conditions like age-related macular degeneration.

21.P18
Survival of subretinally implanted human fetal and adult retinal pigment epithelium monolayers on cell carriers in rabbits

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A large-eye animal model was developed with chinchilla-bastard rabbits for cost-efficient tissue engineering studies of retinal pigment epithelial (RPE) replacement on healthy tissue. Fetal & adult human RPE cultures were grown for at least 6 weeks on biostable polyester membranes(PET); epithelial polarity was verified with transepithelial resistance above 250 Ωcm^2. Bullet-shaped implants were made with a trephine.Vitrectomy was performed in 2–2.5 kg rabbits and a small retinal detachment(RD) created. The implant was then passed through an enlarged opening of the retina with a custom-made shooter instrument. The RD over acellular implants resolved within 4-7 days, but resulted in an outer retinal atrophy without inflammation. RD alone without implant placement induced neither retinal atrophy nor inflammation.Subretinal implantation of fetal hRPE on PET in non-immunosuppressed rabbits ($n > 30$) resulted after 4 days in neural retinal edema above the RPE implant on SD-OCT and funduscopy. After 1 week, an atrophy of the retina overlying the fetal xeno-transplant was observed, remaining stable thereafter. Histology obtained 4 weeks after implantation showed an almost continuous xeno-RPE monolayer on PET. These findings were largely similar in five consecutive rabbits with adult hRPE cells. These data demonstrate successful subretinal delivery of cultured RPE on PET carriers. The xeno-RPE seems to survive over 4 weeks and may actively evade destruction through its immune privilege.

21.P19
Silk fibroin as substratum in ocular tissue engineering

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Fibroin and sericin are the main protein components of silk. While sericin was identified as an allergenic substance, the fibroin was explored as a potential biomaterial for tissue engineering applications. In the past recent years, we developed membranes and scaffolds made of silk fibroin produced by the domesticated silkmoth Bombyx mori (BMSF) and assessed them as substrata for growing successfully various types of ocular cells, including corneal limbal epithelial cells (presumed stem or progenitor cells), limbal mesenchymal stromal cells, corneal endothelial cells, and retinal pigment epithelial (RPE) cells. BMSF presents a series of characteristics (good mechanical properties, transparency, and slow biodegradability) that makes it suitable as a biomaterial for the reconstruction of ocular tissues. Our results presented here, and published so far in about a dozen of papers, indicate the potential of using BMSF as a substratum to create tissue-engineered constructs for the restoration of ocular surface, for repairing the corneal stroma, for endothelial keratoplasty procedures, and for the transplantation of RPE cells in the management of age-related macular degeneration. Currently, we are investigating the wild silkmoth (Antheraea pernyi) fibroin (APSF), which contains the RGD ligand sequence and can lead to enhanced cell attachment.

21.P20
Adhesiolysis after fractures of the orbital floor by membranes laminated with amniotic membrane

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Introduction: We have investigated the usefulness of amniotic membrane in reconstructive surgery of the orbital floor. Because of its proposed anti-adhesive qualities, amniotic membrane was used to inhibit complications such as diplopia or limitations of ocular motility, as are often caused by postoperative cicatrization.
Patients and methods: The study included eight patients (mean age 37 years, all male) with deficient ocular movement after orbital floor fracture. Five patients had been previously operated upon, whereas three had not been treated surgically. To be included in the study, time from trauma had to be at least 4 months, and MRI scans had to indicate the presence of cicatricial tissue at the fracture location. Patients were treated with surgical adhesiolysis and insertion of allogenic human amniotic membrane laminated on PDS foil functioning as the carrier material. During 3 months follow-up time, the patients were examined ophthalmologically, especially for diplopia or limited ocular bulb motility.
Results: Disorders of ocular bulb motility disappeared completely in five patients. Two patients showed improved motility and a reduction of objective and subjective symptoms. One patient showed no improvement.
Conclusion: Allogenic human amniotic membrane has anti-adhesive effects when used in reconstructions of fractures of the orbital floor.

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22. Vascular Tissue Engineering (in coop. ESAO)

22.01
Keynote: Challenges and outlook of vascular tissue engineering

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A clinical shelf-ready small-calibre vascular prosthesis is still a quest. Research in Vascular Tissue Engineering (VTE) is fast-growing and may represent a good option. Challenges range from 3D architecture, mechanical characteristics, compliance, biocompatibility, thrombogenicity, growth potential, degradation and tissue regeneration (specific cells, ECM and angiogenesis) to biologically induced long-term alterations such as occlusions, aneurysms, intimal hyperplasia and calcification. Several VTE methods have shown promising results:in vivo VTE using degradable synthetic, natural or decellularised 3D scaffolds with/without growth factors;in vitro VTE using the above scaffolds seeded with autologous or allogenic cells matured in a bioreactor; in vitro cell sheet technologies;organ printing of scaffolds with living cells. Our studies using synthetic biodegradable micro/nanofibre electrospun 3D porous polycaprolactone scaffolds have shown superior results to the clinically used ePTFE in the rat aortic replacement model at 1.5,3,6,12 and 18 months with regard to patency, compliance, endothelialisation, cell invasion, ECM, angiogenesis, intimal hyperplasia and calcification. Some of the above cell/bioreactor-based methods have already been introduced in clinical protocols but are cost and labour intensive. Therefore synthetic 3D scaffolds using the body as a bioreactor may be an easier, cheaper and clinically widely applicable method for future cardiovascular applications.

22.02
Keynote: New designs in electrospun vascular prosthetics

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Cardiovascular disease is the leading cause of death in the United States. Electrospun non-woven, seamless tubular structures have shown potential to design and fabricate bioresorbable vascular prosthetics, promoting tissue regeneration in situ, as an effective treatment for vascular trauma and disease. However, the lack of controlled porosity severely limits the degree of 3D tissue regeneration, and, more importantly, the transmural angiogenesis required in humans to develop a neointima. Thus, we have developed an air-impedance process where the solid mandrel is replaced with a porous mandrel that has pressurized air exiting the pores to impede fiber deposition. With the proper air flow rate, pore size, and pore distribution, we can fabricate a tubular structure with similar mechanical characteristics to an ideal vascular prosthetic, and porosity conducive to the desired regeneration. More specifically, we are engineering the porosity, fiber diameter, and structure composition to regulate the macrophage phenotype (M2 versus M1) interacting and residing within the prosthetic upon implantation which will be required to promote transmural angiogenesis and regeneration as well as defined degradation rates. The results of the associated design and validation will be presented in the quest to develop an ideal small diameter vascular prosthetic by air-impedance electrospinning.

22.03
Keynote: Compliant degradable electrospun nano-fibre structures for cardio vascular engineering

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Vascular bypass grafting is a one of the major treatments for ischemic heart and peripheral vascular diseases, both being main causes of mortality. Limitation in the number of preferred autograph implants creates a need for manufacturing of replacement grafts. Main challenges of the engineered vascular grafts are to create structure which is highly porous to ensure in-graft cells growth, impermeable (blood leakage), mechanically matching native tissues and promotes fast endothelialization. Larger diameter grafts are commonly manufactured using non-biodegradable materials but are plagued by high thrombogenicity due to lack endothelium layer inside the graft. This makes them less desirable in case of smaller diameter (<5 mm) vascular grafts.As a promising technique to create small diameter vascular grafts is electrospinning allowing to adjust mechanical properties of the grafts, as well as creation of high porosity with high surface area/volume structures – through this simulating the structure and dimensions of the native extracellular matrix. Using biodegradable materials gives also clear advantage over synthetic materials.Recently a precise electrospinning technique has been developed in our lab allowing producing multilayered nanofibrous structures with controlled porosity and tailored mechanical properties. Obtained structures closely mimics structure of the extracellular matrix of the native vessels and may promote particular cells growth matching natural one.

22.04
Keynote: Acellular non-degradable nano-scaffolds

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Tissue repair with synthetic matrix grafts that replicate the architecture of the vasculature at the nanoscale is currently one of the most auspicious approaches to overcome the limitations of conventional, small-bore vascular prostheses. Using the technique of electrospinning and with the selection of appropriate elastomeric biomaterials, compliant grafts with mechanical properties resembling decellularized matrix grafts can be created, which benefit cellular reconstitution by the host. In previous studies we focused our work on the development of low-porosity, polyurethane fine mesh conduits which reveal a minimum of biomechanical mismatch to the host artery. However, as reported by other groups low porosity grafts show limitations in cell infiltration and cell viability. In order to reduce these limitations and to benefit long-term host cell survival, we fabricated grafts with increased porosity and increased pore sizes, which would aid in cell infiltration and nutrient transport. The maximum of porosity and pore size have been tuned in dependency of sufficient tensile strength and suture resistance. Coarse

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mesh grafts improved cell repopulation and long-term cell survival especially in the inner sections of the graft wall. This template should allow the fabrication of biodegradable scaffold-candidates with essential prerequisites (cellular ingrowth and function) for successful natural host vessel reconstitution.

22.05
Keynote: Differentiating between transanastomotic and trans-mural endothelialisation: the 'isolation-loop-graft' model

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Introduction: Vascular graft models have yet to distinguish between transanastomotic and transmural endothelialisation. We describe an isolation loop-graft model that clearly separates these distinctly different events.
Methods: High-porosity polyurethane (PU) grafts (ID1.7 mm;150 μm pore) were interposed 'welded' between low-porosity ePTFE (ID1.7 mm; IND 15–25 μm) and implanted in the abdominal aorta of Wistar rats for 6,8,12 and 24 weeks (n = 8/time point). Looping the graft increased their length to 8 cm. Analysis by light, immune-fluorescence (CD31) and scanning electron microscopy.
Results: The transanastomotic outgrowth edge was clearly defined never traversing the endothelial-free isolation-zone (24.72 ± 9.85 mm at 6 weeks and 8.21 ± 4.87 mm at 24 weeks) separating it from transmural midgraft endothelium. Transanastomotic endothelial outgrowth slowed down between week 2 and 24 (from 920 ± 420 μm to 457 ± 128 μm/week proximally [p = 0.034] and 650 ± 240 μm to 501 ± 359 μm distally [NS]). Transmural midgraft endothelialization reached pre-confluence (55 ± 45%) at 6 weeks and 100% confluence between week 12 and 24. There was significant regression of neo-intimal tissue between 6 and 24 weeks (from 70.57 ± 75.44 μm to 51.14 ± 27.50 μm; p = 0.0269).
Conclusion: Transmural endothelialisation can be clearly distinguished from transanastomotic outgrowth in a high throughput rat model. A looped interposition-graft model provides sufficient isolation-length to separate the two events for up to half a year.

22.P01
Creating a functional vascular tunica media: adding transforming growth factor beta one to vascular smooth muscle cells in deep microchannels

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Transformation of vascular smooth muscle cells (vSMC) phenotype from synthetic to contractile at the correct development stage is crucial for producing a functional tunica media.The vSMCs go through cytoskeletal restructuring and line-up in the direction of micropatterns, triggering a phenotype switch from synthetic to contractile at confluence. Transforming growth factor beta one (TGF-ß1) is known to promote differentiation of vascular smooth muscle cells towards a contractile phenotype. The combined effects of micropatterning and TGF-ß1 on vSMC culture have not been studied. We examined the combined effect of a microchanneled scaffold and TGF-ß1 on the contractile phenotype of vSMCs. We also investigated whether temporal addition of TGF-ß1 before or after cell confluence affects vSMC culture in micropatterned scaffold.Culture of vSMCs in a microchanneled PUR scaffold with TGF-ß1 addition was found to align vSMCs parallel to microchannels and increase contractile protein expression thereby upregulating the contractile phenotype of vSMCs. Contractile phenotype upregulation was maximal when the TGF-ß1 was added to the micropattern-vSMC culture before cell confluence was achieved.This novel synergistic effect of culturing vSMCs in a microchanneled scaffold combined with TGF-ß1 addition before confluence to maximize vSMC contractile phenotype might be fully exploited to create a functional tunica media for engineering a small-diameter blood vessel replacement.

22.P02
Novel approach of engineered artery transplantation

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Degenerative vascular diseases represent the first cause of death in Western Countries and the present surgical techniques, based on the implant of prostheses, tend to loose efficacy with time. The aim of this project was to create a functional and not immunogenic engineered vascular graft for small diameter artery substitution. Working on a swine model, we tested different sources of endothelial and mesenchymal cells (bone marrow, blood, vein, skin) to identify the one that best fitted our aims. We tested various methods to extract (conditions for enzymatic digestion), isolate (immunomagnetic separation or immunofluorimetric assays) and expand these cells. Once characterized and expanded, these cells were seeded on a decellularized artery (used as a scaffold) to recostituite the tunica media and the endothelial layer. Implantation of cells was then evaluated with immune-istological assays. The graft built in this way was finally transplanted in a swine model. After fifteen days, the graft was explanted and studied. The transplantation of the pilot graft was successful, and the results of this experiment suggested the feasibility of this approach and the need for further studies to clarify the mechanisms of acellular vascular prosthesis repopulation. Although many parameters must be still defined, we are presently working to improve the technique described above with the final aim to rapidly translate this research project from basic research into clinical practice.

22.P03
Nano and microtopography enhance differentiation of human mesenchymal stem cell into endothelial-like cells for vascular repair

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A small population of circulating cells termed endothelial progenitor cells (EPC) has been hypothesized to be a potential therapeutic for vascular repair to its vascular healing properties. However, its use is limited by the difficulties in isolating sufficiently large numbers of EPC required for the desired therapeutic effect. The derivation of vascular healing cells from alternative source of adult stem cells would therefore

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be in demand. Hence, in this study, the induction of human mesenchymal stem cells (hMSC) into EPC for vascular repair was studied. The hMSC were induced to differentiate in vitro into EPC through the provision of biochemical cue, vascular endothelial growth factor (VEGF), and/or topographical features in the micro- and nanometer scale. Preliminary study via immunofluorescence staining of CD133, CD29, VEGFR2, and CD34 suggested anisotropic microtopography such as 1 μm pillar enhanced the differentiation process, while isotropic pattern such as 2 μm line impeded differentiation. Flow cytometry analysis of the CD133 and CD29 marker expression suggested that the provision of both topographical and biochemical cues could synergistically induce the in vitro differentiation of hMSC into EPC.In conclusion, these findings have helped gained a better understanding of the possible favorable conditions surrounding the induction of hMSC differentiation into EPC with the provision of topographical and biochemical cues.

22.P04
Poly lactic acid based scaffolds as graft for small-diameter arterial replacement

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Vascular Tissue engineering (VTE) has emerged as a promising approach to develop blood vessel substitutes. Investigators have explored the use of arterial tissue cells combined with various types of natural and synthetic scaffolds to make tubular constructs in order to develop a functional small-diameter arterial replacement graft. The grafts must mimic the unique viscoelastic nature of an artery and be non-disruptive to blood ?ow. Moreover, after implantation, the scaffold must be gradually populated by cells and replaced by extra cellular matrix; with this respect, it is crucial that this replacement takes place with a well-defined timescale. In this work tubular scaffolds for VTE were produced via Diffusion Induced Phase Separation. Several PLA/PLLA blends (100/0, 90/10, 75/25 wt/wt) were utilized in order to tune the crystallinity of the scaffolds and thus the rate of biodegradation. In-Vitro biological tests were carried out in order to estimate the non-cytotoxicity of the scaffolds and endothelial cell proliferation inside them. The results showed that the scaffolds present an open structure across the thickness of their walls, a high level of porosity (over 70%) and a homogeneous internal surface with micropores 1–2 μm large. Biological tests showed that scaffold do not induce cell toxicity; cells are able to grow and proliferate into the scaffold covering its internal surface, so they can be considered suitable for the application for the designed aimed.

22.P06
Functional tissue engineering of small diameter vascular graft using circulating sheep endothelial and smooth muscle progenitor cells

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Biodegradable polymers are good scaffold material for vascular tissue engineering. Since many of these polymers have poor cell adhesion properties, biodegradable scaffolds may be modified with extra cellular matrix (ECM) proteins to get good cell adhesion and survival. The cues in ECM may influence the maintenance of cells in the optimum phenotype. Fully functional endothelium and smooth muscle layers are critical for the patency of vascular grafts. Most desirably, ECs should be antithrombotic and SMCs contractile for proper tissue function. We did functional tissue engineering to construct small diameter vascular graft (SDVG) using fibrin-coated PCL and circulating sheep progenitor cells grown under dynamic flow condition. Cells in the constructs were analyzed by scanning electron microscopy after in vitro culture. The EC on the luminal surface got aligned to the direction of flow under shear stress. The nitric oxide (NO) released into the medium indicating functional EC phenotype. The hemocompatibility of EC-seeded lumen was tested by perfusion of human platelet rich plasma (PRP). The results were promising with no platelet adhesion to EC monolayer. On the outer graft surface, smooth muscle cells aligned across the direction of flow and indicated contractile phenotype. But with increased walll thickness SMC also aligned towards the direction of flow. Prolonged culture of TEVG was found to induce ECM synthesis suggesting generation of media-like layer of blood vessel.

22.P07
Engineering chitosan using sebacic acid: A novel wound dressing material

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Introduction: Transformation of biopolymers to biomaterial faces challenges like solubility, stabilizers, strength, biocompatibility, thermal stability. Though the biomaterials prepared till date may possess various properties, biocompatibility property has not yet achieved. In the present study, an attempt was made on to engineer chitosan using sebacic acid (SA) and evaluated its potential as wound dressing material in animal open wound models.
Materials and Methods: Chitosan was mixed with SA in water and the resultant homogenous solution was transformed to 3D scaffold and subjected to test as wound dressing material in animal models. Wound contraction, collagen synthesis, histological examination of granulation tissue with respect to days was the experimental parameters studied.
Results: Wound contraction and complete healing of wound was observed within 16 days for the SA engineered chitosan (SAEC) compared to chitosan alone (19 days) and Control (22 days). H& E staining of granulation tissue displayed recruitment of neutrophils during initial phase in the experimental groups. Further, the quantity of collagen deposition during remodeling phase substantiates the early healing and could be reasoned to the healing environment provided by the SAEC. Examinations on tensile strength of the healed skin suggested, the skin gained its original tensile properties.
Conclusion: SAEC acts as a potential biocompatible wound dressing material and tissue engineering research.

22.P08
Genipin cross-linked poly(epsilon-caprolactone)/collagen scaffolds for vascular graft applications

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Introduction: Glutaraldehyde (GA) used for cross-linking has a major disadvantage due to its toxicity upon scaffold's degradation. Genipin (GN) is an effective cross-linker that exhibits significantly less cytotoxicity than GA. The purpose of this study was to compare vascular scaffolds cross-linked with GA and GN.
Methods: Electrospun scaffolds, fabricated from poly (epsilon-caprolactone) (PCL)/collagen, were cross-linked with GA and GN. Bi-layered scaffold cross-linked with GN was also fabricated. MS-1 was seeded on the denser side of the bi-layered scaffold (fiber diameter 0.30 μm) and 10T1/2 on the porous side (fiber diameter 2.80 μm). The penetration of 10T1/2 was measured over a 3-week period.
Results: The mechanical properties of the scaffolds cross-linked with GA or GN were both stable for a month, without a significant decrease in modulus. Compared with GA, scaffolds cross-linked with GN displayed higher tensile stress and burst pressure. MS-1 and 10T1/2 cells

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seeded on both types of scaffolds, showed better proliferating on GN cross-linked scaffold. MS-1 seeded on one side of the scaffold, significantly enhanced 10T1/2 penetration into the scaffold, when compared scaffolds seeded without MS-1.
Conclusion: These results indicated that GN is a promising and improved cross-linking chemical for fabrication of composite vascular graft.

22.P09
An entirely biological tissue-engineered human blood vessel available off-the-shelf

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There is presently a lack of available synthetic small diameter vascular graft. Therefore, we created a self-assembly tissue-engineered vessel from human dermal fibroblasts. Briefly, fibroblasts were cultured with ascorbic acid to induce the secretion of collagen and the ensuing formation of cellular sheets. The sheets were rolled around a 1.5 mm mandrel and allowed to fuse during a maturation phase. The vessels were decellularised in deionized water with no other agent and the decellularised fibroblast vessels (DFV) were then conserved at 4 °C. We assessed the mechanical properties including the burst pressure. The human DFVs were implanted as infrarenal aortic interpositional grafts in Sprague-Dawley rats over a 4-month period. The DFVs had a burst pressure of 2257 ± 325 mm Hg, a failure strain of 90 ± 12%, a Young's modulus of 1.3 ± 0.2 MPa, and an ultimate tensile strength of 0.7 ± 0.1 MPa. Interestingly, the properties were maintained following conservation of the graft at 4 °C for 4 months. Grafted rats did not show any signs of ill health and the grafts were still functional 4 months post-implantation. Histological analysis revealed that DFVs supported the growth of an endothelium and a media. We believe that the simplicity of such a graft and the low immunogenic properties of fibroblasts allowed human DFVs to be implanted in rats without immunosuppression. A conservative estimate suggests that a single skin biopsy could be sufficient to produce thousands of DFVs.

22.P10
Three-dimensional collagen/elastin scaffolds for vascular tissue engineering: influence of static and dynamic conditions on bio-mechanical performances

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Introduction: Due to the ideal biological properties, the use of natural materials for vascular tissue engineering applications represents the gold standard.We present a collagen/elastin three-dimensional scaffold, with ideal biological properties, able to maintain the contractile phenotype of smooth muscle cells. Moreover, the bio-mechanical performances have been improved by mechanical stimulation in a bioreactor.
Materials and methods: Collagen type I was mixed with a suspension of water soluble elastin obtained by recombinant DNA technology. Murine myoblasts (C2C12) and human aortic smooth cells (HAEC) were added to the collagen/elastin matrix and the suspension was poured in a cylindrical mold.A bioreactor (Bose Corp, USA) was used to set up the shear stress. Proteins expression involved in cells proliferation, differentiation and extracellular matrix remodeling was investigated. The Instron 5564 testing Instruments (Instron Corporation, USA) was used for mechanical tests.
Results: Cells seeded into the 3D scaffold were constantly viable.A myogenic differentiation (Myf5, Myogenin and MHC) was enhanced in presence of elastin. Moreover, the dynamic environment considerably improved the mechanical properties of the scaffold showing a significant ECM remodeling.
Conclusions: Collagen/elastin 3D scaffolds are a viable way to recreate the structure of native vessels, able to maintain the cellular functions and remodel the ECM structure when in presence of a dynamic environment

22.P11
Application of topography to enhance endothelialization in small diameter vascular graft of poly(vinyl alcohol)

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The occlusion of peripheral arteries often leads to ischemia in the distal limb, thus requiring arterial replacement. However, bypass of small diameter (<6 mm) arteries is hindered by thrombogenicity and low patency of synthetic grafts. Topography is known to improve adhesion and proliferation of endothelial cells in vitro. Thus, it is hypothesized that topography will aid endothelialization of synthetic grafts and tissue remodeling, improving functionality and long term patency. Poly vinyl alcohol (PVA) is a biocompatible and nonthrombogenic water soluble polymer. Preliminary study demonstrated good mechanical properties and short-term patency of PVA in the rat aorta, though without endothelialization. In our study, we aim to create a small diameter PVA scaffold with topography to improve in situ endothelialization and patency.PVA crosslinked with sodium trimetaphosphate and NaOH was patterned with micro (2 μm width, gap, height) and nano (250 nm) gratings through solvent casting. When tested with human umbilical vein endothelial cells, enhanced cell adhesion and higher cell density were observed on micro- and nanopatterned PVA scaffolds compared to unpatterned PVA. Also cells on patterned PVA appeared to be more spread, which is a potential precursor of a confluent endothelial monolayer. Patterning was also done on PVA to create a tubular scaffold which show promise as a small diameter vascular graft. In vivo studies must be done to verify its efficacy and long-term patency.

22.P12
Initial wash-out of antimicrobial cocktails from acellular vascular grafts

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Graft infections are a severe complication of vascular surgery with significant morbidity and mortality. To minimise infection risk, surgeons may soak synthetic conduits in an antimicrobial (AM) cocktail prior to implantation. We have developed acellular porcine arterial conduits with a view to future clinical translation. The aim of this study was to load Dacron (synthetic material) and the acellular vascular conduit with clinically relevant antimicrobial (AM) cocktails including rifampicin/tobramycin, vancomycin/levofloxacin, rifampicin/levofloxacin and investigate the elution of AM from the materials. Conduit segments

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(1 cm) were submerged in 1 mg ml^{-1} of AM cocktail for 5, 15 or 30 min. Following loading, the segments were washed thrice in saline. Antibiotics remaining in the cocktail after loading and in each wash were quantified using high performance liquid chromatography. Disks of each material after loading and after each wash were tested using the disk diffusion assay with *Staphylococcus epidermidis* and *Escherichia coli* over 3 days to determine the antimicrobial activity remaining in the disks. Small quantities of antibiotics were washed out (less than 1 μg at each wash) with the majority retained in the materials. There was a slight difference the AM agents released when comparing different loading times, however this was not significant. Future studies will investigate the longer term elution from and antimicrobial activity of AM from vascular material.

22.P13
Ester based degradable thermoplastic polyurethanes for electrospun vascular prostheses

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Thermoplastic polyurethanes (TPU) are widely used in a variety of medical applications including dialysis tubing and pace maker casings. Good biocompatibility and elastomeric mechanical properties make TPUs a viable replacement for plastics currently used as vascular grafts (ePTFE, PET). Use of ester based chain extenders provides TPUs with sites for biodegradation, a potentially useful feature when rate of decomposition can be matched to that of tissue regeneration. A series of aliphatic and cycloaliphatic isocyantaes have been polymerized with ester based chain extenders to give TPUs with mechanical performance (modulus, tensile strength, percent elongation) comparable to that of native blood vessels. Rate of degradation was approximated at elevated temperature and found to be slower for the cycloaliphatic based TPUs. Degradation products were synthesized and tested in assays with HUVECs. Materials with promising performance were electrospun and collected as narrow bore tubes. Electrospun prostheses were implanted into rats to replace the infrarenal aorta. The grafts were explanted after six months and found to be compliant in all cases. Good endothelialization indicates that slow biodegradation may be a useful feature for vascular graft materials.

22.P14
Biologically modified bilayered antimicrobial polycaprolactone scaffolds for small diameter vascular graft applications

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Biodegradable polymers have been investigated in vascular tissue engineering as scaffolds with tailored mechanical properties, degradation kinetics, optimum porosity supporting cell growth and tissue regeneration capacity enabling interaction with the host tissue facilitating healing process withstanding infection. We have investigated the feasibility of silver nanoparticle incorporated poly(ϵ-caprolactone) scaffolds as small diameter vascular grafts. Bi-layered tubular scaffolds of 4 mm diameter were fabricated by solvent casting of polymer-porogen solution and particulate leaching on the lumen side followed by electrospinning on the outer surface. Porosity characteristics of the scaffolds were quantitatively analyzed using microcomputed tomography technique and optimized for tissue integration. The morphological, antimicrobial and mechanical properties of the developed Ag-PCL scaffolds were also evaluated. The ability of biologically modified SNP-PCL to improve tissue integration was demonstrated by growing sheep smooth muscle cells and fibroblasts. The mechanical property of the antimicrobial tissue engineered scaffold was found to be superior when evaluated using Universal Testing Machine (UTM).

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22.P15
Influence of fiber diameter and surface roughness of electrospun vascular grafts on blood activation

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Electrospun grafts are widely investigated for vascular graft replacement due to their ease and compatibility with many natural and synthetic polymers. Here, the effect of processing parameters on scaffold's architecture and subsequent blood reactions blood triggered by contacting these topographies were studied. Degrapol electrospun fibrous scaffolds were characterized with regard to fiber diameter, pore area and scaffold roughness. The study showed that electrospinning parameters greatly affect fiber diameter together with pore dimension and overall scaffold roughness. Coagulation cascade activation, early platelet adhesion and activation were analyzed after two hours exposure of blood to the biomaterials. Blood reactions were observed to be dependent on the fiber diameter and scaffold roughness. Scaffolds composed of thin fibers (diameter <1 μm) triggered very low coagulation and almost no platelet adhered. On the other hand, scaffold with bigger fiber diameter (2–3 μm) triggered higher thrombin formation and more platelets adhered. The highest platelet adhesion and activations rates as well as coagulation cascade activation were found for the scaffolds with the biggest fiber diameter (5 μm).These findings might indicate that electrospun grafts with small fiber diameter (<1 μm) could perform better with a reduced early thrombogenicity.

22.P16
Evaluation of the interaction between decellularized artery structures and cell behavior

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Vascular grafting is a common surgical procedure, which has problems such as deficiency in supply and lack of blood compatibility. So, the development of more riskless grafts is eagerly anticipated. As a candidate of the new graft material, decellularized arteries were widely investigated. In this study, we focused on the interaction between vascular structure and cell infiltration. The component ratio of collagen and elastin become varied the position of artery. To investigate the interaction between the structure difference and cell interaction, we prepared decellularized arteries (aorta, carotid artery and radial artery) and evaluated by in vitro and in vivo experience test. The fibroblast seeded on the decellularized arteries infiltrated into the radial artery

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after 24 h, but the cells seeded on the carotid artery and aorta didn't infiltrate the vessels. The endothelial cells seeded on the intima side of the artery, the cells were covered the artery surface after 24 h, meanwhile the cells seeded on the adventitia side of the arteries attached but did not spread. In vivo behavior of the cells followed a similar pattern of in vitro experiment. These results indicated that the elastic lamina of the arteries obstructed cell infiltration, and the artery surface side was important to the cell behavior. This knowledge appears to be a promising contribution with regard to prepare decellularized vascular grafts.

22.P17
Electrospinning fibers with adjustable orientation

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Introduction: Conventional electrospinning setups suffer from random fiber deposition. Controllable fiber orientation is particularly of interest for tissue engineering to mimic the mechanical and dimensional properties of the natural extracellular matrix.
Materials and Methods: A basic electrospinning setup was upgraded by two rotatable plate-like auxiliary electrodes, operated with adjustable high voltage potential in order to generate a gradient electric field. Polyurethane was electrospun on a rotating conductive aluminum mandrel and fiber deposition was investigated at various speeds, with and without the use of auxiliary electrodes.
Results: By introducing the auxiliary gradient field it was possible to diminish jet instabilities and improve fiber alignment. At collecting speeds of v = 3.6 m/s ninety-five percent of the fibers had a fiber deviation of less than 1° in comparison to 60° at the same speed without the use of auxiliary electrodes. The electrospinning jet was constrained in a focusable plane between the auxiliary electrodes also facilitating a fiber orientation perpendicular to the rotational direction of the target. Nanoscopical alignment and straightness was demonstrated with rotating targets at 4.5 m/s.
Conclusions: This technique offers considerable improvement of fiber placement and alignment. The bending instability, one shortcoming of electrospinning's controllability, could be substantially diminished.

22.P18
Electrospun vascular scaffold for engineering cellularized small diameter blood vessel

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Vascular tissue engineering strategies have been successfully employed to create functional small-diameter tubular grafts by combining cells with a scaffold material. Addition of vascular cells, such as endothelial cells, onto scaffolds has been shown to maintain patency of implanted grafts. While it is evident that cells play a major role in achieving patent vessels, vascular scaffolds, which serve as cell carriers and provide structural support, contribute to the ultimate success of engineered grafts. We previously have shown that an electrospun vascular scaffold, composed of a poly (ϵ-caprolactone) (PCL)/collagen is able to withstand physiological vascular conditions. In this study we examined whether fully cellularized vascular grafts could be created. Smooth muscle (SMC) and endothelial cells (EC) were seeded at various concentrations on the exterior and internal surfaces of vascular graft, respectively. The cell seeded vascular scaffolds were preconditioned in a programmed perfusion bioreactor under various flow conditions for up to 14 days. SMC seeding at a concentration of 10^8 cells/ml resulted in a robust, confluent multicellular layer on the outer surface of scaffold. Uniform coverage of EC was achieved when the cells were seeded multiple times with the rotation of vascular scaffold. These results show that our system is capable of generating fully cellularized vascular constructs for potential in-vivo applications.

22.P19
Selective grafting of amines by microwave plasma on PVA scaffolds promotes cell growth

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Synthetic grafts are successfully used to replace large-diameter damaged blood vessels. However, small-caliber substitutes are still needed, mainly due to the lack of lumen endothelialization. We developed a PVA hydrogel with mechanical properties close to those of arterial vessels, but this scaffold did not support cell growth. The purpose of the study was to demonstrate the efficiency of amine grafting by nitrogen plasma to promote cell colonization on PVA. PVA substrates were prepared by chemical crosslinking with sodium trimetaphosphate, using a casting process. Resulting dried films were subjected to a H2/N2 microwave plasma treatment for 5 min. Surface modifications were characterized with contact angle, XPS and AFM analyses. Human endothelial cells (EAhy.926) viability on films were investigated using a calcein assay and assessed with fluorescence microscopy.Surface analyses evidenced amines on the plasma-modified films and an increase in wettability. AFM results indicated no etching nor changes in roughness. Few cells were observed on PVA films and they did not proliferate. On the contrary, higher cell densities were observed on treated PVA. Moreover, cells on PVA remained discoid while cells cultured on treated PVA were highly spread.Amine functionalization of PVA substrates was efficiently achieved using a nitrogen plasma treatment and resulting films allowed successful cell culture.

22.P20
Non-contact measurement of blood vessel stiffness and its utility in vascular tissue engineering

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Introduction: Monitoring mechanical properties is one of the yet unresolved problems of vascular tissue engineering. We implemented and experimentally verified a non-contact method to determine vessel stiffness during perfusion.
Materials and Methods: A dynamic signal analyzer (DSA) generated filtered white noise (0–200 Hz) to drive a linear motor which in turn actuated the perfusion compartment of a vessel chamber via a gas-tight syringe. Vibrations of native or decellularized human umbilical veins (HUV, 6 cm in length) and the effective motor movements were recorded by triangular lasers and Fourier-analyzed by the DSA. Mode shapes were established, and vessel stiffness was monitored during proteolysis for 120 min.

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Results: HUV showed one or two natural frequencies (NF) in the range of 5–50 Hz. Modal analysis indicated that signal amplitudes are best measured at approx. 20% of the length, although NF determination was independent of the location of measurement. NF of HUV decreased over time during collagenase (-35%) or elastase (-45%) treatment, but remained stable in the absence of proteolytic enzymes. NF of decellularized HUV decreased more rapidly initially due to a quicker onset of matrix degradation.
Conclusions: NF can be utilized to monitor vessel stiffness in a non-destructive way. The required equipment is small and robust enough to be integrated into perfusion bioreactors. This will facilitate engineering vessels with specific mechanical properties.

22.P21
Healing characteristics of nanostructured, bioresorbable vascular substitutes

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The success of novel approaches using biodegradable materials for small diameter vessel reconstruction is often limited by insufficient biomechanical properties and by host immunological reactions. We investigated a new, biodegradable thermoplastic polyurethane for application as graft material. Grafts have been manufactured considering pore size and porosity optimization to facilitate vascular specific host cell infiltration and survival. After evaluation of biocompatibility in-vitro (HUVEC seeding, XTT assay) we evaluated short (1 week) and long term (1, 6, 12 months) performance of these conduits in-vivo in a rat model and compared it with ePTFE controls. Retrieved specimens were analyzed by biomechanical analysis and various histological techniques. ETPU grafts showed significantly higher endothelial cell attachment and proliferation than ePTFE grafts ($p < 0.01$) in-vitro.At all time points eTPU prostheses revealed significantly higher numbers of vascular specific cells in the conduit wall ($p < 0.01$.All eTPU grafts stayed patent without evidence of aneurysms or clots. Degradable grafts showed wall sections with complete remodeling. Tensile strength of eTPU grafts showed a decrease within 12 months but stayed within the physiological biomechanical values of host vessels. Degradable eTPU grafts reveal good long term performance. Biomechanical properties of the grafts seem to be sufficient to guarantee safe temporary vascular replacement during the remodeling period of the prostheses.

22.P22
Polymer blends for vascular tissue engineering

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Blending has been widely used to control the properties of polymers. Poly(L-lactic acid) (PLLA) is a FDA approved polymer used in many tissue engineering applications, such as bioresorbable stents. Previous clinical trials showed that PLLA stents have a bioresorption time of around 24 months and lose radial strength after 3–6 months depending on stent design. Blending PLLA with a suitable polymer could improve the material mechanical properties and increase its bioresorption rate. Blends between PLLA (Mw 258.7 kDa) and poly (DL-lactide-co-glicolide) (PLGA; LA: GA 75:25, Mw: 76–115 kDa) were prepared at various compositions and characterized for their physicochemical properties. PLLA/PLGA 75/25 wt/wt. blend was selected due to its superior mechanical properties. The physicochemical properties of PLLA/PLGA 75/25 blend compatibilised with either 1–3% polycaprolactone (PCL), PCL-co-PLA or PGA-co-PLA were compared. Compatibilised blends generally showed a more ductile mechanical behavior and a higher compatibility between the phases as compared to the uncompatibilised blend. The blend compatibilised with 2%PCL was selected for its superior mechanical properties. Tacrolimus was incorporated into the blend and in vitro release tests were performed. The effect of drug release on in vitro endothelial cell proliferation was studied. The blend is a promising material for drug eluting bioresorbable stents.NANOSTENT (Regione Piemonte Project) and FIRB2010 projects are acknowledged.

22.P23
Rapid host remodeling transforms synthetic grafts into arteries in situ

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Host remodeling is important for the success of medical implants including vascular substitutes. Synthetic and tissue-engineered grafts have yet to show clinical effectiveness in arteries smaller than 5 mm. We designed a synthetic vascular graft that degrades rapidly to yield neo-arteries nearly free of foreign materials 3 months after interposition grafting in rat abdominal aorta. This design focuses on enabling rapid host remodeling. The synthetic graft has a highly porous tubular core made of poly glycerol sebacate and a thin fibrous sheath made of polycaprolactone . We coated the grafts with heparin and implanted them directly without cell seeding and culture. Three months post-implantation, the neo-arteries resemble native arteries in the following aspects: regular, strong and synchronous pulsation, a confluent endothelium and contractile smooth muscle layers, co-expression of elastin, collagen and glycosaminoglycan, and tough and compliant mechanical properties. This approach represents a philosophical shift from the prevailing focus on cells in vascular tissue engineering, and may impact regenerative medicine in general.

22.P24
Influence of cyclic strain on endothelial colony forming cells; relevance for in-situ vascular tissue engineering

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In situ vascular tissue engineering aims at capturing cells that can differentiate inside a scaffold into vascular phenotypes. Endothelial Colony Forming Cells (ECFCs) express endothelial markers but, under the influence of TGFβ1, they can transdifferentiate into smooth muscle-like cells. Here we test whether ECFC transdifferentiation can also be achieved via cyclic strain.Human ECFCs were conditioned for 5 days on FlexCell plates with uniaxial cyclic strain (5%, 1 Hz). Control groups were cultured statically in the presence or absence of TGFβ1. Cell fate was assessed by analyzing CD31, αSMA, cytoskeleton organization, and collagen production. Strained ECFCs showed a typical strain-avoidance behavior by developing a well organized cytoskeleton aligned orthogonally to the strain direction. Collagen type III was organized in thick bundles parallel to strain direction, while static controls showed randomly oriented fibers. Collagen type IV was similarly distributed on the substrate for each group.In all the samples, ECFCs expressed CD31 at cell membrane and perinuclear unorganized αSMA. Flow cytometry assays showed no differences in the expression of these markers between groups. These preliminary results indicate that ECFCs

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are mechanosensitive but transdifferentiation was not achieved. Future studies should determine the possibility to induce ECFC transdifferentiation by extending the conditioning time or by combining mechanical and biochemical cues.

22.P25
Rat peritoneal cavity 'bioreactors' and hyaluronan modified scaffolds to grow vascular replacements

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Strategies to overcome inherently poor elastic matrix synthesis by adult smooth muscle cells are critical to engineer functional small-diameter vascular grafts. Thus, we aimed to recruit young, autologous cells within peritoneal cavity 'bioreactors.' We determined the role of scaffold composition and elastogenic hyaluronan oligomer (HA-o)–functionalization on recruited cell phenotype and matrix synthesis. Conduits were electrospun from solutions of 22% w/v poly(ϵ-caprolactone) (PCL) and (75/25) PCL/collagen blend. They were incubated with or without HA-o ($n = 6$), inserted into a pouch and then rat peritoneal cavities for 14, 28, or 42 days, and removed for analysis (e.g. TEM, PCR) or intra-aortal grafting. Recruited peritoneal cells produced highly cellular, HA-rich fibrous capsules. Cells infiltrated within PCL conduits, with higher infiltration ($p < 0.001$) and total cellularity ($p = 0.08$) for the largest fiber diameters conduits (2.1 ± 0.8 vs. 1.0 ± 0.5 μm). Macrophages, α-smooth muscle actin expressing fibroblastic cells, and an outer layer of mesothelial cells were present. Cellular collagen synthesis after 14 day was higher ($p = 0.015$) on the PCL vs. blended conduits, but elastin deposition was limited. HA-o modification provided a noticeable but insignificant ($p = 0.39$) decrease in cellularity at 14 day, though matrix generation was unaffected. Remodeling after aortal grafting was demonstrated. We expect more pronounced changes over longer implantation times, which we are now investigating.

22.P26
Possibility of cell therapy for aortic aneurysm

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Aim: The pathogenesis of aortic aneurysm (AA) is degradation of extracellular matrix with increased matrix metalloproteinase (MMP) and loss of structural integrity of the aortic wall. We have reported that implantation of 'cell sheet' composed of bone marrow derived mesenchymal stem cell (BM-MSC) which have immunosuppressive, anti-inflammatory and tissue repair properties, inhibits AA progression in mice (J Vasc Surg 2012;54:1743). In this study, we investigated the effect of adipocyte derived mesenchymal stem cell (AD-MSC) and dermal fibroblast (D-FB) for treatment of AA in comparison with BM-MSC.
Methods: BM-MSC, AD-MSC, D-FB and aortic tissue were obtained from 6–8 weeks old C57BL/6J mice. These cells are investigated cell surface markers and pluripotency. We established ex vivo AA model by aortic tissue culture. The aortic tissue was co-cultured with BM-MSC, AD-MSC or D-FB and measured the content of elastin and expression of MMP-2, -9 in aortic wall.
Results: BM-MSC and AD-MSC were positive for Sca-1, CD44, CD73, CD106 and negative for CD11b, CD29, CD31, CD34, CD45, CD117, and they have pluripotency. However, D-FB was negative for CD73, and it dose not have pluripotency. In an ex vivo AA model showed that aortic tissue was significantly preserved elastin and decreased MMP-2 by co-cultured with BM-MSC, AD-MSC or D-FB.
Conclusion: Our results suggested that AD-MSC and D-FB, which can be obtained easier than BM-MSC, might be alternative cell sources for treatment of AA.

22.P27
Endothelial differentiation of human dermal fibroblasts

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Vascular tissue engineering strives for the production of functional grafts for clinical use. The addition of a cellular component will aid in obtaining functional constructs. Using adult stem cells for this purpose introduces several difficulties in regards to efficiency of both isolation and expansion, as well as the morbidity and discomfort associated with the harvest procedure. Fibroblasts can differentiate towards several distinct lineages, and given the standardized isolation procedure and favorable growth kinetics, may serve as an alternate source to autologous endothelial cells. The present study investigates the endothelial differentiation of human dermal fibroblasts in regard to genome-wide expression, protein expression and function. Differentiation was induced by subjecting fibroblasts to human serum. Differentiated fibroblasts displayed increased expression of proteins and genes associated with endothelial differentiation and function. 1038 genes were significantly up-regulated, including fundamental endothelial cell markers such as angiotensin II receptor, vascular endothelial cadherin, prostaglandin E synthase, vascular endothelial growth factor receptor 2, von Willebrand factor. Moreover, increased uptake of low-density lipoprotein and the formation of capillary-like networks were shown. The results of this study imply that endothelial differentiation of dermal fibroblasts is possible, and may have an impact on cell sourcing for vascular tissue engineering.

22.P28
Bilayered electrospun vascular grafts for improved tissue regeneration and reduced blood leakage

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Shelf-ready synthetic biodegradable vascular grafts are promising alternatives to autologous vascular material for small diameter vascular replacements in the clinic. In this study, bilayered vascular grafts with two distinct micro-architectures were prepared and evaluated. The bilayered grafts were made by electrospinning polycaprolactone into a high porosity graft and adding a low porosity barrier layer either on the luminal or the adventitial side. Grafts were characterized in vitro for fiber size, pore size, total porosity, water and blood leakage, mechanical strength, burst pressure, and suture retention strength. The two types of grafts were then evaluated in vivo in the rat abdominal aorta replacement model for 3 and 12 weeks. The in vitro blood leakage through these barrier grafts was significantly reduced compared to the single layer high porosity graft. In vivo, the cell invasion at 3 and 12 weeks ($6.4 \pm 2.3\%$ and $12.5 \pm 0.7\%$ for the outside barrier grafts vs. $23.5 \pm 5.5\%$ and $35.3 \pm 5.3\%$ for the inside barrier grafts) and

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neovascularization at 3 weeks (3.2 ± 1.1 vs. 14.7 ± 3.1 capillaries/field of view) was significantly reduced in outside barrier grafts, but there was no significant difference between the grafts for endothelialization rate or intimal hyperplasia. Multi-layered grafts are therefore promising approaches for achieving optimal tissue regeneration in synthetic biodegradable vascular grafts, while avoiding the problem of blood leakage during surgery.

22.P29
Subcutaneous implantation of polymer rods for the development of an in vivo tissue engineered blood vessel for hemodialysis vascular access

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Introduction: Vascular access is the Achilles heel of hemodialysis due to stenosis and thrombosis. The development of an autologous tissue engineered blood vessel (TEBV) may circumvent this. We present a novel *in vivo* tissue engineering approach, in which the tissue engineered blood vessel is grown directly in the body.

Materials and methods: Cylindrical polymer rods were developed that upon implantation evoke a foreign body response leading to the formation of a fibrocellular capsule around the rod. After extrusion of the rod and implantation into the vasculature this tissue engineered structure will transdifferentiate into an adequate TEBV. By modulating the characteristics of the implantation material, the tissue response can be tailored. Different polymer rods composed of 300PEOT55PBT45, 1000PEOT70PBT30 and PCL as well as surface modified rods and rods dip coated with TGF β were implanted subcutaneously in male Wistar rats. After 3 weeks the tissue capsules were harvested and analyzed by (immuno)histochemistry and -morphometry.

Results: The engineered tissue capsules were composed of an inner layer of circumferentially aligned αSMA positive myofibroblasts and an outer layer of Sirius red positive collagen showing circumferential alignment as well. The various implantation materials and surface modifications resulted in distinct histological differences. The most pronounced increase of wall thickness and amount of αSMA positive cells was achieved with surface modified and TGF β coated rods.

Conclusion: By modifying the characteristics of implantation material, the tissue formed around this material can be tailored, thereby creating a solid basis for an autologous *in vivo* tissue engineered blood vessel.

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23. Ear/Nose Area

23.01
Keynote: Comparison of novel biomaterials for cartilage tissue engineering in the head and neck region

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Providing an adequate microenvironment to ensure chondrocyte migration and de-novo synthesis of cartilage specific extracellular matrix (ECM) is crucial for the development of bioimplant matrices for facial cartilage reconstruction. The aim of this study was to compare three novel and promising biomaterials with respect to specific requirements of the head and neck region.The following biomaterials were evaluated for their application with human nasal chondrocytes: Bacterial cellulose, marine biocollagen and decellularized cartilage. Scaffolds were seeded with 2.0×10^5 human septal chondrocytes per scaffold and cultured for up to 42 days. Cytotoxicity, cell adhesion and migration as well as matrix production were evaluated. Cartilage specific ECM was examined on protein level by immunohistochemistry and on gene expression level by real time PCR for presence of collagen type I and II, aggrecan and versican. None of the materials was cytotoxic, cell adhesion, stability and matrix production in 3D culture differed clearly between the materials due to respective substrate origin, manufacturing process and different porosities.Rapid cell migration and matrix formation was found in softer biomaterials while stiffer materials led to reduced cell adhesion and migration. Therefore each material has specific advantages and disadvantages with regard to its use for reconstruction of facial cartilage defects. Supported by the Euronanomed - EU and BMBF, FK 13N11075. Project EAREG.

23.02
Keynote: EAREG - Ear tissue regeneration using human cells and novel nano-cellulose scaffolds

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In about 1:10 000 births the external part of the ear, the auricle, is severely malformed or absent. Furthermore, tumours and trauma can cause defects to the auricle. This research focuses on auricle reconstruction using a novel biomaterial, bacterial nano-cellulose (BNC), generated in dynamic culturing conditions using bioreactors, and co-culture of human chondrocytes and stem cells. 3D models of patient-specific ears are printed from clinical MR images. These models are used to create a bioreactor in which BNC is grown. The biomechanical properties of human auricle cartilage are then measured and used as a benchmark for tuning BNC properties. The porosity of the BNC biomaterial is designed to host co-culture of auricle chondrocytes and bone-derived mesenchymal stem cells. Studies of cell differentiation and proliferation have been performed. The promising BNC-cell constructs are evaluated in in vivo animal studies. The primary focus is on auricle reconstruction, however, the methods and results developed are also applicable in the regeneration of nose, trachea, spine and articular joints.
Acknowledgment: EuroNanoMed (EAREG-406340-131009/1).

23.03
Migration mechanisms of human chondrocytes into a novel xenogenic implant matrix

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Using a novel xenogenic biomatrix for cartilage tissue engineering, consisting of decellularised porcine nasal septum cartilage, we showed migration of human chondrocytes into the matrix in vitro. Histological analysis revealed channel formation and active migration of single cells presumably based on enzymatic activities. Potential candidates are matrix metalloproteinases (MMPs) regulating development and physiologic events. Aim of this study was to detect and to identify MMPs responsible for migration mechanisms. Scaffolds were seeded with human chondrocytes and cultured for up to 42 days. Relative gene expression levels of MMP-1, MMP-8, ADAMTS-1, -2, -3 and -5 were evaluated over time and compared to native human nasal septum cartilage (NSC).Expression levels of MMP-1, ADAMTS-1 and -3 were significantly decreased while the mRNA level for MMP-8 and ADAMTS-5 increased up to 14 days. Until day 42 the expression of MMP-8 and ADAMTS-5 stabilised on an average level similar to NSC. These results indicate that MMP-8 referred to as collagenase-II and ADAMTS-5 as aggrecanase are most likely responsible for active migration of chondrocytes whereas the other tested MMPs do not directly influence channel formation. These mechanisms enable chondrocytes to migrate into the matrix and to reach an optimal surrounding. Subsequently the expression of examined MMPs is down-regulated to a normal level compared to healthy NSC.

23.04
Cell source determines the biochemical properties of the generated matrix in tissue-engineered auricular cartilage

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A major challenge in auricular cartilage Tissue Engineering (TE) is defining an appropriate cell source. The aim of this work is to compare the functional properties of TE constructs produced by several cell sources. With an overall goal of external ear reconstruction, in this work these functional properties are evaluated against the properties of native ear cartilage. Isolated human chondrocytes from three sources (ear, nose, knee) and mesenchymal stem cells (MSCs) from bone marrow and adipose tissue, were expanded for four passages and subsequently cultured in alginate to assess chondrogenic potential. Matrix components (glycosaminoglycan (GAG), collagen, elastin) were measured using biochemical assays. Signs of hypertrophy were evaluated by gene expression analyses of collagen type X, MMP13 and alkaline phosphatase. Each cell type was able to generate a cartilage matrix. Ear and nasal chondrocytes produced the highest amount of GAG and

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collagen, which was equivalent to only 10% of the matrix content normally found in native ear cartilage. Hypertrophic genes were highly expressed by MSCs, considerably expressed by ear and nasal chondrocytes and barely expressed by articular chondrocytes. To evaluate the capacity of the TE constructs for ongoing TE efforts, further work will involve implanting the constructs subcutaneously in nude mice, followed by histological, biochemical and mechanical analyses. *Funding*: Senter Novem and ERA-NET/EuroNanoMed.

23.05
Bacterial nano-cellulose is a promising mechanically tuneable biomaterial for regenerative tissue engineering of the outer ear

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Bacterial nano-cellulose (BNC) is a novel non-degradable material that is biocompatible and functionally competent for blood vessel tissue-engineering (TE) applications. It has also been shown to promote chondrocyte adhesion. To determine its potential for ear cartilage TE, this work investigates (1) whether BNC mechanical properties can be tuned to match native ear cartilage, and (2) whether BNC scaffolds can be produced in complex shapes; ie the human auricle. BNC samples ($n = 78$) with varying nominal cellulose content (2.5–15%) were compared biomechanically with human ear cartilage ($n = 22$) using stress-relaxation indentation. Additionally, a patient-specific BNC prototype was produced from an MRI scan of a human auricle. The results show that equilibrium modulus (Eeq) of BNC was correlated to cellulose density ($R^2 = 0.6$); and despite significantly different characteristic relaxation times (τ; $p < 0.05$), Eeq of BNC at 15% cellulose content reached the mechanical properties of ear cartilage (BNC: 2.4 ± 0.4 MPa and cartilage: 2.4 ± 1.1 MPa). This work shows that BNC can be tuned to match native ear cartilage Eeq, and fabricated in to patient-specific shapes. Future work will be focussed on further enhancing BNC mechanics; specifically relaxation characteristics. Alongside positive progress in cell culture efforts, BNC provides promise for engineering ear implants with good mechanical properties and patient-specific shapes. *Funding*: SNSF and ERA-NET/EuroNanoMed.

23.P01
Cartilage reconstruction from clinical and biomaterials perspective: Processed xenogenic cartilage as novel biomatrix for cartilage tissue engineering

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Providing an adequate microenvironment to ensure chondrocyte migration and neo synthesis of cartilage specific extracellular matrix (ECM) is crucial for the development of bioimplant matrices for facial cartilage reconstruction. Xenogenic collagen biomatrices from porcine origin (pNSC) could be the solution for several clinical problems.For in vitro biocompatibility studies human chondrocytes (hCh) were isolated from fresh human cartilage. pNSC scaffolds were seeded with 5×106 hCh/cm^2 and cultivated for up to 42 days. Migration behaviour of hCh and production of ECM proteins was examined on protein and gene expression level. In vivo biocompatibility, adaptability to defect region and form stability of pNSC were examined. Seeding experiments demonstrated that processed pNSC provide excellent environmental properties for hCh with respect to cell adhesion, migration and neo synthesis of ECM proteins like collagen type II and aggrecan. In vivo evaluation of biocompatibility revealed that pNSC is biocompatible and provides adequate stability and plasticity for surgical application. In vivo and in vitro no shrinkage or deformation of scaffolds was detectable.The naturally structured and porous bioimplant matrices are characterized by an optimal pore size and individual ductility. pNSC matrices from natural origin offer biological characteristics ideal for clinical application providing adequate stiffness and form stability for surgical handling and implantation.

23.P02
Development of a porous polymer scaffold for mastoid bone regeneration

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In order to remove severe infections or growths in the mastoid bone a mastoidectomy is performed, where a portion of the bone is removed. A common problem with these procedures is the long-term morbidity associated with the resulting bone cavity, including hearing impairment or loss and continuous discharge. To avoid these issues the cavity can be filled ('obliterated'), however this does not restore the unique physiology of the mastoid bone. Mastoid bone contains mucosa-lined air cells which have vital functions including sound conduction, maintaining pressure regulation, gas exchange, material secretion, waste excretion and middle ear cavity aeration. These functions can only be restored by regeneration of the mastoid bone. Here we describe the development of a highly porous, biodegradable polymer scaffold for mastoid bone regeneration based on poly(lactic-co-glycolic acid) (PLGA)/poly (ethylene glycol) (PEG) microparticles and alginate beads. When the PLGA/PEG particles are mixed with liquid this creates a paste which can be injected to fit a cavity and solidifies at 37 °C. The alginate beads degrade rapidly resulting in a highly porous PLGA/PEG scaffold structure. The composite scaffolds are capable of delivering antibiotics to aid in the treatment of infection, and support cell growth. An in vitro model of mucosa-seeded scaffolds is currently being developed to demonstrate the potential for repair of mucosa-lined mastoid bone with this system.

23.P03
BNC in combination with alginate as a cell compatible material for cartilage replacement

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The application of tissue engineered cartilage in the head and neck is challenging since no degradable material was able to fulfil the requirements of daily physical strains so far. Based on own preliminary results we hypothesise that non degradable bacterial nano cellulose (BNC) in combination with biocompatible alginate is a potential scaffold for cartilage replacement. Four different BNC prototypes, differing in structure, porosity and production process, were tested. The BNC scaffolds

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were seeded with human primary auricular chondrocytes (hpaCh). Cell seeding experiments were performed with and without 1% alginate. Seeded BNC scaffolds were cultured with 3D culture medium for up to 28 days. Expression of chondrogenic marker proteins (aggrecan, collagen II) and dedifferentiation markers (versican, collagen I) was examined on gene expression level by real time PCR as well as on protein level by immunohistochemical stainings. Cytotoxicity assays using Hoechst, MTS and fluorescent live/ dead stains were performed to analyse cytotoxic effects. In all tested BNC scaffolds cell uptake and adherence was found and no material showed cytotoxic effects. The combination with alginate enhanced cell recovery and expression of chondrogenic makers. We conclude that the application of alginate in BNC positively influences chondrogenic expression on gene and protein level. Supported by the 7th framework programme of the EU – Euronanomed and BMBF, FK 13N11075. Project EAREG.

23.P04
Evaluation of macroporous bacterial nano-cellulose scaffolds for ear cartilage tissue engineering

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Bacterial nano-cellulose (BNC) is a promising new nano-biomaterial which has shown to possess impressive tuneable biomechanical properties and excellent biocompatibility for the use in the field of regenerative medicine. BNC has already been evaluated as a biomedical implant in several applications. For tissue engineering of an auricle no suitable materials exists today. Macroporous BNC is a promising candidate. Macroporosity can be induced by incorporation of porogens during the bacterial culture. Particles of paraffin and alginate have been evaluated as porogens. Freeze drying of homogenized BNC blended with non-cross linked alginate has also been evaluated as a method of inducing macroporosity in BNC. The three different scaffolds were evaluated in vitro with human auricle chondrocytes, human stem cells and co-cultures thereof. Adhesion, spatial distribution, proliferation and production of ECM were evaluated with histological and immunohistochemical staining methods. The three different scaffolds provide varying macroporous architecture and pore interconnectivity. Scaffolds produced by freeze drying displayed the best results in regard to cell adherence, migration and production of cartilage specific ECM proteins such as collagen II and aggrecan. This study shows that macroporous BNC and BNC / alginate composite scaffolds provide a suitable environment for the cultivation of human chondrocyte / stem cell co-cultures. Acknowledgement: EuroNanoMed – EAREG programme.

23.P05
Bioprinting of 3D patient-specific auricular scaffolds

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Bacterial nano-cellulose (BNC) is a promising non-resorbable biomaterial for auricular cartilage TE. It has been shown that by increasing the cellulose content (cc) of BNC to 15%, compressive mechanical properties similar to those found in ear cartilage can be obtained. Furthermore, we have developed a bioprinter that allows for the biofabrication of patient-specific BNC auricular scaffolds with 1% cc. This work investigates two biofabrication methods to produce BNC auricular scaffolds with 15% cc by (1) culturing BNC pellicles (n = 72) in media with varying concentrations of corn steep liquor (2, 4 and 8 v/v% CSL) as a means to increase the cellulose production, and (2) printing of culture media into a negative ear mold elongated in the z-direction to produce enlarged BNC auricular scaffolds (n = 4), followed by post-treatment to increase cellulose density; i.e. compressing the enlarged scaffolds to original ear size and stabilizing the 3D structure by physical crosslinking. The results show that a threefold increase in cellulose production occurs when the culture media is supplemented with 8 v/v% CSL compared to the reference media (2 v/v% CSL: 1.01 ± 0.02% cc and 8 v/v% CSL: 3.24 ± 0.16% cc). Furthermore, enlarged BNC scaffolds can be compressed and cross-linked physically to obtain stable BNC scaffolds with 9% cc. This work shows the feasibility of combining different biofabrication methods to engineer patient-specific BNC auricular implants. *Funding*: ERA-NET/EuroNanoMed.

23.P06
Mechanical and biochemical maps of ear cartilage to set a benchmark for auricular reconstruction

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Adequate mechanical competence and extracellular matrix production are critical aspects for the success of regenerative medicine endeavours in ear reconstruction. It is hypothesized that for successful outcomes, a comprehensive understanding of the structure-function relationships of the native ear is required. This work aims to create preliminary mechanical and biochemical maps of native human auricles, and examine the structure-function relationships between them. Instantaneous and equilibrium moduli (Ein and Eeq), maximum stress (σmax) and relaxation characteristic (t) for 20 fresh human ear cartilage biopsies (4 males; middle-aged 49 & 58 and elderly 85 & 93 years old) were measured using stress-relaxation indentation. Samples were labelled according to their location in the ear; concha, scapha, anti-tragus. Additionally, for the 93 year-old, glycosaminoglycan (GAG) and collagen content was quantified. Initial results show a significant difference in Eeq, Ein and σmax for age (middle-age versus elderly) and location (scapha and concha versus anti-tragus). GAG and collagen content show variation with location, but experimental power is limited. This preliminary map must be extended to a larger cohort in order to elucidate the true relationships in ear cartilage. Local mechanical and biochemical properties, and the relationships between them, are required for auricular reconstruction efforts in order to ensure functional outcomes. *Funding*: SNSF and ERA-NET/EuroNanoMed.

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24. Current Clinical Applications in Veterinary Medicine (in coop. IVRMS)

24.01
Keynote: Regenerative veterinary medicine – an overview

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Veterinary medicine is pioneering in the application of regenerative therapies. Disorders of the musculoskeletal system represent a major part of all cases in veterinary clinical practice. Especially the horse as a high performance athlete is prone to injuries of the musculoskeletal system, which represent a substantial cause of early termination of an equine career - a frequently occurring problem in the equine athlete. The prognosis for patients suffering from musculoskeletal disorders such as tendon or joint injuries is poor. Therefore, major emphasis has recently been put into regenerative therapies for orthopaedic conditions. Different kinds of regenerative concepts have been researched and used in veterinary practice. This applies for the use of cells (chondrocytes, MSC) and modifications of these cells through tissue engineering or genetic engineering as well as for the use of autologous blood products like platelet rich plasma or autologous conditioned serum, the latest research efforts going into ES and iPS cells and their potential use. The focus of attention in veterinary science is currently drawn to mesenchymal stromal cells and their potential in regenerative medicine. Several therapies utilizing MSC for animal patients are being developed (e.g. treatment of spine injury in the dog, treatment of osteoarthritis in the horse) and some, like the treatment of equine tendinopathies or cartilage degeneration in dogs have already successfully entered the market.

24.02
Keynote: Experimental and clinical application of BMSCs for the treatment of large bone defects in animals

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Objective: The aim is to refer the results of the experimental and the clinical application in veterinary orthopaedics of bone marrow mononuclear cells (BMMNCs) and cultured bone marrow stromal cells (cBMSCs) for the treatment of some orthopaedic lesions in animals. The experimental study was performed in a sheep model where the performance of a resorbable bioceramic based on silicon stabilized tricalcium phosphate (TCP) and with a titanium scaffold and BMMNCs, in promoting the repair of a large-sized experimentally induced defect was evaluated. The clinical cases instead regarded 40 dogs with different orthopaedics lesions.
Materials and methods: Bone formation and scaffold resorption were evaluated by sequential x-ray studies, CT scans, histology, immunohistology, microradiography, and quantitative analysis of x-ray studies and microradiographs .The clinical study regarded 40 dogs of different breed, age and size with the different lesions: bone cyst, nounion, large bone defect, Legg-Calvé-Perthés disease.
Results: The data of the study in the sheep model showed an excellent implant integration and significant bone regeneration within the bone substitute. As to the results of the clinical cases, till now the treated dogs have shown very good clinical and X-ray results.
Conclusion: The obtained data showed that the cell therapy seems to be an effective therapeutic treatment for the orthopaedic lesions in animals.

24.03
Tracking of mesenchymal stem cells in tendon injuries following in vivo administration

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Mesenchymal stem cells (MSCs) are increasingly used to treat tendon injuries. They persist for several months but survive in low numbers when implanted intra-lesionally. It is unclear when this loss occurs, how many survive initial implantation, and if alternative injection routes can utilise the 'homing' ability of MSCs. Labelling efficiency of MSCs with technetium-99m and HMPAO was initially determined in vitro. 10 million labelled MSCs were then implanted into 13 horses with naturally-occurring tendon or ligament injuries intra-lesionally (IL), intravenously (IV), and by regional perfusion under a tourniquet (RP) on separate occasions 3 days apart and traced for up to 48 h by gamma scintigraphy. Maximum in vitro cell labelling was 55% with >90% viability but this varied from 1.8–18.5% (mean 9.3%) in clinical cases. Cells were retained in the lesion after IL injection but only 24% were still present at 24 h. After IV injection, cells largely distributed to the lung fields and were undetectable in the tendon lesion. Significant, but lower than IL, labelling of lesions was observed in 11/12 horses after RP. Highest cell numbers were retained after IL injection, although with substantial early-stage cell loss. RP may be a viable alternative if there is no central deferct for cell implantation. The absence of cells seen after IV administration may be due to of low labelling efficiencies, but cells still did not 'home' to the lesion in large numbers within 24 h of injection.

24.04
Serum increases the adhesion of bone marrow derived MSC to articular cartilage in vitro

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Cellular therapies for Osteoarthritis (OA) using mesenchymal stem cells (MSC) were shown to provide beneficial outcomes for patients. Nevertheless, several reports suggest that just a small fraction of injected cells adheres to damaged cartilage and therefore contributes to tissue regeneration. In the current study we developed an in vitro test system allowing us to compare the effect of different application vehicles on MSC adhesion with the aim to improve cell adhesion to damaged cartilage and therefore OA therapy.Saline, PBS, and increasing serum concentrations (2, 10, 20, 50 and 100%) were used as suspension vehicles for bone marrow derived MSC. Labeled MSC were seeded on osteochondral explants from rat tibia plateaus with an intact surface or with a full-thickness defect. Adhering MSC were stained and the area fraction covered by labeled cells was analyzed using imageJ

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software. Results were confirmed in a human model seeding labeled human MSC on OA cartilage explants.MSC adhesion to healthy rat cartilage was significantly lower than to defective- or OA cartilage. In all three models the area fraction covered by labeled cells was significantly higher in the presence of high serum concentrations than with saline or PBS used as vehicles. A maximum coverage of 23% could be reached with 50% serum. In vitro attachment of MSC to healthy, damaged and OA cartilage is increased by factors present in serum.

24.05
Tissue engineering for cartilage repair: in vitro development of an osteochondral scaffold

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Objectives: The aim of the present work is the in vitro optimization of the chondral phase of an osteochondral scaffold and the analysis of the effect of the fibrin glue as embedding scaffold for the seeded chondrocytes.
Methods: Fresh chondrocytes were seeded onto the scaffold by embedding them in fibrin glue or in medium as control. In the second part of the study, chondrocytes were isolated and expanded in the presence of specific growth factors; they were resuspended in fibrinogen and seeded onto the scaffold that was cultured in vitro for 1, 3 and 5 weeks in a chondrogenic medium.
Results: histological and immunohistochemical data demonstrated that the presence of fibrin glue ameliorated cell distribution and survival into the chondral composite. Data from the second part of the study showed that chondrocytes' phenotype was rescued after 3 weeks of in vitro culture and maintained for the following weeks; the biomechanical properties improved during time but they started to decrease between week 3 and 5.
Conclusion: The in vitro data demonstrated that chondrocytes can grow and promote the formation of a mature cartilaginous tissue when seeded on the chondral scaffold proposed in this study; their survival and activity are ameliorated by the presence of fibrin gel as embedding scaffold and by maintaining the vitro culture for 3 weeks in the presence of specific growth factors.

24.P01
Comparison of equine mesenchymal stromal cells derived from different sources

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Stem cell based therapy of orthopedic disorders, especially tendon injuries, is being used with favourable success in the equine patient. Most commonly, mesenchymal stromal cells (MSC) derived from bone marrow or adipose tissue are applied for this purpose. Yet, multipotent MSC can be isolated from various tissues in the body. The aim of this study was to compare basic, clinically relevant characteristics and tendon marker expression level of equine MSC derived from different sources.MSC yields were 222-fold higher after cell recovery from adipose, tendon or umbilical cord tissue compared to bone marrow or umbilical cord blood. Furthermore, adipose and tendon derived MSC proliferated more rapidly than umbilical cord blood and bone marrow derived MSC. All MSC were capable of migration, whereat tendon derived MSC migrated fastest. Osteogenic differentiation was most distinct in bone marrow MSC, but weak in umbilical cord blood or matrix derived MSC. In contrast, chondrogenic differentiation was most prominent in MSC from umbilical cord blood. Collagen 1A2 expression was highest in adipose and tendon derived MSC, while Scleraxis expression was highest in umbilical cord blood derived MSC.Features of equine MSC highly depend on the tissue from which they have been isolated. This could be advantageous for specific therapeutic applications. Furthermore, adipose and tendon tissue derived MSC appear to be good alternatives to bone marrow derived MSC.

24.P02
Dog adipose-derived mesenchymal stem cells (ASC). Characterization of adherent and non-adherent culture conditions: influence of autologous serum enriched with platelet-rich plasma on ASC self-renewal

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Osteoarticular pathologies very often require a regeneration process for bone, cartilage and/or tendon with de novo vascularisation. Clinical approaches performed on osteoarticular complications in dogs constitute an ideal model for human clinical translational applications. The multipotency of adipose-derived mesenchymal stem cells (ASCs) can be maintained in vitro and they can be differentiated to osteocytes or chondrocytes offering a good tool for cell replacement therapies in human and veterinary medicine. Here we have compared the efficiency of ASC isolation from direct inguinal adipose biopsies either on adherent-dependent cultures with bovine serum (group 1, $n = 4$) or in non-adherent conditions with autologous serum supplementation (group 2, $n = 4$). Two weeks after fat biopsy acquisition, flow cytometry analysis of group 2 showed better yield on the percentage of cell-population for the primary stem cell marker CD90: (group 1) 24 + 8% vs (group 2) 80.6 + 17%. In adherent conditions, the proliferation rates of both groups in the presence of 10% bovine serum or canine serum was not different. Platelet-rich plasma (PRP) treatment in a dose-dependent manner significantly induced the proliferative activity in the absence of serum. Overall, the use of autologous serum and PRP for isolation and amplification of the stem cell population from adipose tissue represents a safe alternative to bovine serum for cell replacement therapies. Work supported by Fundac.García-Cugat and MICINN.

24.P03
Effect of matrigel on the osteogenic potential of canine adipose tissue-derived mesenchymal stem cells

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Adipose tissue-derived mesenchymal stem cells (Ad-MSCs) are a promising source of cells for bone tissue engineering. Matrigel is a basement membrane extract containing multiple extracellular components. This mixture may promote the osteogenic differentiation of MSCs and provide a more appropriate microenvironment for the transplanted cells.

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Here we investigated the effect of Matrigel on the osteogenic potential of Ad-MSCs. Canine Ad-MSCs were cultured in 2D and 3D matrices, and implanted into subcutaneous pouches of dogs either with or without Matrigel. Culture mineralization, cell adhesion efficiency, cell proliferation, osteoid matrix production, and alkaline phosphatase (ALP) and tartrate-resistant acid phosphatase activities were quantified and compared. Ad-MSCs grown in 2D cultures with Matrigel showed higher levels of calcium deposition and ALP activity than those grown in the absence Matrigel under osteogenic conditions. In 3D cultures, the cells cultivated with Matrigel showed greater attachment, proliferation, and osteogenic differentiation than those grown without Matrigel. In vivo, Ad-MSCs implanted with Matrigel showed higher osteogenic potential than those without Matrigel. In conclusion, these data suggest that the use of Matrigel can increase the osteogenic potential of canine Ad-MSCs.

24.P04
Efficiency of adipogenic differentiation methods in mesenchymal stromal cells from diverse sources

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Adipogenic differentiation is one feature of the multipotency of mesenchymal stromal cells (MSC). Different protocols for adipogenic differentiation have been described. However, dependent on the MSC source, especially the most commonly used methods do not always lead to sufficient results. Therefore the aim of this study was to evaluate the suitability of different differentiation protocols for MSC. Equine MSC derived from diverse sources were subjected to different adipogenic differentiation protocols. These based on a standard differentiation methods, which were then modified in terms of cycles, incubation times and media supplements. After incubation period, the intensity of adipogenic differentiation following Oil Red O staining was assessed by the percentage of differentiated cells and size of intracellular lipid vacuoles.Using the standard differentiation method, a sufficient adipogenic differentiation could not be achieved in all MSC samples. Incubation in media supplemented with rabbit serum resulted in significantly higher adipogenic differentiation scores, whereat supplementation with 15% rabbit serum and incubation over a shortened period of time led to the highest score points. However, after differentiation with high rabbit serum concentrations and prolonged incubation time, massive cell detachment was observed. Protocols using rabbit serum over a short period of time are suitable to induce a distinct adipogenic differentiation in equine MSC.

24.P05
Effective treatment of a traumatic tendon lesion within the tarsal sheath of a horse with PRP in combination with isolated bone marrow mononucleated cells

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New therapy methods of tendon lesions with platelet rich plasma (PRP) or stem cells have shown a more rapid and higher quality of tendon healing.In the present case, a mechanical trauma of the deep digital flexor tendon (DDFT) within the tarsal sheath was diagnosed in a horse. The lameness was evaluated as 3/5 on the left hindlimb. The sonographic examination showed a severe tendinitis of the DDFT. Our aim was a regenerative therapy approach using a combination of bone marrow mononucleated cells (BMMNCs) and PRP. We applied a one-step procedure to enrich the concentration of stem cells within the bone marrow aspirate. A tenoscopy of the tarsal sheath was performed and the lesions were treated with PRP. Thirteen weeks after the operation, sonography still showed lesions in the tendon. At this point of time the tendon was treated with PRP in combination with BMMNCs. Bone marrow aspirated from the sternum was incubated with hypotonic buffer to lyse the red blood cells. After centrifugation, the pellet was washed with PBS, resuspended in 2 ml PRP and mixed with thrombin 30 seconds before application. Eight weeks after the PRP/BMMNCs treatment,a lameness grade of only 1–2/5 was observe. The sonographic examination showed clearly improved echogenicity of the tendon. The implantation of stem cell-enriched BMMNCs in combination with PRP was an effective treatment of the lateral digital flexor tendon within the tarsal sheath and demonstrated new possibilities of treating tendon lesions.

24.P06
Efficient bone regeneration by means of human adipose derived stem cells which reduce callus volume upon BMP-2 administration

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Since the number of patients with degenerative diseases is continuously growing the demand for new therapeutic approaches to treat bone defects and fractures is increasing in trauma surgery and orthopaedics. Improving and accelerating recovery as well as reducing the risk of delayed bone healing are the main targets especially for long bone fracture healing. 'Tissue Engineering' offers promising new technologies that combine the three components – matrix, cells and growth factors. Upon administration of the growth factor BMP-2 embedded with ASCs in a locally-applied fibrin matrix in vivo bone healing was investigated. In the femur of male rats a 2 mm transcortical drill hole served as a small defect model for fracture simulation. μCT and histology were performed after 2 and 4 weeks to determine the bone and callus volume. After only a short period of time (2 and 4 weeks), our animal model disclosed comparative information about bone regeneration and the osteogenetic potential with little effort, without osteosynthesis. The most significant result found in this model is the fact that the combination of ASCs and BMP-2 in a fibrin matrix essentially reduces callus formation after 2 weeks compared to BMP-2. The callus formation was significantly increased just by BMP-2. ASCs embedded in the fibrin matrix did not lead to increased bone regeneration. The callus induction by BMP-2 was modulated by transplantation of ASC to a normal volume.

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25. Fibrin (and its Modifications)

25.01
Keynote: Fibrin – a biomaterial with a long history and a bright future

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Fibrin has 30+years history of safe clinical application with one of the lowest adverse event rates of any medication registered. At the same time it is one of the most versatile tools used in tissue regeneration. As a biomaterial it is non-toxic and fully cell and tissue compatible. Fibrin formation represents the final step in the coagulation cascade, hence fibrin lends itself to its classical applications in hemostasis and sealing across a plethora of surgical procedures. The speed of solidification can be easily tailored to the needs of the surgeon. Fibrin is fully biodegradable and the rate of fibrinolysis can be adapted to the area of its use. Fibrin can be used as a delivery matrix for cells and bioactive substances, the latter either being physically mixed in or bound in a covalent or non-covalent fashion to achieve sustained release kinetics. Furthermore, fibrin can be used as a gene-activated matrix for gene therapy or as a scaffold in 3D-organ culture.

25.02
Keynote: Cell-compatible fibrinogen-based hydrogels designed for tissue regeneration

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In tissue regeneration, the regulation of cellular morphogenesis and differentiation via the provisional extracellular matrix (ECM) is one of the hallmarks of a successful repair process. Our group has been working towards developing hydrogel biomaterials that mimic the inductive repair characteristics of the natural provisional ECM, fibrin, using protein-polymer chemistry. We apply a semi-synthetic fibrinogen-based hydrogel as a provisional ECM analog for the repair of various focal defects, including in cartilage and bone injuries. These dense hydrogels, which displace the blood clot in the injury site, are designed with an in vivo resorption rate that is synchronized with the natural repair process. Immobilized growth factors, such as bone morphogenic protein (BMP), can also be used to accelerate the tissue repair process. As the implant degrades, the liberation of bioactive factors in a sustained manner recruits progenitor stem cells to the injury site, guides the differentiation of these progenitors into mature specialized cells, and dramatically improves the efficacy of clinical therapies aimed at restoring the damaged tissues.

25.03
Thrombin in fibrin sealants –A critical factor in vitro and in vivo

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Fibrin sealants are a well established surgical tool for hemostasis and sealing. Over the last years, they have been additionally used to medicate burn wounds or as delivery matrix to enhance wound healing. The objective of this study was to compare two commercial distinct fibrin sealants Artiss and Evicel regarding their effects on cells and wound healing in vitro and in vivo. Keratinocytes show significant differences in adhesion, viability and morphology when getting in contact with these fibrin sealants in vitro. In this and previous studies we could demonstrate that those differences were mainly driven by the thrombin concentration in the sealant. To verify these results in vivo we used an excisional wound healing model in rats. We found a more rapid wound closure (day3 and day7) and less wound severity (day7) with Artiss containing a lower thrombin concentration compared to wound healing with Evicel. Further, less newly built functional vessels (SMA staining) were counted in Evicel treated wounds after 7 days which fits the result that a lower level of VEGF was expressed after 2 days in such treated wounds. These in vivo results may be partially explained by the biocompatibility data found in vitro. At last, also fibrin degradation was observed. Artiss fibrin sealant was lysed after 10 days (Evicel: 15 days). As shown in previous publications thrombin is a very important player not only for clotting kinetics but also for cell compatibility and wound healing.

25.04
Fibrin loaded porous poly(ethylene glycol) hydrogels induce vascularized tissue formation

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Vascular network formation within biomaterial scaffolds is essential for the generation of properly functioning engineered tissues. In this study, a method is described for generating composite hydrogels in which porous poly(ethylene glycol) (PEG) hydrogels serve as scaffolds for mechanical and structural support and fibrin is loaded within the pores to induce vascularized tissue formation. Porous PEG hydrogels were generated by salt leaching technique with 100~150 μm pore size and thrombin pre-loaded within the scaffold. Fibrinogen was loaded into pores with varying concentrations and polymerized into fibrin due to the presence of thrombin, with loading efficiencies ranging from 79.9 to 82.4%. Fibrin was distributed throughout the entire porous hydrogels, lasted for greater than 20 days and increased hydrogel mechanical stiffness. A rodent subcutaneous implant model was used to evaluate the influence of fibrinogen concentration on in vivo response. At weeks 1, 2 and 3, all hydrogels had significant amount of tissue invasion, but no difference in the depth of invasion was found. Fibrin loaded porous PEG hydrogels induced more vascular formation, with significantly higher vascular density at 20 mg/ml (week 1) and 40 mg/ml (week 2 and 3) fibrin loaded hydrogels than without fibrin. In conclusion, we have developed a composite hydrogel that supports rapid vascularized tissue ingrowth and thus holds great potential for tissue engineering applications.

25.05
Tenascin C binds morphogens with its fibronectin type III domain five

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Abstract not available.

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25.06
Mechanical and biochemical evaluation of mussel-mimetic glue for fetal membrane repair

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Introduction: Iatrogenic preterm prelabour rupture of membranes (iPPROM) is the main complication after invasive interventions into the uterine cavity. A variety of sealing materials have been recently employed to prevent iPPROM. Here, the sealing properties and the biochemical stability of mussel glue, a catechol functionalized PEG-based adhesive, have been evaluated under physiological conditions and compared with those of a fibrin sealant.
Methods: Sealing properties of mussel and fibrin glue were evaluated ex vivo using a custom made inflation device. Defects with different sizes were created on samples of caesarean delivered fetal membranes and sealed under wet or dry conditions. Sealed samples were inflated until rupture, monitoring the deformation. The proteolytic degradation of sealants was evaluated in vitro incubating plugs for 22 days in cell culture medium supplemented with collagenase or plasmin. The gluing materials have been tested in midgestational rabbits.
Results: Mussel glue efficiently sealed ex vivo small and large defects both in wet and dry conditions allowing higher membrane deformation than fibrin glue. Mussel glue, in contrast to fibrin glue, was not significantly degraded in vitro by collagenase and plasmin. Moreover, it remained stable and didn't induce a significant inflammatory reaction in midgestational rabbits.
Conclusions: Its good mechanical performance and biochemical stability in physiological conditions make mussel glue a promising sealant.

25.P01
Regenerative and angiogenic capacity of rat bone marrow MSCs encapsulated in fibrin microbeads in a rat muscle injury model: preliminary study

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Tissue engineering approach may be useful to treat skeletal muscle loss by providing an adequate method of delivering regenerative stem cells within a 3D matrix. In this work, rat bone marrow mesenchymal stem cells (BM-MSCs) were encapsulated in fibrin microbeads (FMB) composed of rat platelet rich plasma (PRP) from whole blood samples of healthy rats and alginate (patent application TR-PT 2012/01068) via ionotropic gelation. Pure fibrin microbeads were obtained by extracting alginate using sodium citrate buffer. The histocompatibility of the FMBs containing BM-MSCs was investigated on explants which were transplanted subcutaneously into the epigastric groin fascia of Wistar rats. Histocompatibility was evaluated by following the tissue reaction over a period of 8 weeks. The regenerative and angiogenic capacity of the FMBs with BM-MSCs was evaluated in a muscle injury model in Wistar rats. For this, a $2 \times 3 \times 5$ mm^3-size cylindrical defect was created in the biceps femoris (both sides) of each rat; then, BM-MSCs encapsulated FMBs were placed inside the defects. Explants were retrieved at 1, 4 and 12 weeks post-transplantation for evaluation of the angiogenic and regenerative effect by histology. Results supported the concept that BM-MSCs encapsulated fibrin microbeads were basically histocompatible and induced neovascularization both at the subcutaneous site and at the injured rat muscle.

25.P02
A porous injectable hydrogel system for soft tissue regeneration

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Injectable hydrogel combined with cells could be used as a means to restore soft tissue defects. While this approach presents with several advantages that include higher cell loading efficiency and replacement of multiple surgeries with minimally invasive injection procedures, inadequate nutrient diffusion to cells remains a challenge as injection volume increases. To address this limitation, we hypothesized that hydrogels with porous structure can support cell viability by facilitating mass transfer. In this study we explored the possibility of creating a highly porous injectable hydrogel system that would support cell survival, proliferation and tissue regeneration. To produce a porous hydrogel structure, gelatin microspheres/microfibers were used as pore-generating materials within the fibrin gels. At physiological temperature of 37 °C, the gelatin dissolved and diffused out of the gel forming empty spaces. Cells in the control nonporous fibrin failed to proliferate except at the peripheral boundary adjacent to the host tissue in vivo. In contrast, porous fibrin gel had a wider boundary at the matrix-host tissue than the control. Enhanced mass transfer by the porous structure of hydrogel resulted in increased cell proliferation and tissue matrix formation. These findings suggest that the use of gelatin materials as sacrificial templates for micropore formation improves mass transfer in hydrogels.

25.P03
Development of volume-stable adipose tissue constructs by combining fibrin gels and porous support structures

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Adipose tissue engineering aims to provide novel solutions to the strongly growing clinical need for adequate implants to repair soft tissue defects. One of the major obstacles, however, still is the lack of volume stability of the generated constructs. Therefore, the objectives of this study were to investigate different fibrin gel formulations with regard to adipogenesis in vitro and combine the hydrogels with a biodegradable porous polyurethane scaffold as support structure. Human adipose-derived stem cells (ASC) were obtained from abdominal lipoaspirates. In long-term stable fibrin gels developed in our group as well as in TISSUCOL Immuno (Baxter), strong adipogenic differentiation was observed after induction employing a common hormonal cocktail. Lipid accumulation was visualized using Oil red O staining and upregulation of adipogenic marker genes was demonstrated on mRNA (PPARγ, C/EBPα; qRT-PCR) and protein level (leptin; ELISA). Porous poly-ϵ-caprolactone-based polyurethane scaffolds were manufactured by a gas foaming process (Polymaterials AG). After injection of the fibrin components into the scaffolds, adipogenesis of ASC occurred to a similar extent as in fibrin gels alone. The constructs exhibited an enhanced volume stability, however, only constructs using the stable fibrin gels completely maintained their size and weight for 21 days. The combined constructs offer great potential to further investigate the development of engineered adipose tissue in vivo.

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25.P04
Alginate-human fibrin microcarriers like scaffold for tissue engineering of skin

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Large, full-thickness skin defects resulting from burns, soft tissue trauma, congenital giant nevi, and diseases leading to skin necrosis, represent a significant clinical problem that is far from being solved. Currently, dermal equivalents like AlloDerm®, Integra® or Dermagratf® offer a solution for this problem; however, their high cost make them inaccessible to people with low incomes. The present work describes the production by a microfluidic approach and characterization, of microcarriers constituted of sodium alginate and fibrin from human plasma. Microdroplets were gelled into a CaCl2 solution (3.0%, w/v), freeze-dried, UV sterilized and then rehydrated in DMEM F12 medium. 3T3 fibroblasts were cultured on the microcarriers, first in a Petri dish and later in a spinner. Alginate-fibrin microcarrier's structure was imaged by scanning electron microscopy. Some mechanical properties of the microcarriers were evaluated using dynamic rheology with an elastic modulus prevailing over viscous; the hydrated material seems to be stable for at least a month. Alginate-fibrin microcarriers were found to have good biocompatibility with 3T3 fibroblasts in tissue cell culture: they adhered on the smooth surface and grew in high cell density. Results suggest a good potential of the alginate-fibrin microcarriers as a wound-healing biomaterial.

25.P05
VEGF modified fibrin biomatrix improves wound healing by inducing persistent functional angiogenesis in a dose dependent manner

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In this study we tested ascending VEGF concentrations released from a fibrin biomatrix in the ability to reduce ischemia challenged tissue necrosis and to induce functional angiogenesis.The standard rodent epigastric flap was used to study the influence of fibrin sealant (FS) alone or spiked with rhVEGF (20–800 ng/ml FS) on ischemia challenged tissue in comparison to controls (quilting sutures only). VEGF groups which performed best on day 7 after induction of ischemia were additionally tested for persistent mature angiogenesis 4 weeks after traumatic insult and were compared to FS alone.Flap necrosis was significanly less until day 7 in the 200 and 400 ng VEGF/ml groups compared to controls. Significantly improved flap perfusion was also found then using these two concentrations. Therefore these two VEGF groups were used to test persistent mature angiogenesis. Again, both treatment groups were effective in reducing tissue necrosis after 1 week compared to the FS group. At 4 weeks, sma vessel count showed significantly higher numbers in the 400 ng VEGF group in all tissue layers compared to FS alone as well as 200 ng VEGF group. In summary, we found that flap necrosis was substantially reduced and flap perfusion significantly increased when using sprayed fibrin biomatrix with VEGF in a dose dependent manner. This is due to induction of persistent functional angiogenesis which is again seen dose dependently. Study supported partly by EU grants Expertissues and Angioscaff.

25.P07
A platform photochemical crosslinking technology for use in design and fabrication of biologically-active surface coatings, tissue sealants and scaffolds for tissue engineering

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The structure and properties of material surface coatings or scaffolds intended for tissue engineering are critical for the control of cellular behaviour including cell adhesion, migration, proliferation and tissue formation. Extracellular matrix proteins and protein-based matrices are biocompatible and can self-associate into tissue-like structures. We recently reported the fabrication of a highly elastic insect protein, covalently crosslinked protein biomaterial via a rapid photochemical method using visible light and a ruthenium metal ligand catalyst. We further demonstrated that other tyrosine-rich, self-associating proteins might also be susceptible to dityrosine crosslinking via this approach. We show that these proteins include a wide range of natural biological matrix proteins as well as biological peptide motifs. Depending on the choice of protein and/or peptide, the resulting scaffold can be tailored to suit a variety of applications varying from inert materials to cell compatible materials and scaffolds with selective biological function. Proteins like fibrinogen and gelatin can be photochemically crosslinked to rapidly form a strong adhesive elastic hydrogel that can be applied as a surgical sealant for repair of dura, lung, arterial and colonic wounds. In addition the technology can be used for control of inert hydrogels or surface coatings using suitable inert self assembling peptides combined with biological motifs like RGD.

25.P08
In situ crosslinkable and tissue adhesive gelatin-based hydrogel for tissue regeneration

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In situ forming hydrogels are widely exploited as injectable biomaterials for tissue regeneration and drug delivery based on minimally invasive technique. In this study, in situ cross-linkable gelatin-based hydrogels via enzymatic cross-linking reaction were developed as an injectable bio-adhesive scaffold. The gelatin derivatives were synthesis by EDC/NHS chemistry, and their chemical structures were characterized by 1H NMR and UV measurements. The hydrogels were rapidly formed via the enzymatic reaction under physiological conditions. Their physico-chemical properties were characterized, and could be controlled easily by varying the concentrations of catalysts. The gelation time and mechanical strength were ranged from 5 sec to 2 min, and from 1 to 20 kPa, respectively. The highest adhesive strength was 55 kPa, which was 8 times higher than that of fibrin glue as a control. In vitro cell culture was conducted to confirm cyto-compatibility of the hydrogels, demonstrating that the hydrogels had excellent cyto-compatibility. Obtained results demonstrated that the hydrogels have a great potential as an injectable tissue adhesive material for biomedical applications.

Acknowledgements
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25.P09
Encapsulation of human adipose mesenchymal stem cells in fibrin microbeads from platelet rich plasma and their in vitro evaluation

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Platelet-rich plasma (PRP) is identified as autologous blood with a concentration of platelets in high levels. Platelets contain a variety of growth factors and cytokines that are principal in soft tissue healing and bone mineralization. In this study, fibrin microbeads were developed using a novel technique for encapsulating mammalian cells (patent application TR-PT 2012/01068). PRP was obtained from anticoagulated human whole blood samples. Human adipose derived mesenchymal stem cells (hAdMSCs) were isolated from lipoaspirates collected under ethical approval; then cultured and characterized by FACS. Encapsulation protocol was optimised. hAdMSCs-containing PRP-alginate solution was prepared and fibrin microbeads were dropwise formed in CaCl2 solution. Later, alginate was extracted by the calcium chelator sodium citrate. hAdMSC-encapsulated fibrin microbeads were cultured at 5% CO2-95% air and 37 °C. Microbeads obtained from PRP and platelet poor plasma were used as controls. SEM analyses were performed to characterize fibrin microbeads. In vitro release of TGF-β1 from fibrin microbeads was examined in cell culture medium by using ELISA kit. Viability of encapsulated hAdMSCs was evaluated by MTT assay. In vitro differentiation properties of encapsulated hAdMSCs were investigated histologically. In vitro experiments demonstrated that encapsulated hAdMSCs retained viability, proliferated for >6 weeks and retained tri-lineage differentiation property inside fibrin microbeads.

25.P10
Fibrinogen modified chitosan promotes osteoclastogenesis and substrate degradation by osteoclasts

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Chitosan (Ch) is a widely used biomaterial, biocompatible and biodegradable, as well as easily modifiable and processed into 2D and 3D substrates. However, if Ch is to be used as a scaffold for bone regeneration it is important to evaluate its degradation by osteoclasts (OC). While unmodified Ch has been reported to inhibit OC activity, when modified with collagen the result was increased OC activity, making these attractive candidate cells for promoting modified Ch scaffold degradation. Thus, this work aimed to investigate the influence of Human Fibrinogen (HFG)-modified Ch substrates on osteoclastogenesis and the degradability of these substrates by human primary OC. Buffy Coats from healthy blood donors were used to isolate monocytes by negative selection. OC were induced to differentiate on top of Ch films modified or not with HFG. OC formation and substrate degradation were evaluated by fluorescence and electron microscopy. TRAP production was also analyzed. The results obtained indicate that Ch substrates support the formation of functional OC, but a significantly higher number of bigger and more multinuclear OC were obtained when Ch was modified with HFG. Furthermore, our results show that although both unmodified and HFG-modified Ch substrates can be degraded by OC, Ch modification with HFG led to significantly higher film degradation. In conclusion HFG modification of Ch promotes its capacity to support bigger OC, which have the ability to degrade it.

25.P11
Development of a novel plasma hydrogels for use as a cell delivery scaffold.

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Platelet-rich plasma (PRP) and platelet-free plasma (PFP) provide patients with an autologous source for bioscaffolds, and are currently being used in tissue engineering applications. We have developed novel modifications of PRP and PFP, using polyethylene glycol (PEG), that allows plasma to maintain hydrogel-like characteristics. Fresh PRP was provided by the Division of Hematology located at the USAISR. To obtain PFP, PRP was centrifuged at 4500 xg for 30 min at 24 °C. PRP or PFP was then mixed at a 10:1 molar ratio of PRP/PFP to PEG for 10 min at 37 °C. PEG-PRP/PFP was then polymerized either by adding CaCl2 (1–30 mM) or thrombin (5–20 U/ml) and their physical properties characterized. For some experiments, adipose derived stem cells (ASCs) were added to PEG-PRP/PFP prior to polymerization and maintained in culture for up to 14 days. PEG-PRP/PFP yielded semi-rigid, clear hydrogels, while unPEGylated gels were opaque and easily deformed upon handling. Optimal concentrations that supported ASCs growth without the gels becoming distorted was 10 U/ml of thrombin and 23 mM of CaCl2. At these concentrations thrombin and CaCl2 gels exhibited a storage modulus of ~47 Pa and ~41 Pa, respectively. We have demonstrated that human plasma can be PEGylated to generate a rigid hydrogel that supports stem cell growth. This will allow the development of treatments using autologous patient plasma and ASCs to create a construct that can be used to treat skin and other soft tissue wounds.

25.P12
Production of a composite hyaluronic acid/gelatin/blood plasma gel for soft tissue engineering applications

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Soft tissue engineering-based approaches have numerous potential applications in plastic and reconstructive surgery. The purpose of this study was to evaluate the suitability of 3D scaffolds, composed of human platelet-poor plasma, gelatin and human umbilical cord derived hyaluronic acid, for reconstructive surgery applications through in vitro tests. Fibrinogen was precipitated from human platelet-poor plasma and its composite gels with gelatin and hyaluronic acid (HA/Gelatin plasma gels) were produced afterwards. Mechanical properties of the glutaraldehyde crosslinked gels were determined with compression tests and their porosity and pore size determined from their cryosections stained with commassie brilliant blue. The Young Modulus of the HA/Gelatin plasma gels was determined as 3.5 kPa, which is close to that of soft tissues. The composite HA/Gelatin plasma gels had higher porosity than plain plasma gels (72.5% vs 63.9%) and larger pores. Adipose derived stem cells (ASCs) were isolated from human lipoaspirates and characterised with flow cytometry at the third passage, and also were differentiated into osteogenic and adipogenic lineages. The cell proliferation assay (WST-1) of ASCs on the HA/Gelatin plasma gels revealed the nontoxic nature of the gels and that the number of cells on the gels increase with time. Adipogenic and chondrogenic differentation capability of ASCs on the gels is being investigated.

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26. Collagen Technologies

26.01
Keynote: Strategies for direct engineering strong, native collagen-cell constructs: pre-polymerisation

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Traditionally, we have been satisfied to choose between ending up with either strong collagen materials which are denatured by cell-lethal cross-linking OR native, cell friendly but extremely weak constructs. One aim of ENGIEERING collagen materials is to remove this dilemma, making strong & native a viable option. To increase the strength of collagen matrices controllably in the presence of cells the first strategy has been to develop cell-tolerated (non-lethal) cross-linking. In an alternative, plan 'B', approach we have developed processes for fabrication of tissue-like constructs using native collagen which is extracted and purified as ready-cross-linked fibrils. This uses collagen polymer purification-dispersal techniques described >4 decades ago1 to produce fibril suspensions which can be shear-aggregated along with living cells. In effect this allows component fibrils to be pre-cross-linked for greater strength. Incidentally, these bonds are physiological, so susceptible to cell turnover and the fibrils can be aligned to fabricate strong, native and 'neo-tissues'. 1Steven FS. 1967. The effect of chelating agents on collagen interfibrillar matrix interactions in connective tissue. Biochim Biophys Acta.140:522–8.

26.02
An optimised microgel for maintaining 3D viable microenvironment for human mesenchymal stem cells

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Introduction: Human mesenchymal stem cells (hMSCs) are known to have therapeutic effects by transdifferentiation or through paracrine mechanisms. However, the challenge is to maintain high cell survival invivo. Cell survival and retention can be improved using biomaterials derived from natural extracellular matrix (ECM).In this study, it is hypothesised that crosslinked collagen (type-I) microgel can be used to embed human mesenchymal stem cells. The specific objective of this study is to obtain an optimised microgel platform, which supports cell survival and enhanced release of paracrine factors.This study investigates the effects of collagen (1,2 and 3mg/ml) and crosslinker concentration (1:1 and 1:2) with different cell densities (1, 4 and 8 X 10^6cells/mL) on cellular viability and morphology, cellular phenotype, paracrine secretome and construct size.
Material and methods: Microgels were fabricated by crosslinking type-I collagen with 4S-PEG after the addition of hMSCs. Microgels were stabilised after microdispensing the on a hydrophobic surface for 1h at 37°C. These microgels were maintained in cell culture conditions for 8 to 16 days for analyses.
Results: Over 80% cells embedded in microgels were viable after 16 days. Significant differences were observed in paracrine factors secreted between cells in microgels and cells grown on a 2D surface quantified by multiplex elisa and protein array. The flow cytometry analysis confirmed hMSC phenotype at the end of culture period.

26.03
Cold gas plasma treatment for collagen films to modulate of inflammation

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This study sought to determine the in vitro effect of different plasma treatments on the attachment of macrophages and the expression of inflammatory cytokines from adherent macrophages on collagen films.
Methods: Oxygen (O2) and nitrogen (N2) plasma were used to modify the surfaces of collagen films by an Emitech K1050 X Plasma Asher. The surface topography, chemistry and hydrophilicity were characterized by Scanning Electron Microscopy, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy and contact angle measurement. Macrophage attachment and proliferation were assessed using an FCS assay. The expression of pro/anti inflammatory cytokines and chemokines by adherent U937 cells were assessed by the measurement of secreted proteins by Elisa and Luminex. These secreted proteins include: TNFα, IL-1β, IL-10 and TGFβ.
Results: Contact angle analysis revealed that the surface hydrophilicity significantly increased after both O2 & N2 plasma treatment. There was a decrease in cell attachment and proliferation for U937 cells on both plasma treated surfaces. There were no significant differences in inflammatory cytokine secretion on O2 plasma treated collagen films, however there were decreases in TNFα, IL-1β and an increase in TGFβ on N2 plasma treated collagen films.
Conclusions: Plasma treatment of collagen films may enhance wound healing, by reducing cell adhesion & shifting the cytokine release to an anti-inflammatory profile, especially after N2 treatment.

26.04
Pilot production of novel recombinant bacterial collagens for use as tissue engineering scaffolds

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Collagens have a characteristic triple-helical structure with a (Gly-X-Y)n repeating sequence. Collagen has proven safe and effective in numerous medical products, and is frequently used in tissue engineering studies. Collagen is usually extracted from animals, but there is a concern of transmissible diseases. This introduces the potential use of recombinant collagens. However, recombinant mammalian collagens require the functional co-expression of prolyl 4-hydroxylase (P4H) to achieve collagen stability. Alternatively, collagen-like sequences have been characterised from several bacteria. These collagens lack hydroxyproline, yet form stable triple-helices at 35–38 °C, and can be readily expressed in E. coli. In this study we have used the collagen-like protein Scl2 from S. pyogenes. We have previously shown that this collagen is non-immunogenic and non-cytotoxic, and can be fabricated into sponges suitable as tissue engineering scaffolds. This collagen has limited biological

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interactions so can be used as a blank slate into which selected functional domains, such as heparin or integrin binding, have been inserted. Also, multimeric constructs of the single Scl2 collagen-like domain have been constructed. The present study has shown that these various new constructs can be readily produced in 2L or larger fermentation and that initial yields of better than 6 g/L can be obtained in several cases. Functional studies confirm their suitability as tissue engineering scaffolds.

26.05
Structure and in vivo integration of dense collagen matrices

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Dense collagen matrices have been recently developed in order to perform new tissue substitutes. In vitro studies showed that dermal fibroblasts fully colonized collagen matrices at 5 mg/ml after 15 days, whereas at 40 mg/ml, colonization was incomplete. In the present study, the structure and in vivo integration of dense collagen matrices at 5, 10, 20 and 40 mg/ml were analysed. The analysis by scanning electronic microscopy revealed the presence of a fibrillar network within all matrices. Rheological measurements showed that the elastic modulus of collagen matrices increased with collagen concentration (10 KPa at 40 mg/ml). After subcutaneous implantation in rats, collagen matrices exhibited different degrees of tissue integration. Macroscopic views revealed a good integration of 5 and 10 mg/ml matrices after 15 days and histological analysis showed their complete colonization by host cells. From 20 mg/ml, integration appeared lower. After 30 days, 20 and 40 mg/ml dense matrices were still observed and their colonization by host cells detected up to 200 μm depth. Quite interestingly CD-68 immunodetection revealed the absence of severe host response regardless of collagen concentration. Taken together, our results show that 5 and 10 mg/ml collagen matrices could find applications as dermal substitutes as they rapidly integrate a host organism, whereas 20 and 40 mg/ml matrices could be promising for abdominal wall repair, because of their better mechanical properties.

26.06
Comparison of two gelatin and thrombin combination hemostats

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This study compares two gelatin hemostats. In vitro, the amount of Red Blood Cells (RBC) and thrombin not retained by each were compared. In vivo, a heparinized porcine liver abrasion model was used to compare hemostatic success, degree of bleeding, and blood loss at 2, 5, and 10 minutes after application. In vitro, bovine gelatin retained significantly more thrombin as measured in gelatin-thrombin supernatant (6.81 vs. 10.89 IU/ml; 95% CI: -5.48 to −2.67, P=0.0013). Bovine gelatin retained significantly more RBC as measured in clot supernatant (0.0685 vs. 0.0911 x106/uL; 95% CI: −0.0356 to −0.0096, P=0.0085). In vivo, bovine gelatin provided superior hemostatic success to porcine gelatin at 5 minutes (85% vs. 60%; Odds Ratio, 5.3247; 95% CI: 1.6 to 17.8) and 10 minutes (72.5% vs. 47.5%; Odds Ratio, 5.0; 95% CI: 1.5 to 16.1). Bovine gelatin had significantly lower degree of bleeding at 5 minutes (0.575±0.874 [SD] vs. 1.025±1.121; Odds Ratio, 3.3570; 95% CI: 1.3394 to 8.4138) and 10 minutes (1.125±1.137 vs. 1.650±1.051; Odds Ratio, 3.8653; 95% CI: 1.6216 to 9.2135). Mean blood loss was less with bovine gelatin at 2 minutes (0.07±0.19 vs 0.13±0.63 mL/min), 5 minutes (0.04±0.13 vs. 0.23±0.45 mL/min), and 10 minutes (0.09±0.24 vs. 0.21±0.32 mL/min). An increased ability to retain thrombin and RBC in vitro may explain why bovine gelatin provides superior hemostatic effectiveness, superior control of bleeding, and greater reduced blood loss in vivo.

26.P01
Structural interaction between collagen and di-carboxylic acids (DCAs): An improved platform model for 3D tissue engineering research

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Introduction: Current scenario in tissue engineering research demand 3D scaffolds with high porosity, mechanically and thermally stable, biocompatible and biodegradable. However, bringing these properties in single scaffold material is a challenging task and needs intensive research. In recent years, ionic liquids have been investigated to strengthen the properties of biomaterials. Similarly, α,β-Di-carboxylic acids through its non-covalent interactions may improve and add strength. Thus, an approach was made and the present study explores details and the results obtained.
Materials and Methods: Series of α,β-DCAs were mixed with collagen (type–I) and the homogenous solution obtained was transformed to 3D scaffold. Analyses on degree of cross-linking, strength, CD & FT-IR, morphological observation, thermal stability and binding interactions were made.
Results: Chosen DCAs dissolute and structurally modify the collagen through its non-covalent interactions. An increase in alkyl chain length increases the porosity of the scaffold. Degree of cross-linking was ≍55–60%, thermal stability (>300°C) and exhibit ≥ 3–4 fold increase in mechanical strength compared to virgin collagen. The molecular docking simulation studies proved the chemistry behind the interaction of α, β -DCAs with collagen.
Conclusion: α, β -DCAs played dual role to engineer the collagen based scaffold with improved properties for tissue engineering applications.

26.P02
Engineering anisotropy in mechanical properties by orientated collagen cross-linking

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Biomimetic plastic compressed (PC) collagen gels provide a biocompatible extra cellular matrix. Orientated focal cross-linking can be used to generate surface anisotropy, improve material stiffness and layer integration for the production of a stable 3D construct. Riboflavin (0.25mM) mediated cross-linking of PC collagen gel was assessed by measuring the diffusion depth of riboflavin with time. The dynamic force analyser was used for peal force testing for interlayer cross-linking and material stiffness in perpendicular axis after orientated/topical cross-linking. One minute riboflavin diffusion time on either surface will saturate >12% of the collagen gel. Bonding strength doubled between PC collagen gel layers with a 5 minute increase in cross-linking time and break stress was increased significantly after cross-linking. Importantly, mechanical anisotropy was introduced in the break stress using orientated stripes of riboflavin in cross-linking, almost doubling the break stress parallel to the stripes. Limited riboflavin penetration in one minute means that surface photodynamic cross-linking will enhance deep cell survival within the gel, advantageous for tissue engineering purposes. Riboflavin mediated focal/orientated cross-linking generated new predictable anisotropy at the construct. The increase in

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bonding strength between layers after cross-linking enhances layer integration and graded surface stiffness will impact on cellular/mechanical properties of compressed gels.

26.P03
Layer-by-layer coating of electrospun fibres with collagens

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Electrospinning has become increasingly popular for fabricating nano- to micrometer diameter fibres for use in tissue engineering. Electrospun collagen would be an ideal scaffold, but there are significant concerns that electrospinning results in denaturation into gelatine, potentially losing many of collagen's advantages. This study reports the development of electrospun nanofibre materials coated with non-denatured collagen. Electrospun fibres from both resorbable (polylactic-co-glycolic acid and gelatin) and non-resorbable (polyacrylonitrile) materials were used. The surfaces of synthetic fibres were activated to provide a negative surface charge. The core fibres were coated with collagen, through a layer-by-layer method that used alternating layers of positively charged (native) and negatively charged (modified) type I collagen. Collagen layers were stabilised by crosslinking with EDC. Negatively charged type I collagen was made by various methods: deamidation and succinylation, which are irreversible changes, and maleylation and citraconylation which allow subsequent removal of the modifying groups. Spectroscopy confirmed the collagen coatings. Native and collagen-coated fibres were examined after 16 h in cell culture with L929 mouse fibroblasts. This showed that uncoated synthetic fibres had some attached cells but little or no spreading had occurred, whereas cells seeded on collagen coated fibres consistently showed good attachment and significant spreading.

26.P04
Improvement of collagen immobilization on electrospun PLLA and PCL for better cell attachment and proliferation

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Scaffolds made of electrospun synthetic polymers have been largely used in tissue engineering. Collagen (COL) immobilization is proposed as an alternative to increase their hydrophilicity. The goal of this study was to evaluate how three different methods of COL incorporation affect the cell proliferation in poly-L-lactide (PLLA) or polycaprolactone (PCL) electrospun scaffolds.Solutions of PLLA (5%) and PCL (15%) in chloroform were electrospun. The scaffolds were submitted to aminolysis (A) or hydrolysis (H), followed by COL immobilization with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) or glutaraldehyde (GT, only with A). COL quantification was performed by ninhydrin assay and cell proliferation was determined by MTT assay. The data were submitted to 1-way ANOVA.PLLA-H-EDC showed higher COL incorporation (1.5±0.2%) than PLLA-A-EDC (0.7±0.2%) and PLLA-A-GT (0.3±0.05%). COL concentration was higher in PCL-A-EDC and PCL-A-GT (1.6±0.5 and 1.3±0.07%) than in PCL-H-EDC (0.30±0.02%). Cell proliferation at 8th day in PLLA-H-EDC (3.9±1.1 absorbance unit, a.u) was similar to PLLA-A-GT (2.5±1.0 a.u) and higher that PLLA-A-EDC and PLLA (2.0±0.4 and 1.9±0.1a.u). PCL-A-EDC (4.1±0.5a.u) was similar to PCL-A-GT (3.2±0.2a.u) and higher than PCL-H-EDC and PCL (2.2±0.4 and 2.7±0.3a.u). A minimum of 1.5% of COL incorporation in PLLA/PCL scaffolds was needed to improve the cell adhesion and proliferation in these materials.

26.P05
Development of a novel atelocollagen vitrigel membrane aiming for the utilization in regenerative medicine

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A collagen vitrigel (CV) membrane is composed of high density collagen fibrils equivalent to connective tissues in vivo and possesses excellent strength, transparency and permeability of protein with high molecular weight. It functions as a scaffold for anchorage-dependent cells in both in vitro and in vivo. However, CV membranes have been prepared from bovine native collagen and culture medium containing fetal bovine serum (FBS) so far. In this study, as a first step aiming for the utilization in regenerative medicine, we attempted to develop an immunogenic telopeptide-free atelocollagen vitrigel (ACV) membrane combining excellent strength and transparency equivalent to native CV membrane using ruminant protein-free materials as simple as possible. Here, ACV membranes were prepared using porcine atelocollagen and three different solvents [culture medium containing FBS (CM-FBS), serum free-medium (SFM) and PBS] and were subjected to analyze above properties in comparison to those of traditional CV membranes. As a result, we succeeded in preparing transparent ACV membranes using every solvent and demonstrated that their strength was dependent to each solvent (CM-FBS > SFM >> PBS). Also, we confirmed that the ACV membrane using SFM could provide almost equal properties of traditional CV membrane using CM-FBS.

26.P06
Marine collagen scaffolds crosslinked 'in situ' with genipin for cartilage regeneration

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Nowadays, growing needs for improved treatments has motivated research on the use of marine sources for the development of health-related applications. In particular, a key biomaterial such as collagen can be extracted from several marine resources, namely fish skins, fish bones and others, with clear economic and environmental benefits. In addition, marine collagen has the advantage of presenting reduced risk of posing diseases to humans, in contrast to the one obtained from calf or porcine sources.In this study collagen based porous structures were obtained by freeze-drying of solutions of collagen extracted from shark (Scyliorhinus canicula) skin crosslinked 'in situ' with genipin, a natural crosslinker, after pre-determined reaction times. The ninhydrin assay showed that collagen structures with different crosslinking degree were produced, depending on the reaction time. This crosslinking of the structures was important to promote their stability, demonstrated in PBS and culture medium up to 4 weeks, which is needed to support cell culture and proliferation.The morphology analysis of the produced scaffolds showed a porous structure with 150–200um of pore size, which could be adequate for cell culture. Moreover, the characterization of the mechanical and biological performance of marine collagen structures suggested their use as scaffolds for cartilage regeneration.

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26.P07
A simple, rapid method to form biomimetic tissues with defined properties

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Plastic compression (now known commercially as RAFT) of cell-seeded collagen type I hydrogels is a simple, rapid way to make biomimetic tissues; liquid is removed by a combination of compression and absorption. The process is well suited to making tissues for cell therapy or drug discovery. A well controlled process is needed to make tissues with defined properties. However, multiple interdependent variables (collagen volume and concentration, compression and absorption parameters) can make it difficult to optimise. We now can control liquid removal, which allows the process to be streamlined, standardised and simplified and it can now be reproducibly and conveniently carried out in standard multi-well plates. We will present data mapping the independently varied critical process parameters including the volume of collagen, concentration and compression time in relation to the resultant tissue properties. We will show it is now possible to pre-select the desired tissue characteristics. This is a significant advance, as the technology can be simply adopted into routine use for research or for the development of novel cell-based and regenerative medicine therapies.

26.P08
Highly controlled parallel production of collagenous tissues of variable size and shape

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The plastic compression process, known commercially as RAFT™, has been in use in tissue engineering and regenerative medicine research since 2005. During that time it has been applied to a number of areas including cornea, skin, nerve, ureter and bladder which indicate a broad suitability for ongoing research. To support expansion of the RAFT™ technology we have carried out an extensive study which investigated various absorbent materials, whose function is to gently remove liquid from the hyperhydrated collagen gel. Techniques applied were gravimetric methods and scanning electron microscopy to characterise basic material properties, and a novel, non-invasive optical measuring system for thickness measurements of the produced RAFT™ tissues. This instrument also allows in situ tissue thickness measurement as liquid is removed, enabling monitoring of tissue formation in real-time. A range of absorbing materials has been tested and rules defined for pore size, porosity and surface tension of the absorber. We can now control liquid removal, which allows the process to be streamlined, standardised and simplified. It can now be reproducibly and conveniently carried out in parallel in standard multi-well plates or in a range of shapes and sizes for tissue engineering and regenerative medicine therapies.

26.P09
Characterization of collagen I scaffolds

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This work compared physical and chemical properties of collagen I unidirectional (UDS) and multi-directional (MDS) scaffolds and evaluated the effect of collagen concentration and crosslinking method in the following scaffold properties: water vapor sorption (WVS), water vapor permeability (WVP) and surface water contact angle (WCA). Data show that uncrosslinked scaffolds had the highest WVS followed by glutaraldehyde, dehydrotermal and genipin crosslinked scaffolds, respectively. We found that an increase in collagen concentration (2–8 mg/mL) resulted in a decrease of WVS and WVP. Also, that WVS was not affected by fiber orientation and that WVP was significantly lower in UDS than in MDS. The WCA values of the scaffolds tested were characteristic of hydrophobic materials. Dehydrotermally treated scaffolds showed the highest WVP followed by uncrosslinked, glutaraldehyde and genipin crosslinked scaffolds. Genipin treated scaffolds exhibited the highest denaturation temperature (Td). Overall, glutaraldehyde crosslinked scaffolds displayed the best WVS, WVP, WCA and Td. The scaffold thermal stability assessed by DSC analysis and the integrity of collagen triple helix evaluated by FTIR analysis indicated that after crosslinking collagen kept its native form.

26.P10
Novel technology for simple assembly of aligned 3D cellular collagen materials for tissue engineering

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Many tissues (e.g. nervous system, cardiac and musculoskeletal) have anisotropic structures and cellular alignment is fundamental to their function. Mimicking tissue anisotropy is important in tissue engineering, but achieving robust alignment of cells in a hydrogel matrix is challenging. Here we report a new technique, combining cellular self-alignment in collagen gels with a simple method of stabilising the aligned cellular gels, to produce biomimetic aligned tissues without the use of pre-formed scaffolds. Cells are seeded in 2 mg/ml type-I collagen gels and tethered at opposite ends of a rectangular mould. After 12–24 hours of incubation uniaxial tension develops within the collagen gel, resulting in cells aligning parallel to the axis of principal strain. We stabilise the aligned cellular construct using controlled compression and absorption (RAFT Real Architecture for 3D Tissue). The resulting tissues have physiologically relevant collagen concentrations and cells remain highly aligned even after removal of tethering. This approach, which can be scaled-up and automated, provides a powerful new way to produce aligned cellular biomaterials. We have developed and tested it using nervous system cells to provide Engineered Neural Tissue for repair and modelling of both the CNS and peripheral nerves. Our data demonstrate its broad potential for tissue engineering, where robust and stable cellular alignment is required for repair and as better tissue models for research.

26.P11
Collagen gel with aligned fibers modulate electric field-directed migration of ligament fibroblasts

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Many cells reside in complex and anisotropic microenvironments in vivo. A major extracellular matrix component, type I collagen forms fibers in vivo via a nucleation process which can be controlled with flow. We established a microfluidic system to generate collagen gels with aligned fibers and cultured porcine anterior cruciate ligament (ACL) fibroblasts on the gel. 65.2% of the cells exhibited alignment to the direction of the fiber (compared with 11.7% in the random group). When subjected to DC electric fields (EFs, 6 V/cm), cells in the random collagen group exhibited enhanced motility (increased migration speed) and directionality (toward the cathode). Interestingly, when EF

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was applied parallel to cells seeded on aligned collagen fibers, this directionality was abolished while speed remains improved. This finding contradicts previous studies in corneal epithelial cells [Rajnicek et al., 2007]. When RhoA activity was attenuated with C3 transferase, EF-induced directionality was restored on aligned collagen fibers without changes to cell motility. Our results indicate the involvement of RhoA in contact guidance and EF-induced directionality. Furthermore, as collagen gel is considerably softer than the quartz substrate used in the previous corneal studies, material stiffness may also play a role in these interactions. EFs promote wound healing clinically and the current study present possible insights into the healing mechanisms.

26.P12
A 3D synthetic model of collagen fibers with nanohydroxyapatite grafted with SPARC for bone regeneration and prevention of cancer related bone metastasis.

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Synthetic hydroxyapatite (HA) nanoparticles are interesting scaffold materials in bone tissue engineering due to their structural similarity with biological apatite and to their osteogenic properties.[1] SPARC (secreted protein, acidic and rich in cysteine) is a glycoprotein that works as a modulator of cell-matrix interactions and is associated with bone tissue development, remodeling and repair, cell turnover and bone mineralization. [2] Additionally, SPARC controls important mechanisms involved in cancer development and progression and seems to be relevant in metastatic dissemination capacity of both prostate and breast cancer cells into bone and lung tissues. However, its specific role is still not fully understood. [3–4] The novelty of this work relies on the design of a 3D synthetic model that mimics bone (collagen fibers with nanoHA) capable of being grafted with SPARC peptides involved in bone metastization. This may be promising in bone regeneration and in the development of counter adhesive treatment strategies against metastatic bone tumors.

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26.P13
A novel material of high density collagen fibrils: A collagen xerogel membrane and its application to transplantation in vivo and a culture chamber in vitro

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A collagen vitrigel (CV) membrane is composed of high density collagen fibrils equivalent to connective tissues in vivo and is easily handled with tweezers. Also, it possesses excellent transparency and permeability of protein with high molecular weight and consequently the various researches utilizing it as a cell culture substratum advances so well. However, the thickness of CV membrane is 15–20 micrometer and it required a nylon frame to avoid self-twisting. In this study, we aimed to develop a CV membrane with an excellent handling ability and to examine its utility in cell culture and transplantation experiments.Here, we developed a mass fabrication technology of not only a thin but also thick CV membrane as a novel material attached to a parafilm in a dried state that easily peel off. The dried CV membrane detached from the parafilm was considered as a collagen xerogel (CX) membrane with an excellent handling ability. Such a CX membrane was easily converted into a CV membrane by the rehydration. The thick CX membrane is transplantable to a rat extensive skin defect by directly suturing it with surgical ligature. Also, we developed a CV membrane chamber by pasting the thin CX membrane onto an edge of plastic tube. The CV membrane chamber was useful for reconstructing culture models such as 'tissue sheet' composed of epithelial cells alone or endothelial cells alone and 'organoid plate' composed of epithelial cells, mesenchymal cells and endothelial cells.

26.P14
Adipogenesis using human adipose tissue-derived cells combined with collagen/gelatin scaffold sustained release of basic fibroblast growth factor

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Introduction: We have developed collagen/gelatin scaffold (CGS) that can provide the sustained release of basic fibroblast growth factor (bFGF). We proved that CGS impregnated with the appropriate dosage of bFGF accelerates dermis-like tissue formation two or three times earlier than existing artificial dermis. In this study, we disseminated ASCs on CGSs impregnated with bFGF to aim at developing efficient measures for adipogenesis.
Method: Human ASCs were primarily isolated from human adipose tissues that were obtained in breast cancer surgery with informed consent at Kyoto University Hospital. ASCs were isolated from collagenase digests of adipose tissue. We impregnated CGSs (8 mm in diameter, 3 mm in thickness) with bFGF (0.1, 1, 7,14 $\mu g/cm^2$) or normal saline solution. Then, we disseminated cells (passage 3) on CGSs at a seeding density of $1x10^5$ cells/cm2 and implanted them into the back subcutis of nude mice. Six weeks after implantation, adipogenesis at the administered site was evaluated.
Results: Newly-formed adipose tissue was observed in all groups macroscopically and histologically. The weight of regenerated adipose tissue was largest in the 1 $\mu g/cm^2$ of bFGF group.
Conclusions: In this study, we showed that our CGS impregnated with low-dose bFGF could be used as a scaffold with ASCs for adipogenesis.

26.P15
Quantifying spatial and temporal variation in cell function in collagen constructs for tissue engineering applications

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The successful design of any tissue engineered construct requires: biocompatibility, integration once implanted in vivo, material support of the cell population, mechanical integrity and ability to mimic features of native tissue architecture. These criteria present a significant challenge that we are incrementally addressing through the use of plastic compression to produce dense collagen scaffolds that can mimic the fibril density found in native tissues. The matrices produced have good biomimetic and functional mechanical properties where compliance and strength are concerned.This study quantifies cell fate (death,

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proliferation and migration) at different spatial positions throughout the plastic compressed collagen type I scaffold over 10 days. Three segments of the compressed collagen matrix (core, middle and outer) were analysed by quantifying cell death and proliferation, using live/dead and proliferation assays. Through combining this information with oxygen tension data through the 3D construct, quantitative information on the relationship between cell fate and oxygen tension is obtained. Moreover these results validate and test a mathematical model, which allow the extraction of continuous data on cell fate in time and space.The impact of these findings on construct design will be explored; in particular, the potential for introducing channels to mimic a vascular supply will be investigated to augment nutrient delivery to the core for larger constructs.

26.P16
Long term evaluation of laminar absorbable prosthetic materials in the regeneration of the abdominal wall

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Introduction: Laminar absorbable prostheses have been added to the materials available for the repair of hernial defects in the abdominal wall. They are gradually degraded in the host resulting in the formation of a neotissue. Aim: assess the long term behaviour of collagen and synthetic (polyglycolic acid/trimethylene carbonate) absorbable prostheses.
Methods: Hernial defects were created in the abdominal wall of 24 rabbits and repaired using collagen (Tutomesh®/Strattice®) and a synthetic (BioA®) prostheses. 180 days post-implant, specimens were taken for light and scanning electron microscopy to determine tissue regeneration. The macrophage response was assessed by immunohistochemistry.
Results: Tutomesh, the thinnest bioprosthesis, became easily infiltrated by host tissue and at 180 days was almost complete degraded and substituted by a loose connective tissue. The thickest prosthesis (Strattice), revealed encapsulation of the material, showing cell infiltration only in the outermost area. However, the synthetic one (Bio-A), similar to Strattice thickness, showed great degradation, with small traces of copolymer in a rich adipose neoformed tissue. A significant increase of the number of macrophages was observed in the synthetic prostheses.
Conclusions: Thickness of bioprostheses determines tissue infiltration. Synthetic absorbable meshes showed higher rate of degradation and tissue regeneration, but more intense inflammatory reaction at long term. DPI2011-27939/FMM2008

26.P17
The influence of collagen density in cellular distribution

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Adequate cellular in-growth into biomaterials is one of the fundamental requirements in regenerative medicine. Type-I-collagen is the most commonly used material for soft tissue engineering, because it is non-immunogenic and a highly porous network for cellular support. However, adequate cell in-growth and cell seeding has been suboptimal. Different densities of collagen scaffolds (0.3% to 0.8% (w/v)) with/without polymer knitting (poly-caprolactone (PCL)) were prepared. The structure of collagen scaffolds was characterized using scanning electronic microscopy (SEM) and HE staining. The mechanical strength of hybrid scaffolds was determined using tensile strength analysis. Cellular penetration and interconnectivity were evaluated using fluorescent bead distribution and human bladder smooth muscle cells and urothelium seeding. SEM and HE analysis showed the honeycomb structure and the hybrid scaffolds were adequately connected. The hybrid scaffolds were much stronger than collagen alone. The distribution of the beads and cells were highly dependent on the collagen density: at lower densities the beads and cells were more evenly distributed and penetrated deeper into the scaffold. The lower density collagen scaffolds showed remarkably deeper cellular penetration and by combining it with PCL knitting the tensile strength was enhanced. This study indicated that a 0.4% hybrid scaffold strengthened with knitting achieved the best cellular distribution.

26.P18
Autologous keratinocyte transplantation in recombinant human collagen III for treatment of full thickness porcine wounds

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Introduction: Full thickness wounds require rapid filling of the defect in order to support dermal regeneration. Cultured keratinocyte auto grafts (CEA) are used to deliver large amount of autologous cells to skin wounds. Recombinant techniques have enabled various fibrillar structures such as the wound healing-associated collagen type III to be produced feasibly in large quantities offering alternatives for materials of animal origin. Study aim: Our aim was to evaluate biocompatibility of recombinant human collagen type III (rhCol-III) in CEA treatment using a porcine full thickness biopsy-wound model.
Material and methods: Full-thickness skin wounds were created with a punch biopsy on the back of two domestic pigs, fourteen wounds per pig. Before the operation, autologous fibroblast and keratinocyte cultures were established from skin biopsy. Treatment was initiated by application of a rhCol-III hydrogel (n=8) with pre-cultured keratinocytes (n=8) or keratinocytes and fibroblasts (n=8). Untreated wounds were used as control for the treatment groups (n=4).Two EGF-receptor tyrosine kinase inhibitors; PD153035 and AG1478 were used to show EGF-linked effect of fibroblast.
Results: RhCol-III was biocompatible for transplantation did not delay wound healing or cause a marked inflammatory response. In conjunction with autologous keratinocytes it improved wound healing through increased granulation tissue.

26.P19
Sequential-release of nucleic acid from a collagen scaffold with hollow microspheres that avoid phagocytosis by activated macrophages

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Precise control over the temporal and spatial presentation of signaling cues is essential to understand the role these factors play in dynamic cellular processes. Hollow microspheres offer new opportunities to study anisotropic signaling and direct tissue morphogenesis. However, a major issue with microspheres is macrophage clearance. The purpose

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of this study is to design a sequential-release system to avoid macrophages. The specific objectives were to fabricate hollow microspheres, identify a size to avoid phagocytosis, embed microspheres within a scaffold, characterize the release profile, and assess the delivery of anti-inflammatory and pro-angiogenic signalsin vivo. Collagen hollow spheres (CS) were fabricated (100nm, 1 and 10μm) using a novel template method. Microscopy showed CS weremonodispersed, while pDNA complexes showed efficient loading andprolonged release. Transfection studies showed the ability of released complexes to transfect fibroblasts, and reduced toxicity when unloaded polyplex doses were compared. FACS and microscopy analysis of uptake by THP-1 cells identified 1 and 10μm CS as appropriate reservoirs and hence used to study the release from the CHS/hydrogel system.Initial analysis of in vivo samples shows minimal inflammation and signs of increased angiogenesis in a temporal manner matching the delivery profile. Further analysis will determine the effect on inflammatory and angiogenic signals.

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27. Extracellular Matrix and Cells from Natural Tissues (in coop. IPLASS)

27.01
Keynote: Hepatic cell-human amniotic epithelial cell (hAEC) interactions leading to ECM degradation

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Background: Chronic inflammation leads to hepatic cirrhosis with extensive ECM deposition (fibrosis). Infusion of placenta derived hAEC into cirrhotic mice reduces hepatic inflammation/ECM deposition, but the mechanisms are unknown. Objectives: Determine whether infused hAEC reduce inflammation/fibrosis via secreted factors that act on hepatic macrophages and ECM depositing hepatic stellate cells (HSC).
Methods: C57BL/6 mice were given carbon tetra chloride(CCl4) over an extended period to induce cirrhosis. hAEC were infused IV and mice culled 4 weeks later. Effects on hepatic macrophages and HSC were studied in excised livers and in vitro.
Results: Some hAEC remained engrafted in mouse livers.Treated mice had significantly lower numbers of hepatic macrophages that may be due to altered levels of monocyte recruiting chemokines. Macrophage phenotype changed from classically activated M1 to alternatively activated M2 that has been linked to fibrolysis. HSC showed reduced proliferation, activation, pro-fibrogenic cytokine and collagen output and increased apoptosis.In vitro studies showed that factors secreted by hAEC including activin A,BMP-1, PGE2 and HLA-G may regulate the effects on hepatic cells.
Conclusion: hAEC infusion induces a fibrolytic macrophage phenotype and together with multiple effects on HSC contributes to increased ECM degradation.These findings suggest that hAEC may have therapeutic potential for treating established fibrosis resulting from chronic inflammation.

27.02
Mimicking the properties of fetal cardiac extracellular matrix to stimulate cardiomyocyte proliferation

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Children born with serious congenital heart defects (CHD) require major reconstructive surgery to survive. In contrast to mature cardiomyocytes (CMs), fetal CMs are highly proliferative, and this behaviour is likely modulated by the environment of the developing heart. We hypothesized that recapitulation of the fetal cardiac extracellular matrix (ECM) environment would stimulate the proliferation of CMs. Hearts were harvested from rats at fetal (E15–E19), neonatal (P1–P3), and adult (3–6 mo.) time points and ECM properties were investigated using Western blot (composition) and uniaxial tension tests (mechanics). ECM was isolated from hearts via perfusion of sodium dodecyl sulfate (SDS), solubilized in pepsin-HCl, and adsorbed onto culture dishes. Neonatal rat CMs were seeded onto ECM-coated substrates, and assayed for proliferation (PHH3 & cardiac α-actinin double staining) after 24 hr and 1 wk. After 24 hr, CM-specific proliferation was significantly higher on fetal ECM compared to adult. After 1 wk, CM-specific proliferation was significantly higher on fetal ECM compared to both adult ECM and bare culture dishes, although proliferation overall was decreased. Significant changes to ECM composition (notably Col V and periostin) throughout the age range studied are currently being investigated as potential mechanisms for the observed effect as is the role of stiffness in this process. Our results suggest that ECM promotes CM proliferation in an age-dependent manner.

27.03
Decellularised bone gels – novel materials for bone regeneration

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The objective of this work was to develop new material platforms from decellularised bone that can be used to successfully bridge and heal critical sized bone defects. Although demineralised bone has been used as graft material, the use of decellularised bone for bone regeneration has not been attempted. Scaffolds composed of extracellular matrix (ECM) have been shown to provide cues for in vivo cell migration, proliferation and differentiation. The advantages of maintaining tissue specificity in repair and regeneration has been demonstrated with scaffolds derived from liver and respiratory tract ECM but not yet for bone. Bovine femurs were sorted into cancellous fragments prior to treatment with in-house developed demineralisation and decellularisation protocols. Histological evaluation showed that cellular fragments were present in demineralised bone (DBM) but were removed by the decellularisation process. A pepsin digest and solubilisation procedure was undertaken with the DBM and decellularised bone (bECM) to form gels. Characterisation of the gels included assessment of turbidimetric gelation kinetics and rheological behaviour; the surface morphology was examined with scanning electron microscopy. The proliferation of human osteosarcoma cells on collagen, DBM and bECM gels of different concentrations (3 and 6 mg/ml) was also assessed. Cellular proliferation on DBM and bECM gels was equivalent or greater to that observed on collagen gels.

27.04
Catheter deliverable extracellular matrix derived hydrogel for treating myocardial infarction

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Injectable materials present the exciting opportunity of minimally invasive therapy to prevent heart failure by providing a temporary scaffold to replace the damaged extracellular matrix (ECM) that occurs post-myocardial infarction (MI). To date, no material appropriately mimics the ECM composition and structure and most have failed to translate to catheter-based delivery. We have developed an injectable form of myocardial ECM that retains native proteins, peptides, and polysaccharides, and self-assembles into a nanofibrous gel upon injection into the myocardium. We have recently demonstrated that this injectable myocardial matrix preserves left ventricular (LV) volume and cardiac function post-MI in a rat model, as well as increases post-MI endogenous cardiomyocyte survival. We have also shown that the myocardial

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matrix can be delivered via a percutaneous transendocardial approach using the NOGA guided Myostar catheter and improves LV volume and cardiac function in a porcine MI model out to 3 months. Furthermore, we did not observe an increase in arrhythmias in either the rodent or pig model. This acellular scaffold has the potential to be an effective minimally invasive treatment for MI, providing the appropriate extracellular environment to replace the damaged ECM. The material has also been shown to increase maturation of human embryonic stem cell derived cardiomyocytes, and thus also has potential as a scaffold for cell-based cardiac tissue engineering.

27.P01
Human placenta derived extracellular matrix scaffolds for skin regeneration

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Human placenta extracts and amniotic membranes have been reported to reduce inflammation, promote epithelialization, and prevent scarring because they contains abundant extracellular matrix (ECM) components and growth factors that are important during pregnancy. Herein, human placenta-derived ECM (HPECM) is presented as a promising material for regeneration of serious wounds. HPECM was extracted from whole placenta via homogenization and decellularization. The final product was fabricated into a sheet by molding and freeze-drying. HPECM sheet with porous structure exhibited good mechanical properties. In addition, the sheet retained the ECM component and various growth factors. The structure and composition feature of HPECM scaffolds dramatically supported the growth of human adipose-derived stem cells in vitro. In full-thickness wound of rat, the degree of wound healing with the HPECM scaffold implantation was higher than that without the scaffold. Moreover, HPECM scaffolds produced skin substitutes with similar cellular organization of normal skin and reduced wound contraction, which may lead to reduced morbidity in patient outcomes. These results suggest that the HPECM scaffold provides a good microenvironment and accelerate healing of the wound site.

27.P02
The relationship between the molecular and ultrastructural properties of amniotic membrane scaffolds and urothelial cell growth

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Various strategies have been proposed over the years for the regeneration of the urothelium. Many potential scaffolds have been trialed, including amniotic membrane (AM), but understanding of the relationship between molecular and ultrastructural properties of scaffolds and urothelial cell (UC) growth as well as knowledge of UC interactions with scaffolds is still in its infancy. Cryopreserved human AM was used as scaffold in three different ways. Normal porcine UCs were seeded on the amniotic epithelial monolayer (intact AM), the basal lamina (denuded AM) or the stromal matrix (AM stroma) and were cultivated for 3 weeks. UC growth on AM scaffolds was monitored daily. Molecular and ultrastructural properties of AM scaffolds were determined by histochemistry and electron microscopy. Molecular and ultrastructural analysis confirmed presence of collagens, neutral and acid proteoglycans in the basal lamina and the stromal matrix of AMs. After 3 weeks, AM scaffolds were mainly acellular, but retain the specific molecules and morphology. The growth of UCs was the fastest on AM stroma scaffold, where the basal lamina between stromal matrix and urothelium was formed de novo. Using AM, we introduced a new approach for stimulation of UC growth. De novo formed basal lamina on AM stroma provides a natural substrate for UC growth and further differentiation. By promoting UC attachment, proliferation and differentiation, AM presents a promising scaffold for use in reconstructive urology.

27.P03
Injectable micronized amniotic membrane attenuates the progression of osteoarthritis

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Amniotic membrane is a promising intervention for osteoarthritis (OA) due to its anti-inflammatory and anti-fibrotic properties along with low immunogenicity. The objective of this study was to quantitatively assess the efficacy of injectable micronized human amniotic membrane (AmnioFix Injectable, MiMedx Group, Inc.) as a disease modifying intervention in a surgically induced rat model of OA. Medial meniscal transection surgery was performed in Lewis rats. 24 hours after surgery, amnion or saline was injected intra-articularly into the stifle joint. Animals were euthanized at 72 hours or 21 days. Cartilage attenuation, thickness, and lesion volume were quantitatively assessed using EPIC-uCT and H&E and Saf-O staining. At 21 days post-surgery, amnion treatment completely inhibited lesion formation and reduced fibrillation occurrence as compared to saline treated animals. Similarly, amnion treatment reduced cartilage attenuation, indicating increased proteoglycan content, by 78% compared to saline treatment. Histological staining of treated stifle joints showed the presence of amnion in the synovium at 3 and 21 days post-surgery. This tissue incorporation and residence time make injectable micronized amnion a promising delivery vehicle for additional pharmacological interventions or in vivo contrast agents. This study demonstrated that injectable micronized amniotic membrane is an effective treatment which attenuated the progression of OA.

27.P04
Decellularized human placental vascular scaffold provides a platform for tissue and organoid engineering

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Introduction: Decellularized natural vasculature is a suitable material for tissue and organ engineering. Human placenta is a large vascular organ. We established a method to decellularize whole placentas to produce decellularized human placental vascular scaffold (DHPVS) and examined its vascular integrity.
Methods: Placentas stored at –80°C were thawed and perfused with Triton X-100 and SDS. Decellularization was assessed by DNA content and H&E staining. Fluid and cell conductivity was examined by infusing dye or luciferase labeled cells into DHPVS. Cell distribution was analyzed using a Xenogen IVIS Spectrum.
Results: DHPVS was a semi-transparent tissue matrix retaining anatomical characteristics of a placenta with few remaining intact nuclei. DHPVS had significant reduction in DNA content compared to untreated placentas. Trypan blue dye infused into DHPVS distributed from the umbilical cord to the edge of the placental disc, indicating that large, small and micro vessels were intact. Luciferase labeled cells infused into DHPVS distributed through most of the placental vasculature. Dynamic cell distribution was observed when incubating DHPVS for two hours after cell infusion. DHPVS was used as an incubator to culture cells by circulating medium with a peristaltic pump through the vascular scaffold.

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Conclusions: DHPVS efficiently distributes cells and fluid and can be used as a platform for tissue engineering to generate constructs requiring cell repopulation.

27.P05
Anti-fibrotic effects of fresh vs. cryopreserved amniotic membrane

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The human amniotic membrane (hAM), fresh or preserved, has been successfully clinically applied to treat ocular diseases and ulcers, due to its anti-scarring, anti-inflammatory, and wound healing properties. We recently demonstrated that fresh hAM, used as bandage on the liver surface, is able to reduce liver fibrosis induced by bile duct ligation (BDL) in rats. The aim of this study was to explore if hAM retains the ability to reduce liver fibrosis after cryopreservation, which allows for a long-term storage and biological safety. hAM was used either fresh, within 24h from peeling from placenta, or preserved, frozen onto a carrier material, in cryomedium with DMSO. Pieces of fresh or thawed hAM were applied on the liver surface of rats after BDL. After six weeks, the degree of liver fibrosis was assessed histologically by the Knodell and METAVIR score systems. Image analysis was used to quantify the liver area occupied by ductular reaction, extracellular matrix (ECM), activated myofibroblasts, and hepatic stellate cells (HSCs). Although cryopreservation reduced viability of hAM cells, fresh and cryopreserved hAMs displayed similar microscopic features and comparable anti-fibrotic effects. In all treated rats, liver fibrosis, ductular reaction, ECM deposition, and the number of activated myofibroblasts and HSCs were significantly reduced.Our findings demonstrate that the ability of hAM to reduce liver fibrosis could be maintained after appropriate cryopreservation.

27.P06
Heat-dried human placental ECM supports human amnion derived adherent cell (AMDAC) growth and induces secretion of growth factors and cytokines

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Introduction: Amnion Derived Adherent Cells (AMDAC) are isolated from the amnion and expanded in culture (US Patent Application 2010/0124569). AMDACs are CD200+, CD105+, CD10+, OCT4- and CD34- and are immunosuppressive as shown in a mixed lymphocyte reaction (MLR). Their secretion profile suggests relevance in wound healing applications. We previously described the isolation of an extracellular matrix from human placenta (ECM) which can be formulated as sheets (Bhatia MB, Wounds 20, 29, 2008). Here we examine a combination of ECM with AMDACs as a potential wound healing product.
Methods: ECM was isolated from placentas using methods described previously. The resulting ECM paste was vacuum dried into sheets. AMDACs were isolated from the amnion via enzymatic digestion and culture expanded. AMDACs were seeded on ECM sheets and cultured 4–6 days. Cell proliferation, morphology and secretion profile were examined using the MTS assay, Calcein AM/PI staining and multiplex ELISAs.
Results: AMDACs demonstrated strong binding and proliferation on the ECM sheets. AMDACs bound to ECM secreted high levels of growth factors and cytokines including VEGF, PDGF, b-FGF and IL-8, which are relevant in wound healing cascades.
Conclusion: We have demonstrated the combination of a placental ECM formulated as a stable sheet with AMDAC; a possible utility of this construct is being examined in wound healing animal models.

27.P07
Wharton's jelly mesenchymal stem cells differentiation towards hepatocyte-like cells: in vitro evidences

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Wharton's jelly mesenchymal stem cells (WJ-MSC) can differentiate into diverse cell types, showing a unique ability to cross lineage borders. This, together with their in vitro proliferative potential and their immunomodulatory features, renders these cells promising for regenerative medicine applications in different pathological settings. We exposed WJ-MSC to differentiative factors at different time points several parameters were evaluated: markers expression at both protein and mRNA level, PAS staining for glycogen, Glucose-6-phosphatase activity, CYP4503A4 activity, Cardiogreen uptake.WJ-MSC were successfully differentiated towards hepatocyte-like cells expressing mature hepatocyte functions. We demonstrated that the differentiative protocol applied led to the expression of several markers specific of the hepatocyte lineage, as demonstrated by the expression of albumin, alpha-1 antitrypsin, and the cytokeratin switch. Moreover, at all the timepoints, hepatocyte-like cells showed cytoplasmatic glycogen accumulation, were able to metabolize glucose-6-phosphate, showed inducible CYP4503A4 activity following rifampicin challenge, and were able to uptake indocyanine green from the culture medium.The results obtained so far strongly suggest that WJ-MSC can constitute extrahepatic candidates for cellular therapy strategies aiming at liver regeneration and repopulation. Further work is underway to apply these cells in a preclinical model of swine liver failure.

27.P08
Expressions of pituitary tumor transforming genes (PTTGs) family in placenta-derived stem cells (PDSCs) control self-renewal through regulation of p53 expression

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Pituitary tumor transforming gene (PTTG) is an oncogen highly expressed in a various tumors. Placenta-derived stem cells (PDSCs) are characterized by their capacity to differentiate into hepatic lineages as well as mesenchymal lineages. However, the expression and the function of PTTG family in PDSCs have not been explored. So, we analyzed the expression of PTTGs including PTTG1, -2 and -3 in PDSCs and evaluated their function. Expressions of PTTGs by transfection for PTTGs plasmid or siRNA were analyzed by RT-PCR and Western blot in PDSCs. All of PTTGs family expressed in naïve PDSCs, however, their expression were decreased in differentiation of PDSCs into three germ layers.

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PTTG-2 and PTTG-3 expressions more dramatically decreased in hepatogenic differentiation of PDSCs. Otherwise, PTTGs expressions were increased in PDSCs transducing human TERT gene using lentiviral gene delivery system. Interestingly, the expression of PTTG2 was shown to increase in late passage of PDSCs. Down-regulation of PTTG1 and PTTG2 in PDSCs by siRNA inhibited self-renewal activity through decreased p53 expression. Taken together, the dynamic expressions of PTTGs in PDSCs regulate self-renewal as well as potential for differentiations. Therefore, these results not only further our understanding of the potential roles of PTTGs in PDSCs, but also contribute to the foundation for a potential new treatment strategy of cell therapy.

27.P09
Preparation of decellularized tissues: The importance of source animal age and thoroughness of decellularization upon the remodeling outcome

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Extracellular matrix (ECM) derived from decellularized tissues is increasingly being used for the reconstruction of injured tissues. Despite known variations in tissue remodeling outcomes, there is a paucity of quantitative studies which relate the source tissue properties or method of preparation to the remodeling outcome. The objectives of the present study were to evaluate: 1) the effects of source animal (pig) age (3, 12, 26 or >52 weeks), and 2) the effects of ineffective decellularization of source tissue (small intestine (SIS)) upon the in-vivo remodeling response. ECM strength, thickness and degradation resistance all increased with age. SIS-ECM from younger animals promoted progenitor cell chemotaxis while SIS-ECM from >52 week old animals promoted cell proliferation. More aggressive decellularization promoted a shift in macrophage phenotype from M1 to M2. Increasing SIS-ECM age was associated with an increasingly M1 host response and less constructive tissue remodeling. SIS-ECM from younger animals promoted the formation of new, innervated and vascularized, skeletal muscle tissue while SIS-ECM from old animals formed dense collagenous tissue in a rat model of abdominal wall reconstruction. It is clear that the age of the source animal and extent of decellularization are important determinants of a constructive tissue remodeling outcome.

27.P10
Optimization of physical tissue decellularization methods. A histological study

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Introduction: Decellularization consists on cell elimination without mechanical disruption of the extracellular matrix (ECM). The aim of this work is to evaluate two physical methods of tissue decellularization based on ultraviolet light (UV) and ultrasounds (US) exposition.
Methods: Mouse tissues were excised and washed in PBS. UV or US exposition during 10, 20 or 30 minutes were used as decellularization methods, with agitation. Histological analyses were performed by using 4',6-diamidino-2-phenylindole (DAPI), Gomori reticulin and Picrosirius staining. Statistical analysis was carried out by using student's t-test.
Results: Results of DAPI assay revealed that both methods were able to remove most of the cells from the tissues (95.10% for US and 96.87% for UV). Picrosirius stain demonstrated that US was able to preserve more efficiently the ECM collagen fibers (84.98%) than UV (73.46%) ($p=0.000$). Gomori reticulin staining showed that reticular fibers were better preserved using US (100%) than UV (83.54%) ($p=0.000$). No significant differences were found for different times (10, 20 and 30 minutes) for US or UV techniques.
Conclusion: US and UV techniques demonstrated to be efficient decellularization agents. The use of US seems to preserve more adequately the fibers of the ECM and should therefore be preferentially used in tissue engineering.
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27.P11
Preparation of biological tissue-polymer complex for percutaneous device

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Peritoneal dialysis is usually executed by implanting the flexible catheter directly the skin and peritoneal cavity. However, the problem with the flexible catheter is that the compatibility of the polymer and the skin tissue is too low. This often causes the down-growth of the epithelial cells which eventually causes the infection or even avulsion of the device. We tried to overcome this problem by preparing a percutaneous device based of a 'biological tissue-polymer complex', which possesses tissue compatibility and maintainability of the flexible catheter within the device at the same time. For the base material, the decellularized dermis was prepared by high hydrostatic pressure (HHP) method. By soaking the methyl methacrylate (MMA) monomer, benzoyl peroxide and N,N-dimethyl-p-toluidine into the decellularized dermis, we polymerized MMA at room temperature to obtain a dermis-poly(methyl methacrylate) (PMMA) complex. Scanning electron microscope images showed that PMMA formed a stable complex with the collagen fiber within the dermis. This complex induced the increase in the mechanical strength upon compression. It was possible to control the polymerization area limiting the central part of the decellularized dermis. So we prepared a tissue-polymer complex with the flexible catheter firmly held by the stiff PMMA in the middle of the dermis. We believe this complex is a promising material for percutaneous device which can be brought to the clinics in the near future.

27.P12
Marine sponges as natural scaffolds: decellularization by supercritical fluid technology and cellularization with osteoblasts for tissue engineering applications

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Marine sponges possess remarkable structures, with highly interconnected pores, making them excellent candidates as nature made scaffolds with potential use in tissue engineering strategies. In addition they also exhibit interesting high swelling degree and a skeleton composed mainly of collagen fibres, in some species with embedded siliceous or carbonate spicules. In the present study, several marine sponges (Dysidea avara, Chondrosia remiformis, Axinella damicornis,

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Petrosia ficiformis, Agelas oroides, Sarcotragus spinosulus, and Psammocinia sp.) were decellularized by supercritical fluids, for further use as natural scaffolds for osteoblasts. Supercritical fluid extraction was performed at 200 bar and 40 °C for 6 hours, in the presence of ethanol (40%), which was used as a co-solvent. The morphology of sponges, namely porosity interconnectivity and mean pore size, was analysed by SEM and micro-CT. To evaluate decellularization, sponges before and after treatment were analysed in sections stained with Hematoxylin-Eosin. Cellularization experiments were performed in S. spinosulus, using Saos-2 cell line. Cells were seeded on the sponge, with further evaluation of cell morphology by SEM, cell viability by Live/dead cell staining together with phalloidin.and cell proliferation by DNA quantification.The results of in vitro tests demonstrate the success of the proposed methodology for the production of natural scaffolds from marine sponges, namely for Sarcotragus spinosulus.

27.P13
Effect of urinary bladder matrix on the in vitro performances of human mesenchymal stem cells

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Biomaterials, including natural extracellular matrix (ECM), have been proposed as scaffold for tissue engineering. It is important to consider that tissue morphogenesis is influenced by the interaction of cells with the complex architecture/composition of natural ECM. Simple polymers such as collagen, gelatine and fibronectin, have been used for cell seeding/entrapping and in vivo implantation, providing mechanical support to cells, but do not adequately mimicking the interactions between cells and ECM. On the contrary, ECM-based biomaterials can provide a native framework for cell adhesion, at the site of a tissue deficit, allowing local cells to migrate into the matrix, adhere and differentiate. The use of urinary bladder matrix (UBM) as scaffold material can provide the structural support and dynamic exchange signals to local cells leading to tissue infill. The major constituents of UBM are collagen, glycoproteins, glycosaminoglycans and various growth factors. This study describes the ability of UBM based scaffolds to support the adhesion, proliferation, 3-D colonization and osteogenic differentiation of human mesenchymal stem cells form human umbilical Wharton's Jelly (hWJMSCs), when compared to traditional two-dimensional (2-D)cultures. Our data strongly suggest the combination between UBM and hWJMSCs represents a promising approach to the use of natural and biocompatible ECM-based biomaterials for cell culture of mesenchymal stem cells.

27.P14
Extracellular matrix derived from kidney regulates the growth and metabolism of kidney stem cell with regional specificity

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Extracellular matrix (ECM) provides cells with an ideal scaffold with specific cues that mediate cell function. Stem cells are known to reside on specialized ECM niches where they remain quiescent until needed, such as stem cells in the papilla region of the kidney. Currently it is not possible to re-create the complex environment of the kidney using synthetic materials. Therefore, our objective was to use the native ECM to investigate if there were region specific effects on the growth of kidney stem cells (KSCs) and mouse mesenchymal stem cells (MSCs). We derived hydrogels from three kidney regions: cortex, medulla and papilla. MSCs were purchased and KSCs were isolated from the renal papilla of mouse kidneys. Porcine kidneys were sectioned into: cortex, medulla and papilla regions. Each region was decellularized with 0.02% trypsin, 3% Tween, 4% deoxycholic acid, and 0.1% peracetic acid solutions followed by enzymatic digestion. Cells were cultured on the hydrogels or in media supplemented with digested ECM. Metabolic activity, image analysis and DNA quantification were performed. KSCs cultured in the presence of papilla ECM showed higher metabolic activity and lower DNA content when compared to the other ECMs, an effect not observed using MSCs. These results suggest that the hydrogels derived from the native kidney ECM stimulate KSCs but not MSCs. Region-specific ECM may thus provide an optimal substrate for cultivation and delivery of stem cells and their derivatives.

27.P15
Optimizing decellularization of porcine kidneys for whole organ bioengineering

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Kidney transplantation is currently the only treatment that can restore renal function, however, donor supply meets less than one-fifth of demand. Regenerative medicine offers an alternative through whole organ decellularization and recellularization. The goal of this study was to evaluate detergents for effective decellularization of porcine kidneys and subsequent sterilization methods. Kidneys from Yorkshire pigs were obtained then decellularized with various detergents (SDS, SDC, Triton X-100, NP-40 or Tween-20) followed by rinsing. Resultant tissue samples were quantified for residual DNA and sGAGs. Whole acellular scaffolds were either gamma-sterilized or perfused with chemical sterilants with biopsies placed in growth medium to determine contamination or processed for histology. Only SDS was capable of removing cellular material as demonstrated by a lack of nuclear staining and a 99% reduction in DNA levels compared with fresh tissue. SporGon treatment or gamma-irradiation ≥1MRad sterilized whole kidney scaffolds, however, this resulted in morphological changes to acellular tissue. Sulfated GAGs were not significantly reduced following 1MRad irradiation. These studies show that SDS detergent perfusion removes all cellular material from porcine kidneys. 1MRad gamma irradiation provided a sterile scaffold which demonstrated human cell biocompatibility. This study represents a significant step towards a transplantable graft from porcine-derived acellular whole kidneys.

27.P16
A critical evaluation of kidney extracellular matrix after perfusion decellularization as a structural basis for renal tissue engineering

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The kidney is the most frequently transplanted organ, yet few studies have focused on kidney tissue engineering. We compared three decellularization protocols in rat kidneys to create extracellular matrix (ECM) scaffolds for tissue engineering applications. We have further analyzed these scaffolds using rigorous methods for: 1) retention of critical matrix structures, 2) efficient removal of cells and 3) presence of structural and growth factor proteins. Three different protocols were used: Triton only (P1), Triton and SDS (P2) and Triton and Trypsin (P3). Hematoxylin & eosin (H&E) staining and scanning electron microscopy

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(SEM) was used to characterize the ECM. The presence of ECM structural proteins was analyzed by immunofluorescence and growth factors by ELISA.After decellularization, kidneys were transparent and cells were not present in H&E staining (P2 and P3). The structure of glomeruli and tubules remained intact and was confirmed by SEM. Immunostaining showed the presence of collagen IV. FGF was detectable in P1 and P2.P2 and P3 showed high efficiency of cell removal while maintaining preservation of the kidney architecture. However, P1 and P2 demonstrated retention of FGF. Taken together, the use of triton and SDS efficiently removes cells and retains FGF after decellularization in rat kidneys. This provides a basis for repopulating the parenchyma with renal progenitor and endothelial cells.

27.P17
Endothelialized vascular tree in decellularized liver for liver tissue engineering

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Introduction: Liver is an important organ which has various functions. There is no medical treatment for severe liver disease except liver transplantation. Therefore, we will create a novel method for the reconstruction of liver tissue equivalent. The structure which consists of gel embedded liver cells and vascular network is expected to become liver tissue equivalent. In this study, endothelialized vascular tree in decellularized liver was developed.
Material and method: The livers from rats were decellularized by detergent and DNase treatment. Human umbilical vein endothelial cells (HUVEC) were seeded into decellularized liver via portal vein and cultured. Histological and functional evaluation of the endthelialized liver was performed. On the other hand, liver cells were put around the endothelialized vascular tree.
Results: Using Triton X-100 and DNase treatment was the optimum condition to obtain complete decellularized liver with a fine framework of blood vessels. The site-specific adhesion of HUVEC was achieved by seeding the cells via portal vein. After circulation culture for 3 days, HUVEC formed monolayer and covered the internal surface of tubular structure in decellularized liver. Furthermore, liver cells were observed by the endothelialized vascular tree. Therefore, this study is valuable for the development of liver tissue engineering.
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27.P18
Solubilized matrix from decellulalized liver as a functional material for tissue engineering

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Introduction: Scaffold and growth factors are important materials for not only cell growth but also formation of functional tissue-like structure. In this study, we focused on decellularized organ, and solubilized extracellular matrix of liver (D-ECM) was developed as a functional material for tissue engineering.
Material and method: Decellularized rat liver was prepared by 4% Triton X-100 and DNase/RNase treatment. Decellularized liver was dissolved in 6N-HCl and obtained D-ECM. Air-dried film of D-ECM was prepared in the well plate and supplied for cell culture.
Results and discussion: The nucleic acid concentration and mass ratio (nucleic acid/protein) of D-ECM were 15 Alt0181g/ml and 0.00067, respectively. So, we considered that decellularization of liver completely performed. Growth factor could be immobilized on D-ECM film, and the immobilized efficiency was more than 70%. Furthermore, mitochondrial activity of HUVEC on VEGF-immobilized D-ECM film increased well even in growth factor free medium. Organoid formation and albumin production of hepatocytes were enhanced on HGF-immobilized D-ECM film. These results indicated that D-ECM is potential material for tissue engineering.
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27.P19
Tissue-specific synthetic ECM hydrogels for 3-D in vitro maintenance of hepatocyte function

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Despite recent advances in biomaterial science, there is yet no culture system that supports long-term culture expansion of human adult hepatocytes, while preserving continued function. Previous studies suggested that acellular liver extracellular matrix (ECM), employed as a substrate, improved proliferation and function of liver cells. Here we investigated whether extracts prepared from acellular liver ECM (liver ECM extract, LEE), or from whole (fresh) liver tissue (liver tissue extract, LTE), could be combined with collagen Type I, hyaluronic acid (HA), or heparin-conjugated HA (HP) hydrogels to enhance survival and functional output of primary human hepatocytes. The liver-specific semi-synthetic ECMs (sECMs) were prepared by incorporating LEE or LTE into the gel matrices. Subsequently, primary human hepatocytes were maintained in sandwich-style hydrogel cultures for 4 weeks. Progressive increase in hepatocyte metabolism was observed in all HA and HP groups. Hepatocytes cultured in HA and HP hydrogels containing LEE or LTE synthesized and secreted steady levels of albumin and urea and sustained cytochrome p450-dependent drug metabolism of ethoxycoumarin. Collectively, these results indicate that customized HA hydrogels with liver-specific ECM components may be an efficient method for expansion human hepatocytes in vitro for cell therapy and drug and toxicology screening purposes.

27.P20
Effect of the decellularization process on the local stiffness of acellular lung scaffolds

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Introduction: Decellularized lung matrices are promising scaffolds for lung bioengineering. Different lung decellularization protocols have been proposed, but whether these procedures modify local stiffness - which may play a considerable role in cell homing and fate- is unknown.
Aim: To characterize the effect of decellularization process on lung matrix micromechanics.
Methods: A custom-made Atomic Force Microscope was used to measure the Young's modulus of alveolar septal segments, septal junctions, visceral pleura, and vessels' tunica intima and adventitia of lung matrices obtained by 3 decellularization procedures (DP1, DP2, DP3;10 rats each). DP1: a) lungs were perfused with phosphate saline buffer (PBS) through the pulmonary artery, b) frozen and thawed (4 cycles), washed with sodium dodecyl sulfate (SDS) through the trachea and pulmonary artery, and c) washed with PBS. DP2: same as DP1 but using a CHAPS solution instead of SDS. DP3: same as DP1 but excluding the perfusion steps through the pulmonary artery.

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Results: For any given decellularization procedure, significant local differences in stiffness were found. On the whole, matrix stiffness did not significantly change when comparing DP1 and DP3. However, local lung stiffness was significantly reduced by 35% for DP2.
Conclusion: A cellular lung micromechanics depends on the decellularization process. Whether these changes could affect cell homing and fate during matrix recellularization remains to be investigated.

27.P21
Lung natural acellular scaffolds can be easily obtained using the detergent-enzymatic treatment in small and large animal models

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Abstract not available.

27.P22
Characterisation and immunogenicity of a decellularised skeletal muscle scaffold for laryngeal tissue engineering

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Introduction: Successful replacement of airways in patients has been obtained using tissue engineered constructs. However, replacing the larynx where active movement is critical, requires functional muscle tissue.
Materials & methods: Decellularised scaffolds were characterised using histological, immunohistochemical and molecular techniques and xenogenically implanted to determine the effect of implantation on scaffold biodegradation time and immunogenicity in vivo. The cellular host immune response to the scaffold was quantified by stereology and by FACS in vitro.
Results: Decellularisation results in total DNA clearance and down-regulation of MHC Classes I/II and myosin heavy chain expression, with relative preservation of the scaffold's structural integrity (collagen, elastin, s-GAG content) and biomechanical properties. Decellularisation altered the host response to the scaffold in vivo, resulting in a prolonged degradation time and increased neoangiogenic potential relative to fresh tissue. In addition, we proved for the first time that decellularised scaffolds trigger a lower cellular mediated immune response, resulting in a reduced T-cell proliferative response in vitro, and alter the cellular immune profile towards the scaffolds in vivo, with a reduction in CD3+ cells and a shift towards the M2-macrophage phenotype.
Conclusion: Decellularised laryngeal muscles are non-immunogenic and may provide the optimal scaffold source for a tissue-engineered larynx.

27.P23
Comparison of denuded and decellularized human umbilical vein as scaffolds for vascular tissue engineering

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Introduction: Endothelium-denuded human umbilical veins (denHUV) can serve as building blocks of small caliber vessel grafts. This study investigated completely decellularized veins (decHUV) as an alternative.
Methods: HUV were endothelium-denuded by luminal dehydration or decellularized using (1) a detergent mixture, (2) peroxyacetic acid, or (3) osmotic lysis, each followed by nuclease treatment and extensive washing. HUVEC were seeded on the scaffolds at densities of 5E5 cells/cm2.
Results: Denudation removed endothelial cells without damaging other wall components or decreasing tetrazolium dye reduction. decHUV showed an almost complete loss of H&E-stainable material. Remnants of degenerate nuclei were removed by nuclease treatment. Decellularization caused a loss of laminin and fibronectin staining, as well as fragmentation of elastic fibers. Failure stresses were not decreased by denudation or by chemical treatments, but by nuclease treatment. Static HUVEC seeding resulted in a confluent neoendothelium on denuded vessels. There was incomplete coverage on day 1 and subsequent loss of viable cells in decHUV. Seeding of denHUV in a perfusion bioreactor resulted in a flow-resistant neoendothelium.
Conclusions: denHUV provide a superior surface for endothelial cell seeding compared to decHUV, apparently due to the preservation of intact basement membranes. Denuded vessels are an interesting alternative to complete decellularized vessels for vascular tissue engineering.

27.P24
Study of endothelial cell behavior using cell-derived extracellular matrix environment

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Extracellular matrix (ECM) plays a critical role in providing cells with signaling cues that direct migration, proliferation, and differentiation. Upon the great benefits of ECM in regenerative medicine, numerous studies have been carried. In this work, in vitro cultured MC3T3 preosteoblasts were utilized in directly obtaining preosteoblasts-derived matrix (PDM) and its effect on endothelial cells (ECs) behavior was investigated. After decellularization, SEM images exhibited a unique surface texture of PDM, as compared to gelatin- or fibronectin (FN)-coated surface. Fibronectin, laminin, and collagen I was also identified in the matrix using immunofluorescence. PDM was further subjected to EDC chemistry, and followed by heparin grafting, then vascular endothelial growth factor (VEGF) immobilization. From the quantitative assay, the amount of heparin was 4.0±0.55 mg (n=3) and VEGF was 23.5±1.63 ng per matrix (n=3). While EC attachment and proliferation was active on the gelatin- or FN-coated surface, the capilary-like network of ECs was significantly better with PDM, as confirmed by positive staining of CD 31. The effect of matrix-tethered VEGF is being

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tested. Furthermore the matrix formulation mixed with ECs in three-dimensional construct was great for microvessel formation. The present study will enhance our understanding of how ECs interact with matrix environment, in which nano-structured surface texture and compositional diversity are featured.

27.P25
Development of an autologous vascular substitute using a decellularized matrix scaffold produced by the self-assembly approach

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Tissue-engineered vascular constructs are a promising approach for the replacement of small diameter blood vessels. The self-assembly technique allow the fabrication of autologous vascular substitutes. We adapted the original method by using a decellularized matrix scaffold (dMS), produced by self-assembly using human dermal fibroblasts, to improve versatility and mechanical properties of tissue-engineered vascular media (TEVM). We compared three decelularization protocols, Na-Deoxycholate, osmotic choc alone or with freeze/thaw, to optimize mechanical and vasocontractile properties of the resulting tissue. After the decellularization step, dMS were seeded with human smooth muscle cells (SMC) to form TEVM with our new method (nTEVM). Cell seeded dMS were rolled around a tubular mandrel and cultured in vitro to form vascular constructs. Mechanical properties and vascular reactivity of these engineered tissues were compared to standard self-assembled media (sTEVM) and adventitia (sTEVA). Constructs from every conditions showed a supraphysiological mechanical resistance and both nTEVM and sTEVM showed a contractile phenotype. Our study identifies osmotic choc as the optimal decellularization approach for our tissue since it gives good mechanical resistance and adequate vasocontractility. This method is currently being adapted with rat SMC for in vivo testing in syngenic rats. This study demonstrates the potential for dMS as a biomaterial for vascular tissue engineering.

27.P26
The development and characterisation of primary calvarial cell-secreted decellularised matrices to direct osteogenic differentiation of embryonic stem cells

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In developing bio-inspired materials for tissue engineering interventions it is important to understand how relevant cell types interact with a range of extracellular environs. In vitro cell-secreted decellularised matrices provide a useful model system over simple protein coats as they closer reflect the complex native microenvironment found in vivo.-Murine primary calvarial (mPC) cells, extracted from neonatal CD-1 mice, are cultured on tissue culture plastic for 7 to 14 days in osteogenic media. They express a characteristic extracellular matrix that may be isolated through decellularisation utilising 20 mM ammonium hydroxide followed by a DNase treatment to remove residual nuclear material, shown to inhibit subsequent cell adhesion.The resulting decellularised matrix (DM) is characterised using a range of imaging, immunohistochemical, histological, and materials analysis techniques. Intact, reproducible matrix sheets, exhibit retention of bone-relevant ECM proteins including Collagen I; and removal of key cellular components including DNA fragments and F-actin.The DM may be reseeded with a range of cell types to examine the influence of native ECM on their behaviour and differentiation. We describe the behaviour of murine embryonic stem (mES) cells on the DM in an osteogenic environment.In vitro cell-secreted DMs provide a useful platform to understand cell-matrix interactions, and may inform the future development of bio-inspired synthetic substrates.

27.P27
Comparison of extracellular matrix-based scaffolds for cartilage tissue regeneration

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Articular cartilage has a limited regenerative capacity. Current cell-based therapies require two hospital stays, invoke high costs and enjoy only moderate clinical success. A one-step procedure capable of generating hyaline cartilage within the defect site would overcome these problems. Extra-cellular matrix (ECM) derived scaffolds have shown potential for use in cartilage tissue regeneration as they consist of complex structural and functional molecules which play an important role in tissue development and repair. This study aims to utilise the bioactive properties of cartilage ECM to fabricate a porous scaffold suitable for use in cartilage regeneration. Specifically, scaffolds fabricated using ECM deposited by chondrocytes in 3D in vitro culture were compared to scaffolds fabricated using native ECM. Both cell-derived ECM scaffolds and native cartilage ECM scaffolds were shown to retain levels of collagen and sulphated GAGs following freeze-drying, generating highly porous ECM constructs. In addition, MSCs were shown to infiltrate into the centre of the constructs after 3 days of culture. RT-PCR carried out on the ECM-based constructs demonstrated an up-regulation of chondrogenic genes compared to MSCs seeded onto freeze dried agarose scaffolds. These results indicate the potential of ECM-based scaffolds for use in a one-step cartilage regeneration procedure.

27.P28
Effect of extracellular matrix on proliferation and adhesion properties of bone marrow mesenchymal stem cells, adipose derived mesenchymal stem cells and hair follicule stem cells: utility for tissue and organ regeneration

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Introduction: Cell-matrix interactions are crucial for the successful repair and regeneration of damaged tissue and organ. The aim of this study was to check how different extracellular matrix (ECM) proteins affect proliferation and adhesion potential of Bone Marrow Mesenchymal Stem Cells (BM-MSCs) Adipose Derived Mesenchymal Stem Cells (AD-MSCs) and Hair Follicule Stem Cells (HFSCs).
Materials and methods: Rat BM-MSCs, AD-MSCs and HFSCs were cultured on dishes coated with 4 types of ECM including collagen IV, fibronectin, laminin and poly-D-lysine for 7 days, and simultaneously cultured on a noncoated dish as a control. Proliferation potential was assesed by MTT assay while adhesion capacity by macroscopic observation.
Results and discussion: All of tested ECM proteins, except laminin, do not decrease the proliferation potential of BM-MSCs and AD-MSCs. The largest increase of proliferation was observed on collagen IV and fibronectin-coated dishes. Additionaly, we observed that 80% and

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90%of BM-MSCs and AD-MSCs underwent adhesion in 30 minutes after seeding on fibronectin-coated dishes, respectively. On the contrary, number of HFSCs was significantly increased on non-coated dishes. However the best adhesion capacity was observed on collagen IV, laminin and non-coated dishes.
Conclusions: These results suggest that the signal transduction of the cell-matrix interaction for the proliferation and adhesion of cells is very important for the successful regeneration.

27.P29
Engineering of customizable devitalized extracellular matrices using clonal, death-inducible, immortalized human mesenchymal stromal cells.

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Devitalized engineered extracellular matrix (ECM) could offer a solution to enhance/induce tissue repair, but requires a standardized cell source and an efficient devitalization strategy. Towards this ultimate goal, we here aimed at generating immortalized human Mesenchymal Stromal Cells (hMSCs) including an inducible death-device to safely devitalize engineered matrices while preserving their structure and composition.Fresh hMSCs were immortalized with hTERT and further transduced with an inducible caspase 9 apoptotic gene. A potent osteogenic clone was selected based on expression of osteogenic genes (PCR and cytofluorimetry) and mineralized matrix production (collagen, calcium content, alizarin red). The killing efficiency was assessed by Annexin-V/PI staining. hMSCs were successfully immortalized (>280 doublings) and stably expressed the inducible death-device. Cells stably differentiated into osteogenic, adipogenic and chondrogenic lineages. The clone selected for highly potent osteogenic differentiation was able to deposit a dense, mineralized collagen matrix both in 2D and 3D culture, while being efficiently inducible toward apoptosis (>95%).The generated cell line is immortal and maintains basic features of primary hMSCs. Further genetic manipulations (VEGFα and BMP-2 transduction) and culture in 3D matrices, followed by induction of apoptosis, is expected to yield customized, cell-free ECM with standardized biological functionality for bone tissue repair.

27.P30
Autologous extracellular matrix scaffolds prepared from cultured cells

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Development of autologous scaffold is highly expected because of its biomimetic property and elimination of negative host response. We developed a method to prepare autologous ECM scaffold by 3D culture of autologous cells in a selectively removable poly (lactide-co-glycolide) (PLGA) template. Intracellular components and PLGA template were selectively removed after cell culture to obtain the ECM scaffolds. Human bone marrow-derived mesenchymal stem cells (MSCs), dermal fibroblasts and chondrocytes were used to prepare the scaffolds. Composition of the ECM scaffolds depended on cell type and cell phenotype used to prepare the scaffolds. Implantation of autologous ECM scaffolds using mouse model showed the minimal host tissue response. These ECM scaffolds were used for in vitro culture of MSCs and fibroblasts to examine their potential as scaffolds for cartilage and skin tissue engineering. The MSCs were cultured in MSCs- and chondrocytes-derived ECM scaffolds. The ECM scaffolds supported cell adhesion, promoted both cell proliferation and the production of ECM and demonstrated a stronger stimulatory effect on the chondrogenesis of MSCs compared with a conventional pellet culture method. Fibroblasts were cultured in the fibroblasts-derived ECM scaffolds. After 2 weeks of culture, a uniform multilayered tissue was generated with homogenously distributed fibroblasts. The cultured cells-derived ECM scaffolds will be useful for tissue engineering and regenerative medicine.

27.P31
Real-time ultrasonic monitoring of the decellularization process of corneal stroma

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Nowadays, they are multiple pathologies that may damage organs of the human organism, being the transplant mostly the unique available therapy. The latter has two mayor concerns, together with the huge cost of the rocess: the missing donators and the possibility of reject of the transplanted organ. Recent works described the advances in the substitutive organ therapy, based on the development of so-called bioartificial organs, that benefit of their extracellular matrix, and whose cells are substituted through a recellularization process via primary cells, that show ability to differentiate themselves in their interior. To standardize and optimize the process, it is necessary to control most of the parameters that may vary its effectiveness. We propose to monitor the changes that may suffer the matrix during the descellurarization process using mechanical and optical parameters. To ensure the viability of this protocol, a bioreactor has been designed. The proposed methodology consists of three elements: An (1) experimental setup based on ultrasound-cornea interactions is monitored in real-time, a (2) model that simulates the ultrasound-cornea interactions is numerically solved by the transfer matrix formalism, and a (3) model-based inverse problem is used to reconstruct the evolution of the relevant mechanical parameters during the descellurazation process. The authors would like to thank the MICINN and SAS through Project grants DPI2010-17065 and PI-0308.

27.P32
A composite of human adipose-derived extracellular matrix and adipose-derived stem cells for cartilage tissue engineering

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Cartilage is a highly specialized tissue comprising a network of extracellular matrix (ECM), which is organized in a gel-like structure with a high collagen and proteoglycan content. Cartilage is avascular and displays a very poor capacity for self-repair; hence, even small defects may require surgery. Tissue engineering approaches using autologous cells and scaffolds fabricated from various biomaterials are becoming a promising alternative to conventional surgery. Especially, ECM harvested from various tissues play a pivotal role in regenerative medicine because of their similarities to the intact ECM. Here, we explored cartilage tissue engineering using ECM scaffolds seeded with stem cells. Both scaffolds and stem cells were isolated from human adipose tissue, which is abundant and easily harvested in the human body. The human ECM scaffold contained a large amount of collagen and various endogenous bioactive factors. A composite of human ECM and stem cells was prepared and cultured in chondrogenic medium for 45 days with 10 ng/mL TGF-β1 or not. The volume and weights of the composites gradually increased during culture and the composites formed a spherical cartilage-like tissue with the synthesis of cartilage-specific proteins. Important chondrogenic markers were expressed including Sox-9, agg-

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recan, and collagen type II, XI. These results demonstrate that an ECM/cell composite could provide biochemical cues for the promotion of cartilage formation.

27.P33
Intrinsic bioactive properties and tissue specificity of the adipose extracellular matrix guide soft tissue regeneration

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Soft tissue defects can arise from traumatic injury and conditions such as lipodystrophy and tumor resection. Human adipose tissue was decellularized and lipids removed using mechanical and chemical processing to produce an extracellular matrix biomaterial that retains its unique composition to guide adipose tissue regeneration. To determine its adipogenic inductive capacity, human adipose-derived stem cells were cultured in the adipose matrix and acellular dermal matrix. After 2 weeks, gene expression of adipogenic markers CEBPA, PPARG, FABP and Oil Red O staining showed the adipose matrix led to improved adipogenic differentiation in comparison to the acellular dermis. Additionally, a migration assay found a dose-dependent increase in the chemoattractant effect of adipose matrix on stem cell migration. A 12 week subcutaneous injection study in athymic mice (n=12) compared delivery of the matrix with and without the addition of adipose stem cells. The acellular matrix facilitated new adipogenesis with development of the unilocular adipocytes characteristic of adipose tissue. When delivered in conjunction with adipose stem cells, the matrix also provided a supportive environment for stem cells and was largely replaced by new adipose tissue, thereby supporting the inductive role of matrix components. The adipose matrix provides a promising biomaterial with the ability to guide migration and differentiation, making it an ideal biomaterial scaffold for soft tissue regeneration.

27.P34
Preparation of bovine small intestinal submucosa and application as a wound dressing alone or seeded with mesenchymal stem cells in a full-thickness rat skin defect

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Decellularized small intestinal submucosa (SIS) is a naturally derived biological material proposed as a DNA free, biocompatible xenogenicmatrix for clinical applications in reconstructive surgery and tissue engineering. We have recently described a new decellularizationprotocol using serial treatment of freeze-thawed raw bovine SIS with solutions of sodium hydroxide, vitamin C, hydrogen peroxide, peracetic acid and sodium chloride (patent application TPE-PT-2011/11399). Bovine SIS was evaluated for cellular components using DNAcontent analysis, SEM and histochemistry. The xenogenic matrix was soft, elastic and could easily be manipulated by hand. Rat bonemarrow mesenchymal stem cells (BM-MSCs) were seeded and cultured on bovine SIS and their interaction was evaluated via MTT assay, SEM and histochemistry at predetermined culture time points. The in vivo study was designed to compare the wound healing efficacy ofbovine SIS to that seeded with BM-MSCs in a 7cm2 full-thickness rat skin defect model. Wound healing was evaluated macroscopically, histopathologically and by IHC. In both groups wounds closed within 8 weeks. However, progress of granulous tissue formation was fasterin the group receiving BM-MSCs-seeded bovine SIS with better attachment of the construct to the wound. In the control group (openwound), wound closure took >20 weeks. Findings support the notion that bovine SIS has potential as a wound dressing alone or incombination with BM-MSCs.

27.P35
Fibroin and sericin from Bombyx mori silk stimulate cell migration through upregulation and phosphorylation of c-Jun

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Wound healing is a biological process directed to the restoration of the skin that has suffered an injury. The generation of a basal epithelium, able to wholly replace the epidermis of the wound, is an important phase of wound healing. A broad range of products derived from fibroin and sericin from Bombyx mori silk are used to stimulate wound healing. However, so far the molecular mechanism underlying this phenomenon has not been determined yet. We have studied the molecular basis that underlie wound healing properties of silk proteins fibroin and sericin using a cell model. We used MDA-MB-231 and Mv1Lu cells to assay fibroin and sericin wound healing properties in a wound-healing assay. Both fibroin and sericin stimulated cell migration. Furthermore, treatment with fibroin and sericin activated key factors of the wound healing process. Among them, the activation of c-Jun seems to be a key event. Moreover, fibroin and sericin stimulated the phosphorylation of ERK1/2 and JNK1/2 kinases. The usage of specific inhibitors revealed that MEK, JNK and PI3K pathways are involved in fibroin and sericin stimulated cell migration. Inhibition of these three kinases prevented either fibroin or sericin c-Jun upregulation or phosphorylation. Stimulation with fibroin and sericin of HaCaT cells produce similar results. Altogether, our results showed that fibroin and sericin initiate cell migration by activating the MEK, JNK and PI3K signalling pathways ending on c-Jun activation.

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28. Silk Fibroin (and its Modifications)

28.01
Keynote: Fibroin: an example of hierarchy and conformation dominated biological behavior

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Silk-fibroin proteins have recently attracted wide attention as bioactive matrices for biomedical applications. In vitro and in vivo studies have demonstrated the ability of fibroin to favourably interact with cells and the advantageous use of fibroin scaffolds to engineer the repair and regeneration of different types of tissues. Cell seeded or unseeded scaffolds have been successfully tested for soft and hard tissues repair, in the form of gels, sponges, fibres or films, adapted to a variety of repair sites and clinical needs.As for other natural polymers, silk possesses a complex hierarchical structure that is assembled from the bottom-up, where each pattern/conformation can be correlated to specific biological functions or physical properties. Self-assembly and the resulting conformation of silk fibroin depends however on silk source and processing conditions, that in turn affect the biological and physical behaviour of the material. This fact adds complexity to the understanding and control of the materials/biological system crosstalk and should be carefully assessed when biologically engineering reproducible scaffold.The presentation will be focused on the interactions between silk fibroin and cells. Specific hints on the effect of processing on silk conformation and function-structure relationships will be discussed in light of fabricating responsive devices applicable in regenerative medicine.

28.02
Keynote: Non-mulberry silk biopolymer as emerging natural biomaterials

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The emerging demand of functional materials in biomedical research leads to the search for suitable biomaterial, which is natural, biodegradable, mechanically robust and biocompatible. Silk is unique natural biopolymer used as multifunctional platform in cell based tissue engineering and regenerative therapy. We are focusing on indigenous non-mulberry silks like tropical tasar silk Antheraea mylitta, muga silk A. assama and eri silk Samia ricini as advanced natural material due to its availability, easy processing, cost effectiveness, superior mechanical properties, thermal stability and cytocompatibility. 2D films, 3D scaffolds, biospun scaffolds, microbeads, hydrogels, and nanoparticles are engineered and characterized as biomimetic systems. Growth of human and rat bone marrow stem cells, human embryonic stem cells, bovine chondrocytes, rat myocytes, fibroblasts, keratinocytes, breast, prostate and hepatocellular cancer are observed, as well as subcutaneous fibroblast adhesion and non-differentiation into myofibroblast. Silk based tumor models are engineered to study drug efficacy. Silk nanoparticles are studied as delivery system for bioactive molecules to study targeted in vitro drug release in tumor cells. Self assembly of silk to develop fractal structures on 2D substrates is also investigated. In addition, designing of fibroin based bio-memristor devices opens up new opportunities in advanced bio-inspired circuit design and energy-efficient neuromorphic computing.

28.03
Repair of critical size cartilage defects in rat xiphoid using adipose-derived stem cells and silk fibroin 3D porous scaffolds

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Previous in vitro studies have shown that silk fibroin 3D porous scaffolds can support chondrogenesis of adipose tissue derived stem cells (ASCs). The present study explored the potential of in vivo cartilage repair using rat ASCs and silk fibroin scaffolds in a rat critical size xiphoid chondral defect model. ASCs were cultured with growth medium or chondrogenic medium for 28 days under static or rotation conditions. No significant cell death was observed during culture. Cell constructs cultured in growth medium under rotation had the highest cell proliferation rate, while those cultured in chondrogenic medium under static conditions expressed the highest chondrogenic markers including col2, acan and comp. Cells produced significantly higher levels of sulfated glycosaminoglycan when cultured in chondrogenic medium compared to growth medium. Growth in chondrogenic medium under static conditions resulted in more intense immunofluorescence for collagen II and aggrecan as well as the highest Young's modulus. Cell/scaffold constructs were implanted after 28 days of culture in 3mm diameter cartilage defects in xiphoids of athymic rats. μCT and histological analyses of tissues harvested 8 weeks later confirmed larger amounts of neo-cartilage in defect sites with cell constructs grown in chondrogenic medium under static culture compared to rotation culture. In conclusion, ASCs/silk constructs can be used to repair in vivo cartilage defect when an appropriate pre-culture is applied.

28.04
Novel preparation of silk hydrogels under high pressure carbon dioxide

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Silk fibroin, the main structural protein of Bombyx Mori silk fibers, is a natural polymer that has been utilized extensively in the biomaterials community for its excellent biocompatibility, robust mechanical properties, and controllable degradation rate. Accordingly, silk fibroin hydrogels have found particular application in the biomedical community as gap filling biomaterials or as vehicles for drug or cell delivery techniques. However, technical processing of silk hydrogels can be rigorous with lower solution concentrations requiring days to reach gelation. Manipulation of the physiochemical environment through reduced pH, submission to thermal treatment or the addition of electrolytes to silk solutions has been reported to significantly reduce gelation time. One potential alternative is to utilize a volatile acid to induce silk protein gelation which can be efficiently recovered at atmospheric conditions after processing. Here we present the novel fabrication of silk fibroin hydrogels using high pressure CO2 as a volatile electrolyte. Successful gelation can be completed without the need of thermal treatments or chemical additives resulting in a remarkably clean process. Kinetic data revealed that processing under high pressure CO2 substantially improves gelation time. Lastly, the physical nature of the silk hydrogels prepared under high pressure CO2 exhibited superior properties and may offer potential as substrates for tissue engineering therapies.

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28.05
Native silk for tissue engineering

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The use of silk as a natural biomaterial for regenerative medicine is growing because of its excellent biocompatibility, remarkable mechanical properties and good biodegradability. Several different types of silk have been used in biomedical research including small peptides of the spider silk protein expressed in bacteria or isolated as regenerated silk protein extracted from cocoons of the silkworm. In contrast, we harvest native silk (2.3 MDa fibroin protein complex) from silkworms, which is used to manufacture membranes, textiles and composites under benign conditions. In combination with transgenic silkworm engineering novel bioactive scaffolds are designed using the silkworms high production capacity as a source for the biomaterial fibroin and as a bioreactor. Data about mechanical properties (elasticity, tensile strength) and biocompatibility will be presented. Additionally, the biological activity of epidermal growth factor (EGF) modified silk from transgenic silkworms will be discussed. Finally, examples of ongoing research projects for vascular regeneration and tissue engineering applications will be shown.

28.P01
To differentiate or not – Adipose derived stroma cells for adipogenesis using fibrin coated fibroin scaffolds

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Soft tissue regeneration comes along with repeat surgeries and long lasting cell cultures. To overcome these issues we wanted to compare adipogenesis based on undifferentiated human adipose derived stroma cells (ASC) and adipogenetically predifferentiated ASCs (diffASC) seeded on fibrin coated 3D silk fibroin scaffolds (Wang et al., 2008) The silk scaffolds were prepared from organic solvent (HFIP) process with a pore size of 400–500 microns. First, to examine fibrin concentration-depending degradation, fluorescent fibrin (100 or 25mg/ml fibrinogen including Oregongreen 488) coated scaffolds were implanted s.c. into nude mice (2 and 4 weeks). In vivo fluorescence imaging revealed concentration-independent degradation over 9 days. Histological evaluation showed a better compatibility of diluted fibrin coated scaffolds. For comparing predifferentiation of ASC, scaffolds with diluted fibrin and ASCs were cultured in either adipose differentiation medium (diffASC) or in regular cell culture medium (ASC) (2 weeks). Scaffolds with fibrin in regular medium served as control. Scaffolds were then implanted s.c. into nude mice (4 weeks). Immunhistochemistry revealed the presence of non-mature ASCs and the support of adipogenesis in ASC and diffASC scaffolds, but not in controls. No fibroin scaffold degradation was found in any of the groups. The data suggest the same ability of ASC for adipose tissue regeneration as diffASC in a slowly degrading and biocompatible scaffold.

28.P02
Effect of silk protein on chondrogenesis

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Silk produced by mulberry silkworm (Bombyx mori) is composed of two types of protein polymers, sericin and fibroin. Biocompatibilities of these silk proteins have attracted attention as biomaterial. In this study, we isolated sericin and fibroin from silk cocoon and prepared regenerative silk protein samples in different methods. We investigated the effect of these silk proteins on chondrocyte growth and chondrogenesis. The molecular weight of silk proteins had 0.5 ~ 300 kD. When the chondrocytes were cultured with silk proteins, the ability of cell adhesion and growth were more excellent than control. Also, expression levels of genes related chondrogenesis such as collagen type II, collagen type X and sox 9 were increased. These results suggest the possibility of silk protein for cartilage regeneration.

28.P03
In vitro chondrogenic differentiation of human mesenchymal stem cells on TGF-beta1 loaded silk fibroin nanospheres

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Polymeric materials are frequently used for tissue engineering and drug delivery. Silk fibroin protein from nonmulberry Indian tropical tasar silkworm Antheraea mylitta, is a potential biomaterial for diverse applications due to its versatility, mechanical robustness, and cytocompatibility as a tissue engineering material. To apply this biomaterial as a potential cell supporting matrix for stem cell delivery, nanostructured 3D porous scaffolds with TGF β1 loaded silk fibroin/albumin nanoparticles were designed. The nanoconstruct induced chondrogenic differentiation of human mesenchymal stem cells (hMSCs) by continuous controlled release of TGF β1. Based on a previous study, fabricated silk fibroin/albumin nanoparticles with diameter <200 nm and - 31 mV zeta potential were stable and easily enternalized by the cells. Mechanical characterization, GAG, collagen content and in vitro release of TGF β1 from nanoconstruct favored the growth and proliferation of hMSCs. The silk fibroin nanoconstruct based growth factor delivery can be used to differentiate hMSCs and engineer cartilaginous tissue in vitro, and it may be a promising implantable biomedical device for cartilage tissue engineering.

28.P04
Development of thick electro-spun silk fibroin scaffolds using polyethylene glycol as sacrificial material

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Electro-spinning produces three-dimensional nanofibrous structures with high surface to volume ratio that are attractive in the field of tissue scaffolding and regenerative medicine. Many biomaterials such as PLGA, PEO, and collagen have been successfully electro-spun and demonstrated their performances as functional tissue scaffolds or drug delivery systems. With recent concern with mad cow disease, silk fibroin (SF) has drawn great interest as a new material to replace bovine collagen. However, the electro-spinning of the SF was limited in producing thick scaffolds. Thus, in this study, we demonstrate the electro-spinning of thick SF scaffolds using a mixture of SF and polyethylene glycol (PEG). SF was extracted from Bombyx mori cocoon by degumming in boiling aqueous Sodium Oleate (0.3% w/w), Na2CO3 (0.2% w/w) solution for 1h. The SF solution was prepared by dissolving the degummed silk into a 9.3 M LiBr solution for 1h at room temperature. This solution was dialyzed against deionized water for 3 days to remove LiBr. Dialyzed solution was freeze-dried for 3 days and dissolved in formic acid (15%, w/v). PEG35,000 was also dissolved in formic acid (5%, 10%, 15%, 20% w/v) and each solution was blended with silk fibroin solution (1:1 v/v). The final solution was electro-spun and 2~4cm thick SF scaffolds were fabricated. The morphology and

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microstructure of the electro-spun SF/PEG scaffolds were investigated by scanning electron microscopy.

methanol treatment or water vapor annealing, to fabricate tubular scaffolds with tunable proprieties. Further mechanical and biological characterization is in progress.

28.P05
Controlling the micro-architecture of 3D silk sericin hydrophilic network for therapeutic purpose

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Therapeutics includes repair and regeneration of diseased tissues, targeted drug delivery and wound managements. Hydrogels are cross-linked networks of hydrophilic polymers, capable to absorb large amount of water or biological fluid. These hydrophilic networks hold significant potentiality because of their structural and compositional similarities with physiological extracellular matrix. We demonstrate effects of protein concentration, polymer to protein ratio, drying processes and pH on pore micro-architectures and interconnectivity of 3D protein hydrogel. The pore size and interconnectivity play significant role in cell viability, proliferation, migration, nutrient diffusion and waste removal within 3D culture system. The fabricated hydrogels of polyacrylamide and Indian tasar silk protein sericin of Antheraea mylitta cocoons exhibit naive polymeric networks after cryo-freezing. Relatively reduced pore microstructure and porosity are obtained in vacuum drying. Higher protein concentration results enlarged pores but reduced porosity and interconnectivity within the network. The repulsion among hydrolyzed -CONH2 polymeric chain entanglements loosen the 3D network at alkaline pH, resulting large pores with more internal communication. Cell growth within this biomimetic network forms monolayer, which is revealed by confocal microscopy. The results indicate the potentiality of the present 3D hydrophilic network as functional system for tissue regeneration and diagnostic tools.

28.P06
Silk tubular scaffolds for vascular tissue engineering

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Introduction: Currently there is a need for substitutes for the replacement of small caliber blood vessels, due to the mismatch between the mechanical proprieties of grafts and native blood vessels and the need to regenerate a functional endothelium. The aim of this work is for the design, production and characterization of silk fibroin small caliber tubular scaffolds. To achieve this goal, a combination of two techniques, electrospinning and gel spinning, are used to optimize the mechanical and biological proprieties of this protein polymer into small diameter grafts.
Materials and Methods: Briefly, an aqueous silk solution was prepared from Bombyx mori silkworm cocoons. This solution (~7–8% w/w) was electrospun onto a rotating mandrel to fabricate tubular scaffolds. A second layer was produced by gel spinning using a concentrated aqueous silk solution (~20–25% w/w).
Results: Electrospun-gel spun silk tubular scaffolds (inner diameter = 3mm) were obtained with suitable adhesion between the layers. SEM analysis showed uniform thickness, equal to ~50um for the electrospun layer and ~150um for the gel spun layer.
Conclusion: Novel silk tubular scaffolds were obtained using a combination of electrospinning and gel spinning. The use of these two techniques makes it possible to utilize post-spinning treatments, such as

28.P07
Silk – a novel biomaterial for the reconstruction of the urinary tract

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To overcome the functional loss of an urinary tract defect, reconstruction is mostly performed by using gastrointestinal segments. The aim of this study was to develop a novel biomaterial from silk which mimics the three-dimensional structure and mechanical properties of urinary tract tissue.Silk was isolated from silkworms and processed to membranes of 25 to 150 μm thickness. These membranes were investigated for their urea leakage by ussing chamber experiments. To imitate the structural properties of the urinary tract tissue, composites of silk membrane and silk fleece were produced and investigated using electron microscopy. Membranes manufactured from silk demonstrated enormous elasticity, tensile strength and seam resistance which is important for the later surgical implantation. Interestingly, the thickness of the membrane did not significantly influence the urea permeability. Electron microscopy demonstrated the different structural properties of the newly developed composites: a smooth side (membrane) for seeding of urothelial cells and a fibrous side (fleece) for the seeding of smooth muscle cells. The combination of different structures made of silk enabled the production of a complex composite as a novel biomaterial for the reconstruction of the urinary tract. Current investigations include the biocompatibility of these materials on cells of the urinary tract as well as the urea permeability after cell seeding.

28.P08
Non-mulberry silk proteins facilitate osseo-integration in orthopedic implants

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Last few decades, knee, hip and other orthopedic implants become a common practice to restore the fractured or damaged bone joints. Metals and their alloys are used widely as orthopedic implant material. Titanium and its alloys remain as standard due to its good biocompatibility and low corrosive properties. Modification or engineering of implant surface topography and chemistry is used to accelerate the rate of bone formation and to slow down the rate of infection. Silk proteins, sericin and fibroin; have well established history of using as bio-compatible material and possess self-assembling, and anti-oxidant properties. Silk fibroin, the core protein, is hydrophobic in nature while sericin is hydrophilic glue like protein. In the present study we immobilize both the silk proteins on Ti surface individually. APTES mediated immobilization of silk proteins is found to improve the hydrophilic nature of the surface. FTIR, SEM and AFM are used to confirm the presence of protein and reveal the surface topography. Proliferation and spreading of osteoblast like cell show well cyto-compatibility of the modified surface on cells. Immune-response against silk coated Ti is investigated with macrophage in co-culture with osteoblast like cells. The results indicate that the silk protein facilitates osteoblast cell adherence and proliferation on Ti.

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28.P09
Development of macro/micro porous silk fibroin scaffolds with nano-sized calcium phosphate particles for bone tissue engineering

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Macro/micro porous silk scaffolds with nano-sized calcium phosphate (CaP) particles were developed for bone tissue engineering. Different amounts of nano-sized CaP particles 4, 8, 16 and 25% (CaP:silk fibroin, wt/wt) were generated into the highly concentrated aqueous silk fibroin solutions via an in-situ approach. Afterwards, the pure silk and silk/nano-CaP porous scaffolds were produced by a combination of salt-leaching/freeze-drying methods. Thermal gravimetric analysis results were able to demonstrated that the silk/nano-CaP scaffolds maintained 64–87% of incorporated CaP after salt-leaching. Dynamic mechanical analysis showed that storage modulus of the 16% formulation was significantly higher than all remaining groups. The porosity of silk/nano-CaP scaffolds assessed using Micro-Computed Tomography decreased from 79.8% to 63.6% with increasing CaP incorporated until 16%. By soaking the scaffolds in Simulated Body Fluid for 7 days, cauliflower-like apatite clusters were observed on the surface of both macro and micro pores of 16% and 25% formulations, which was not observed in 4 and 8% formulations. 16% silk/nano-CaP scaffolds were further chosen for in vitro cytotoxicity and biocompatibility assays. Both silk and 16% silk/nano-CaP scaffolds were non-cytotoxic and promoted cell adhesion and proliferation to a similar extent. These results indicated that the 16% silk/nano-CaP scaffolds could be a good candidate for bone tissue engineering.

28.P10
High strength silk protein scaffolds for bone repair

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Biomaterials for bone tissue regeneration represent a major focus in orthopedic research. In the U.S. alone, 1.3 million people undergo bone graft surgeries each year with skeletal defects either from accidents or disease. However, only a handful of polymeric biomaterials are utilized today due to their failure to address critical issues like compressive strength for load bearing bone grafts. A high compressive strength (~13 MPa hydrated state) polymeric bone composite material was developed based on silk protein-protein interfacial bonding. Towards this goal, a new hydrolysis method to generate silk microfibers with control of length was demonstrated. Micron sized silk fibers (10–600 μm) obtained were used as reinforcement in a compact fiber composite with tunable compressive strength, surface roughness and porosity. The developed 3D scaffold systems provided insight on the role of microfiber dimensions on mechanical properties and cell responses. A combination of surface roughness, porosity and scaffold stiffness favored human bone marrow derived mesenchymal stem cell (hMSC) differentiation towards bone-like tissue in vitro based on biochemical and gene expression for bone markers. Further, minimal in vivo immunomodulatory responses suggested compatibility of the fabricated silk-fiber high strength reinforced composite matrices for bone engineering applications.

28.P11
The hybrid silk fibroin scaffold with BMP2 loaded gelatin microspheres for effective bone regeneration

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The silk fibroin itself is a good candidate for tissue regeneration due to the high biocompability and slow degradation as well as robust mechanical properties. But without any biomolecular cues, the mesenchymal stem cell can lead to multiple lineage of differentiation on the pure homogenous silk fibroin scaffold .Therefore, loading the osteoinductive growth factor BMP2 into the scaffold is necessary for promoting the bone regeneration. While the direct loading faces the problem of retention in the long term, an indirect loading of BMP2 is conducted by imbedding the bmp2 binding gelatin microspheres into the silk scaffold via lyophilization. The hybrid silk fibroin scaffold not only presents more sustainable protein release profile, but also shows well maintained protein bioactivity. Besides, it showed improved compression properties as well as keeping the good interconnectivity. And it was also proven to have high cell viability and good cell attachment. The pig mesenchymal stem cell was used to study the osteogenic differentiation on this hybrid scaffold. And it showed better alizarin red staining results and higher genetic expression level of the osteogenic markers as compared to the direct bmp2 loaded homogenous silk scaffold. To be short, this hybrid bmp2 loaded silk fibroin scaffold has a great potential for effective bone regeneration.

28.P12
Silk fibroin for guided bone regeneration

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Silk fibroin (SF) is a natural protein polymer spun by silkworm Bombyx mori and used as a typical textile fiber and a surgical suture. Recently, cytocompatibility and tissue-compatibility of SF has been investigated and elucidated that there is no severe side effect to cell and tissue. The author examined the feasibility of SF as eardrum membrane and it is good for regeneration of eardrum with high efficiency. To expand the usability of SF, the author examined SF for guided bone regeneration. SF membrane with bioactive materials was pre-pared and examined the morphology, structural characteristics, and thermal properties. And then it was applied to white rabbit to evaluate bone formation. Histomorphology and removal torque were analyzed.

28.P13
Osteointegration of anterior cruciate ligament scaffolds fabricated of bombyx mori silk

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Various tissue engineering (TE) approaches are based on silk fibroin (SF) as scaffold material since its superior mechanical and biological properties compared to other materials. Especially in the field of ligament TE, SF scaffolds were extensively used. Surprisingly, there is still a lack of knowledge on the integration of SF scaffolds into bone. In a rabbit model, the ACL of the right leg was excised and the tibial and femoral bone tunnels were created with a 2.5 mm diameter drill bit.

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Both ends of a custom-made SF scaffold (diameter 2.0 mm) were sutured with 1–0 polyester suture (Ethibond, Johnson & Johnson, USA) in a whip-stitch style. Then the scaffold was passed through the bone tunnel and joint cavity. Finally, both ends were fixed by sutures tied over "endobuttons" in femur and tibia. After defined time-points animals were sacrified and the osteointegration was analyzed via μCT, MR (3 and 7 Tesla) and bone histology. In μCT we could determine new forming bone after 6 weeks adjacent to the scaffold material. MR-analysis demonstrated that after 2 weeks the silk scaffolds were surrounded by soft tissue that further developed to bone tissue (followed for up to 26 weeks). The results of a tight osteointegration demonstrated via μCT- and MR-analysis are further confirmed by histology. To conclude, we could show that SF scaffolds integrated into femural and tibial bone tunnels within 16 weeks without causing adverse reactions to the adjacent bone tissue.

28.P14
A novel tissue engineering bulking agent: adipose-derived stem cells-based silk fibroin microspheres for the treatment of female stress urinary incontinence

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Stress urinary incontinence (SUI) remains a worldwide problem affecting patients of all ages. But an effective treatment, especially for SUI due to severe intrinsic sphincter deficiency (ISD), has not yet been developed. The study aims to develop a novel tissue engineering bulking agent combined adipose-derived stem cells (ADSCs) with Silk Fibroin Microspheres (SFMS). Animal models were established with Sprague–Dawley female rats by completely cutting of the bilateral pudendal nerve. The ADSCs were obtained from inguinal fat pad of rats and were characterized in vitro. SFMS were prepared via self-assembling of silk fibroin molecular chains with sizes ranging from 50 to 500μm. Forty female rats were divided into four groups: Group A (5) had sham operation; Group B (5) had no bulking agent injected after denervation; Group C (15) was treated with only SFMS after denervation; Group D (15) was treated with ADSC-based SFMS after denervation. Histology, leak-point pressure (LPP) and the external urethral sphincter electromyogram measurements were done at 4 and 12 weeks after treatment while bio-mechanical testing was done at 12 weeks. The result shows that both the SFMS and the new bulking agent showed convincing functional effects for the treatment of SUI in a rat model. And the better muscle-like tissue formation induced by ADSC-based SFMS suggested potential long-term function.

28.P15
Tissue-engineered fibroin scaffolds for ocular tissue reconstruction

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The silk protein fibroin (Bombyx mori) provides a potential substrate for use in ocular tissue reconstruction. We have previously demonstrated that transparent membranes produced from fibroin support cultivation of human limbal epithelial (HLE) cells (Tissue Eng A. 14(2008)1203–11). We extend this body of work to studies of limbal mesenchymal stromal cell (L-MSC) growth on fibroin. Also, we investigate the ability to produce a fibroin dual-layer scaffold with an upper HLE layer and lower L-MSC layer. Primary cultures of HLE and L-MSC were established in DMEM/F12 with 10% foetal bovine serum (FBS). Cultures were subsequently passaged onto either transparent fibroin membranes, amniotic membrane (AM; current gold standard), or within fibroin 3D scaffolds. HLE cells and L-MSC were also co-cultured upon dual-layer fibroin. L-MSC cultures were characterised via immunocytochemistry and flow cytometry for CD45, CD73, CD90, CD105, and α-sma. Resulting tissue constructs were analysed via immunohistochemistry for cytokeratin 3 and ΔNp63. HLE and L-MSC cultures grown in 10% FBS were able to adhere to and proliferate on silk fibroin biomaterials. HLE constructs on fibroin expressed cytokeratin 3 and Np63 comparably to AM. L-MSC were 80%+ for the characteristic MSC markers and displayed multi-potency. These results confirm the suitability of membranes constructed from fibroin as substrata for HLE and L-MSC cultivation and encourage progression to studies of efficacy in preclinical models.

28.P16
Preparation and cellular response of silk fibroin nanofibers containing Ag nanoparticles for skin regeneration

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Many researchers have recently investigated silk fibroin (SF) as one of candidate materials for biomedical applications because it has several useful properties including good biocompatibility, good oxygen and water permeability, biodegradability and minimal inflammatory reaction. Silver (Ag) is well known that Ag ions or particles are highly toxic to microorganism and strong effect of inhibition against inflammatory cells. Therefore, Ag, as an antibacterial component, has been applied for various biomedical materials such as wound healing, dental resin and antimicrobial filters. In this study, SF nanofibers containing Ag nanoparticles were prepared by electospinning method. We used silver acetate, silver tetrafluoroborate, silver phosphate and silver sulfadiazine as Ag compounds, and they were dissolved into SF solution in formic acid. The size distribution and dispersion of Ag nanoparticles formed in SF/formic acid solution were characterized by UV-Vis spectrophotometer, nanoparticle analyzer, TEM. The morphology of electrospun SF nanofibers containing Ag was investigated by SEM. Ag nanoparticles on or in SF nanofibers were observed by transmission electron microscopy and confirmed by XPS. The conformational structures of electrospun SF nanofibers containing silver were analyzed by ATR-IR, solid state 13C CP/MAS NMR. The effect of Ag nanoparticles on the adhesion and spreading of normal human epidermal keratinocytes was also investigated in vitro.

28.P17
Influence of silk-fibroin fibers on mechanical and biological properties of PDLLA salt-leached scaffolds

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A major tissue engineering approach is to use scaffolds designed as temporary extracellular matrix (ECM) supports for cells. In this study a new construct was prepared combining a porous scaffold of Poly D, L-Lactic Acid (PDLLA) with silk-fibroin fibers. Constructs comprising

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PDLLA and PDLLA plus fibers were compared for their properties as well as their abilities to support relevant in vivo cell populations.Morphological characteristics were assessed using Scanning Electron Microscopy and Micro Computed Tomography, elastic moduli were measured using a mechanical testing apparatus. Human Mesenchymal Stem Cells (hMSC) and Human Umbilical Vein Endothelial Cells were seeded onto the scaffolds and maintained for four weeks in vitro. Our findings suggest that the porosity of the novel scaffold is comparable to the PDLLA salt-leached scaffold, despite its greater stiffness. However, support of cell populations varies considerably. In particular it seems that endothelial cell localization is strongly associated with fiber presence yet, in the absence of fibers, an early osteogenic differentiation of hMSCs is seen in the scaffolds, leading to enhanced ECM production.This study shows the influence of silk-fibroin fibers on mechanical and biological properties of PDLLA salt leached scaffold. The concept of designing composite scaffolds to integrate beneficial properties of different structures and materials seems a promising avenue to facilitate appropriate biological interaction.

28.P18
Characterization of Antheraea pernyi silk fibroin hydrolysate

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Antheraea pernyi silk fibroin (SF) hydrolysate were characterized using UV/VIS spectrometer, amino acid composition and heavy metal contents to explore its potential sources for food or cosmetic additives. The hydrolyzed A. pernyi SF was separated into two parts: (a) SFA, alanine-rich fraction and (b) SFB, nntyrosine-rich fraction. SFB exhibited strong absorption peaks at 210 and 280nm due to the presence of the tyrosine. Heavy metal analysis showed that arsenic and mercury did not detect. Other heavy metals, which includes lead, cadmium, etc., were recorded only a trace amount. Therefore, A. pernyi SF hydrolysate could be safely used as sources of food, cosmetic and pharmaceuticals.

28.P19
Regenerated silk/ bio-glass composite scaffolds for bone tissue engineering

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One of the major issues in bone tissue engineering(BTE) is to design and fabricate bioactive and bioresorbable scaffolds. One of the several methods to approach this goal is using composite scaffolds. In this study,we present fabrication and characterization of a novel silk-bioglass composite scaffold. Fibroin has been made using mulberry silk cocoons and calcium silicophosphate bioactive was made by sol-gel processing. For fabrication of a unified composite bioglass powder was ground and coated with APTS. Composite scaffolds were fabricated by the freezedrying technique. Silk protein was evaluated by FTIR and XRD methods.XPS analysis demonstrated that the amino groups were established on the surface of the glass powder.SEM images showed a uniformly dispersed bioglass particles in all structures. Scaffolds were seeded with hMSC for 21 days. Cell adhesion, viability and proliferation were assessed confirming cytocompatibility of these scaffolds. An increase of ALP activity during 3 weeks of cultivation implicated that the scaffolds support osteogenic differentiation. In conclusion, appropriate structural and biological properties make the silk/bioglass scaffold a proper choice for use in BTE.

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29. Natural Derived Polysaccharides (in coop. Expertissues)

29.01
Keynote: Polysaccharide based scaffolds for an all range of regenerative medicine approaches

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Natural Derived Polysaccharides have an enormous potential for tissue engineering and regenerative medicine. This very versatile class of materials includes an all range of polysaccharides. The innovative use of its characteristics, taking advantage of the similar structure and composition with respect to native tissues and its extra-cellular matrix, enables for the designing of high performance systems with excellent biocompatibility and fine tuned biodegradability. Many of the aimed applications require increasingly complex and demanding architectures and properties. Furthermore, the processing and characterization of these types of materials often have specific requirements.In addition to these necessities, an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. We have been using different human cell sources. The potential of each type of cells, to be used to develop novel useful regeneration therapies will be discussed. Their uses and their interactions with different polysaccharide based scaffolds will be described. The use of bioreactors to control cell differentiation, as well as the surface modification of the materials in order to control cell adhesion and proliferation will also be illustrated. Some examples of the in-vivo performance of different scaffold/cells constructs will be presented.

29.02
Porous polysaccharide-based scaffolds for human endothelial progenitor cells

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Introduction: Human endothelial colony-forming cells (ECFCs), the progeny of circulating endothelial progenitors, are valuable cell therapy products in ischemic diseases. However, injected cells have limited chances to survive and engraft. This study was aimed at determining whether polysaccharide scaffolds constitute a favorable environment for ECFCs culture and release.
Materials and Methods: Porous scaffolds composed of natural polysaccharides, pullulan and dextran, and sodium chloride as a porogen agent were prepared by chemical cross-linking with sodium trimetaphosphate. Human ECFCs were isolated from cord blood and cultured in complete EGM-2 MV medium. For cell seeding, rehydration of the dried scaffold was performed with 20 μL of cell suspension
Results and discussion: We demonstrated that scaffolds with 40μm pores preserved ECFCs viability and proliferation. After 7 days of culture, ECFCs expressed endothelial markers CD31, vWf and maintained endothelial functions such as Ac-LDL uptake, migration along a VEGF gradient and activation by TNF alpha. The cultured cells are easily retrieved by enzymatic degradation of the scaffolds
Conclusion: The polysaccharide-based porous scaffold constitutes a new endothelial cell delivery system able to release cells displaying critical functions required for vascular regeneration in ischemic tissues: proliferation, migration, capacity to form vascular structures and ability to be activated by pro-inflammatory effectors.

29.03
Physically cross-linked injectable chitosan-derived microgels as a template for cell delivery

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Ischemia is a central problem in cardiovascular disease and vascular complications associated with diabetes. Cell therapies applied to these diseases have yielded modest results. Physically cross-linked injectable microgels are attractive since they may improve the delivery of cells in a minimally invasive and non-toxic manner. We developed chitosan-derived microgels with tunable mechanical and cell responsive properties for supporting transplanted cells. Two injectable materials were developed via ionic cross-linking: a) chitosan-derivative (HTCC) with sodium tripolyphosphate (TPP); and b) pure chitosan (PC) with beta-glycerophosphate disodium salt (βGP). PC-βGP was less viscous (5.5±0.5Pa○s) and more elastic (6.3±0.9kPa) at 37°C compared to HTCC-TPP (11±4Pa○s, 0.05±0.01kPa). Morphology revealed uniform pore distribution with HTCC-TPP having higher (14%) porosity. Gels degraded 4× faster in alpha-amylase (pH 7, 37°C, 250IU/ml) than in PBS at 7d. Cell compatibility was assessed using circulating progenitor cells (CPCs), endothelial cells (HUVECs) and islets up to 7d: the number of CPCs and HUVECs adherent on HTCC-TPP was less compared to PC-βGP ($p \leq 0.001$). Live/dead assays revealed comparable cell viability (>70%) between gels, and WST-1 showed greater cell metabolic activity on PC-βGP ($p \leq 0.05$). Chitosan-derived microgels are promising as delivery vehicles and suitable for supporting multiple cell types for cardiovascular and islet transplantation tissue engineering therapies.

29.04
Learning from the cell-matrix crosstalk to improve a cell delivery system based on RGD-modified alginate

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Natural polymers with the ability of forming 3D hydrogel networks under mild conditions such as alginate, offer an exciting possibility for the in situ delivery of vascular cells. The successful immobilization of viable endothelial cells (ECs) within 3D matrices is dependent of several parameters, such as cell density, amount of cell-adhesion sites and matrix stiffness. In this study, Human umbilical vein ECs were entrapped within RGD-alginate under different conditions, with cell density ranging from 5-30 million (M) cells/mL of alginate and RGD density from 50-500 μM. The effect of the 3D microenvironment on ECs was assessed in terms of viability and morphology (confocal microscopy). It was also analysed how ECs modify the viscoelastic properties (dynamic mechanical analysis) and the microstructure (cryo-SEM) of the alginate matrix. ECs were only able to spread and form multicellular network when entrapped at the higher cell densities (30, 20 and 15 M cells/mL), and presented the highest viability at 15 M cells/mL. Although cells were able to establish cell-cell contact, the viability was different, which could be related to the stiffness measured.

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For instance 30 M presented elasticity higher than the 15 M cells/mL. The obtained data points to the importance of analyzing the reciprocity between cells and matrix for the improvement of cell delivery systems. Acknowledgments: FEDER funds through COMPETE and FCT (project PTDC/SAU-BEB/101235/2008 & FCOMP-01-0124-FEDER-010915).

29.05
Gellan gum-based bilayered scaffolds for application in osteochondral tissue engineering

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Bilayered scaffold and cellular strategies are currently applied to solve the challenging problem of osteochondral defects. In this study, several formulations of Gellan gum were developed to fabricate different scaffolds possessing a cartilage-like layer and a bone-like layer. The bone-like layers were obtained by low acyl Gellan gum (LAGG) at 2 wt% and different amounts of hydroxyapatite powders (HAp) (5,10,15 and 20wt%). The cartilage-like layers were obtained by preparing LAGG formulation at 2wt% and formulations of LAGG at 2wt% and high acyl Gellan gum (HAGG) at 0.75wt% at a ratio of 75:25(v%). The viscoelastic measurements were performed using a TRITEC8000B DMA to characterize the mechanical behaviour of the bilayered scaffolds. The effect of the incorporation of different amount of HAp within the bone-like layer on the mechanical properties of the scaffolds was also investigated. Degradation and water uptake studies were performed by soaking the scaffolds in a phosphate buffered saline solution (pH 7.4) up to 30 days. The bilayered scaffolds were investigated by stereo microscope to evaluate the interface between both layers. The cytotoxicity of the bilayered scaffolds was investigated in vitro using a L929 cell line. In vitro studies regarding adhesion, encapsulation and viability of human chondrocytes (cartilage-like layer) and human osteoblasts (bone-like layer) cultured in the bilayered scaffolds were also carried by performing SEM analysis and Live/Dead assays.

29.P01
Development and optimisation of a growth factor delivery hydrogel for cartilage regeneration

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Engineered hydrogels containing TGF-β loaded microspheres and seeded with mesenchymal stem cells have been under investigation for cartilage repair and regeneration. In this study the influence of crosslinking conditions on TGF-β release from gelatin microspheres was evaluated and two polysaccharide hydrogels (agarose and gellan gum) were compared with fibrin to determine the most suitable hydrogel system for promoting chondrogenesis of progenitor cells. Agarose, fibrin and gellan gum hydrogels were seeded with infrapatellar fat-pad derived stem cells and TGF-β 1 loaded gelatin microspheres. TGF-β 1 release was quantified by ELISA. Constructs were cultured for 21 days and evaluated by biochemical assay and histological staining. TGF-β 1 release was dependent on the crosslinking time used to manufacture the microspheres. After 21 days in culture, sGAG synthesis was higher in both agarose and gellan gum compared to fibrin hydrogels. In addition fibrin underwent considerable contraction after 21 days in culture while agarose and gellan gum maintained their original shape. Histological staining demonstrated the presence of collagen and sGAG in the polysaccharide hydrogels. In conclusion, these results indicate that polysaccharide hydrogels are preferential to fibrin for use as a growth factor delivery scaffold for cartilage repair. The high chondrogenic potential of stem cells cultured in these hydrogels demonstrates the potential of these biomaterials for cartilage regeneration.

29.P02
Chitosan membranes for spatially controlled cell adhesion and specific cell recruitment

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We propose a concept of biomaterials that are able to fix specific cell types onto their surface when in contact with a mix population of cells. Adipose tissue has shown to be an interesting source of stem cells with therapeutic potential. However only a small amount of the heterogeneous mixture of the cells extracted from lipoaspirates are stem cells, and within stem cells there are different populations with different capabilities to differentiate through a lineage. We studied the ability of immobilized antibodies on chitosan surfaces to capture specific types of cells with a spatial micrometer resolution.Antibodies were covalently immobilized onto chitosan membranes using bis[sulfosuccinimidyl] suberate (BS3). X-ray photoelectron spectroscopy (XPS) was used to chemically characterize the surface and quartz crystal microbalance (QCM) to calculate the amount of adsorbed and/or immobilized antibody. Data shown greater immobilization when BS3 was used compared to simple adsorption. Specific antibodies covalently immobilized in a surface, kept their bioactivity and controlled the type of cell that attached on the chitosan surface. Microcontact printing permitted to covalently immobilize antibodies in patterns allowing a spatial control in cell attachment. Cell sorting experiments performed using a mixture of adipose stem cells and osteoblast like cells shown that chitosan surfaces were able to capture a specific phenotype depending on the immobilized antibody.

29.P03
Chitosan-bioactive glass hybrid films and scaffolds: assessment of bioactivity and mechanical behavior

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Many efforts have been made in the development of porous matrices with the objective of increasing its strength for a potential application in bone regeneration. The objective of this study was to investigate a composite material having as reinforcement phase bioactive glass. Hybrid scaffolds and hybrid films were developed with 20% content of bioactive glass (60% SiO2, 36%CaO and 4% P2O5) on chitosan crosslinked with 3% glutaraldehyde, and the introduction of the bioactive phase carried out by the sol-gel method. The films were obtained by solvent evaporation technique and the porous scaffolds were obtained by lyophilization method. The mechanical behavior was investigated by tensile test of the films and compression test of the scaffolds. The hybrid films with 20% bioactive glass showed a significant increase in its tensile strength. The results of in vitro tests with SBF (simulated body fluid) showed the formation of a layer of fibrils on the film surface, upon analysis, that is the characteristic morphology of carbonated hydroxyapatite, reflecting its favorable bioactivity. The porosity and interconnectivity of scaffolds obtained also were confirmed by SEM, which showed a homogeneous structure. The results also showed films with pH-stable after immersion in SBF. The FTIR confirms the presence of characteristic functional groups of the bioactive glass, confirming its introduction into the polymer network of chitosan.

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29.P04
Development of chitosan-based composite nanofibrous scaffolds for tissue engineering application

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Our previous study indicated that the use of electrospinning to prepare chitosan-based composite nanofibrous scaffolds were improved by incorporating polyvinyl alcohol and thus reducing the concentration of acetic acid needed for electrospinning. Nanofibers with uniform morphology were achieved by using a 20% acetic acid aqueous solution to prepare the chitosan-based composite solution and electrospinning at an applied voltage of 15 KV, without the formation of undesired beads on the nanofibrous scaffolds. The stability of fibrous scaffolds could be increased by using a crosslinking reagent glutaraldehyde for 12 hours. We further optimized the electrospinning process by adding other ingredients to prepare various composite solutions. The properties of the solutions including the viscosity, surface tension, and electrical conductivity were measured and compared. However, due to the complex interactions among molecules existing in the solution, it is unclear which factor(s) significantly affect the electro-spinability of the solution. Currently mesenchymal stem cells are used to determine the cytocompatibility of various nanofibrous scaffolds. The differentiation of stem cells is also analyzed. Through this study on the proliferation and differentiation of stem cells, various chitosan-based composite nanofibrous scaffolds suitable for tissue engineering application can be developed. (Supported by the National Science Council, Taiwan: NSC 100-2221-E-002-113-MY2).

29.P05
A particle-agglomeration method for the preparation of 3D chitin-based hybrid matrices

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Hybrid materials have an immense potential for applications in a variety of advanced technologies, both as structural materials, as well as functional materials in biomedical applications. The sustainable development of hybrid materials is moving towards the use of green chemistry and clean technologies, namely by the use of natural polymers, sol-gel processes, ionic liquids (ILs) and supercritical fluids. In this work chitin-based hybrid 3D structures were prepared by particle agglomeration associating the use of an IL and a sol-gel process. Bioactive particles were produced dissolving chitin in an IL and precipitating the solution in a bath containing tetraethylorthosilicate/water/ethanol/hydrochloric acid. Particle agglomeration was promoted by critical point drying as particles were loaded and pressed in a mould together with a gellan gum solution which enhanced the agglomeration and provided mechanical strength to the system. At the same time it is possible to impregnate the particles with an active compound. Ibuprofen was chosen as a model drug and impregnation was successfully achieved. Chitin-based materials were characterized in terms of their morphological and mechanical properties, sustained release ability, bioactivity and biocompatibility. The findings suggest that this strategy is feasible and advantageous to process hybrid chitin 3D matrices with both functional and structural characteristics that make then suitable for regenerative medicine applications.

29.P06
Smart multilayer films for biomedical applications

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Thermo-responsive thin films were developed by layer-by layer sequential assembly of chitosan-graft-NIPAAm and alginate. First, the chitosan-graft-NIPAAm copolymer was synthesized and analysed in order to confirm the introduction of NIPAAm moieties onto the chitosan backbone. The LCST of the synthesized copolymer was around 31-33 °C. Then, the formation of the multilayers was analysed by quartz crystal microbalance with dissipation monitoring (QCM-D) and by UV-Vis measurements. It was found a linear increase of the multilayer film growth and the influence of the presence of salt. Preliminary results showed that, upon reducing culture temperature below PNIPAAm LCST, a gradual detachment of cell sheets from these PNIPAAm-based coatings have occurred. So, the developed films could potentially be used in cell sheet engineering. Furthermore, the easy-to-build and the high tunable features of these LbL coatings make them ideal candidates to be employed for drug delivery and controlled release systems, controllable membranes, filters and sensor devices.

29.P07
Evaluation of ionically cross-linked alginate films as culture substrates for human fibroblasts

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We evaluated the suitability of various ionically cross-linked alginate gel films as culture substrates for cells. Calcium ion-cross-linked alginate is widely utilized in many fields, but is known to be an unfavorable culture substrate for cells. In a previous study, we demonstrated that ferric ion-cross-linked alginate was an effective cell culture substrate. The aim of the present study was to clarify the reasons for differences in the affinity of alginate films for cells when different types of cross-linking ions were used. Typical trivalent (Fe3+ and Al3+) and divalent (Ca2+, Ba2+, and Sr2+) ions known to act as cross-linkage agents for alginate were used to form gel films, and investigated for their ability to support cell growth and their effects on the surface properties of alginate films. We cultured human dermal fibroblasts on each of the different alginate films. We found that trivalent ion- and barium ion-cross-linked alginate films supported good cell proliferation. The protein adsorption abilities of these alginate films were correlated with the degree of cell growth on the films. Surface property analysis revealed that trivalent ion-cross-linked alginates were more hydrophobic than other types, and that only barium ion-cross-linked alginate films had a rough surface. Therefore, the surface wettability and morphology of alginate films may be related to their protein adsorption ability, thus determining their suitability as culture substrates for cells.

29.P08
Synthesis and preparation of konjac glucomannan hydrogel for wound healing

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The chemical functionalities of a hydrogel is of particular importance in wound healing. In this study, we developed a hydrogel from polysaccharide with known properties of biocompatibility, assistance in wound healing and biodegradability. Konjac glucomannan (KGM)is a promis-

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ing material to stimulate cellular metabolic activity via its specific sugar receptors on fibroblast. KGM is a straight chain polysaccharide having component sugars; D-mannose and D-glucose linked by β(1-4) glycosidic chain in a ratio of 1.6:1. Previous study has shown that native KGM is very stimulatory for fibroblasts and is able to support cultivation in unchanged media up to 20 days. Following this, methods for the synthesis of KGM hydrogels were developed using free radical polymerization. KGM hydrogels were synthesized by varying the amount of KGM and Ce (IV) in different ratios. Fibroblasts were seeded on tissue culture plastic (TCP) and the hydrogels were put in direct contact up to 5 days and assessed for cellular viability using MTT assay. Characterizations of the hydrogels were measured using FTIR and DSC. Cell viability assay showed increase in fibroblast viability when the hydrogels with an increase amount of KGM were put in adjacent with cells. However, increased amount of initiator, Ce (IV) caused inhibition to cells, also changing the chemical structure of the hydrogels when measured using FTIR and DSC. The stimulation of fibroblast viability by KGM makes a good candidate for wound healing.

29.P09
In vitro bioactivity of chitosan attached to titanium constructs using a novel electrophoretic deposition method

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Titanium and titanium alloys are frequently utilized in clinical treatment of orthopaedic injuries. Considerable research has focused on improving bone attachment to implants by coating the titanium with biomaterials. Chitosan, a partially deacetylated form of chitin, is a promising coating agent under investigation. Electrophoretic deposition (EPD) is currently being explored as a technique for applying bioactive coatings to implant surfaces. In this study, an EPD method was developed to coat titanium with chitosan. The titanium surface was pre-treated by sand-blasting and/or acid etching to obtain desired surface roughness. Chitosan (DD 87%) was solubilized in acetic acid (1% v/v). EPD was performed using a titanium cathode under constant voltage conditions with generated electric fields ranging from 0.5 to 6 V/cm. Coated surfaces were characterized by water contact angle measurement, SEM, AFM, and μCT analysis. The coated titanium was seeded with MC3T3 mouse pre-osteoblasts; cell bioactivity was analyzed by viability, proliferation, cell attachment, and osteogenic differentiation. Preliminary results suggest EPD is well-suited for depositing chitosan onto titanium implants. No detachment was observed when coated titanium was incubated in cell culture medium during a 3-week study. Obtained coatings were porous due to gas bubble entrapment at the cathode. Future work will focus on minimizing porosity by using pulse-width modulation in place of constant voltage EPD.

29.P10
Investigation of mineralized alginate gels as a scaffold material for stem cell based bone tissue engineering

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Hydrogels have long been recognized for their applications in tissue engineering, either as encapsulation matrices for cells and growth factors or for the use in wound management aids. However, only recently they have shown potential applications in tissue engineering of hard tissues, due to development of strategies for mineralization and cell-matrix interactions. Here, we describe the mineralization of alginate hydrogel beads and encapsulation of human cell types relevant for bone tissue engineering in those materials. Human mesenchymal stem cells (MSCs) derived from bone marrow (BMSCs) and from adipose tissue (AdMSCs) were shown to be compatible with encapsulation in beads mineralized by counter-diffusion, while primary human osteoblasts (OST) showed poor viability post encapsulation. The beads mineralized by counter-diffusion with encapsulated BMSCs were shown to be extensively mineralized after 21 days of culture in a differentiation medium. BMSCs encapsulated in beads mineralized enzymatically showed differentiation towards OST phenotypes when cultured in differentiation medium, and scanning electron microscopy showed that the cells secreted collagen. However, the initial mineralization was necessary for further mineralization of the beads. Thus, the present work shows that a combination of initial mineralization and culture in differentiation medium facilitates secretion of extracellular matrix and further deposition of mineral phase.

29.P11
Characterization of chondroitin sulfate-poly(ethylene oxide) hydrogel

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Chondroitin sulfate (CS), a glycosaminoglycan in the extracellular matrix of all vertebrates, has been used as a component of biomaterials for its applications in tissue engineering and drug delivery. The physicochemical and in vitro properties of CS-poly(ethylene oxide) (PEO) hydrogel were analyzed after synthesis of the CS-PEO gel through a Michael type addition reaction by changing the composition ratios of precursor solutions. Chemical examinations such as NMR and FTIR showed modification of CS as observed by new chemical peaks of CS-acrylate as well as swelling changes of the gel. CCK-8 assay of MC3T3 cells on the gel surface showed initially higher cell adhesion on the higher concentrated CS gel. CCK-8 and Live and dead assays demonstrated that all the cells was spread and aggregated on the surface of the gel, but they were detached from its surface over time and the cells inside gel showed no cell spreading. The hydrogels showed excellent cell viability compared to that of latex, the negative control. All the assays of MTT, BrdU and neutral red indicated excellent cell compatibility of the gel, indicating biocompatibilities of the hydrogel in cell organ levels such as mitochondria, lysosome, DNA. The experimental results showed that the CS-PEO hydrogel was biocompatible for its applications in tissue applications.

29.P12
Polycaprolactone/starch composite with in situ pore formation capability for bone tissue engineering application

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Bone tissue engineering is a new and efficient approach to regenerate bone defects. An ideal composite used for bone tissue engineering scaffold should possess adequate pore size, controllable biodegradability and suitable mechanical properties. This work describes the development of biodegradable polymeric composite, based on polycaprolactone (PCL) and starch with the ability to form a porous structure in situ. By controlling the in situ degradation and the consequent pore formation, the scaffold would have the required mechanical properties

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during the initial stage of implantation. To fabricate SPcl composite with a ratio of 30/70, Starch and polycaprolactone were mixed with Chloroform and cast in moulds. After characterization of composites using FTIR spectroscopy, degradation studies were performed in PBS. The UV-visible spectroscopy's result used to evaluate starch degradation rate as an index for composite degradation. Furthermore the cross-sections morphology, before and after degradation, was observed by scanning electron microscopy. SEM images showed that a porous structure is formed. These results suggest that SPcl composite, with potential to form porous scaffold in situ, is promising for bone tissue engineering applications.

29.P13
Impact of different formulation variables on the biodegradability, surface area and porosity of biopolymeric microparticles

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The influence of different formulation variables including the biopolymer M.W. and concentration, crosslinking density, time and pH in addition to co-crosslinker concentration on the functional properties such as surface area, porosity, chemical and bio-degradability of chitosan microparticles (CS MPs) prepared by ionic gelation technique was studied. The microparticle yield ranged from 8.92 to 38.02% with a particle size of 0.83-1.22 mm. SEM micrographs revealed a spherical shape of microparticles with some cracks and pores. CS MPs showed higher swelling degree in simulated gastric than in simulated intestinal fluid and disintegrated after 6 hr at pH 1.2. Glutaraldehyde co-crosslinked microparticles showed a lower swelling in both media and maintained their integrity for 3-4 days. These microparticles exhibited a surface area of 19.33 - 56.25 m2/g with a pore volume of 28.37 - 77.67 e-3ml/g. They were found to be a susceptible substrate for the hydrolytic action of lysozyme while remained almost structurally stable in HCl and PBS. Conclusively, proper modification of formulation variables can affect the physicochemical properties of these microgel carriers and suggest their usefulness for controlled release of drugs and bioactive substances.

29.P14
Cell adhesion in free-standing multilayer films made of chitosan and alginate

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The method for preparing multilayer ultrathin films by the consecutive deposition of oppositely charged polyelectrolytes has gained tremendous recognition due the user friendly preparation, capability of incorporating high loads of different types of biomolecules in the films, fine control over the materials' structure, and robustness of the products under ambient and physiological conditions. However the preparation of such films needs the assembly on a substrate and, sometimes, cannot be detached from it, which has limited the application of such films in areas as tissue engineering and regenerative medicine (TERM).Thus, the production of free-standing films is of extreme importance once it allows the direct experimental determination of many physical properties of fundamental significance such as ion permeation and mechanical properties that can be tuned for real-world applications. In this work, we investigated the elaboration of free-standing multilayer films made of chitosan (CHI) and alginate (ALG), by detaching a polyelectrolyte multilayer film from its underlying substrate without any postprocessing step. The conditions for optimized film growth were investigated. The adhesion of C2C12 myoblast cells on the CHI/ALG membrane was assessed by cytoskeletal and nuclear staining. A good cell adhesion and spreading was observed all over the surface. The results demonstrate the potential of such biocompatible free standing membranes made of CHI and ALG for applications in TERM.

29.P15
Chitosan/silica homogenous hybrid hydrogels for tissue engineering applications

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The objective of this study was to fabricate a homogenous organic/inorganic biohybrid hydrogel from chitosan and bioglass. Chitosan is a natural polymer with favourable properties of non-toxicity and ability to promote wound healing. Hydrogels of chitosan are considered as a potential candidate for bone replacements; however, lack of bioactivity limits its application. Bioceramics such as bioglass are combined with chitosan hydrogels to address this issue. Bioglass exhibits high bioactivity and capability to bond with living bone in the body without forming fibrous tissues. Addition of bioglass to chitosan improves its bioactivity and cell adhesion properties; however, lack of homogeneity is a major challenge for the development of this composite matrix. Formation of hydrogen bonding promotes the compatibility between organic and inorganic phases in their physical mixtures; however, this bonding was not efficient for the production of homogeneous hybrids. The results of our study demonstrated the feasibility of fabricating a homogenous mixture between chitosan and bioglass by chemical conjugation. FTIR analysis confirmed that functionalization of chitosan with silica-containing molecules resulted in the formation of Si-O-Si bonds between silica groups of bioglass and functionalized chitosan. The physiochemical properties of hybrids such as degradability, swelling and mechanical strength were a function of the degree of covalent bondings of bioglass and chitosan.

29.P16
Evaluation of the effect of addition of wollastonite on the mechanical strength, porosity and cell compatibility of different molecular weight chitosan

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Chitosan is a cationic biodegradable polymer which possesses very good cellular compatibility and tissue growth, but lacks mechanical strength for bone tissue regeneration. The present work describes the effect of wollastonite on various molecular weight of chitosan scaffold. Different composite scaffolds with varying molecular weight of chitosan (109,513, 41,707, 27,175) and % of wollastonite (20%,30%,40%) have been prepared by freeze drying method. The scaffolds were evaluated to find any improvement in mechanical strength, porosity and biocompatibility. The highest mechanical strength was achieved with scaffold containing chitosan of mol. wt. 27,175Da and 30% wollastonite (particle size$<5\mu$m). The compressive stress and compressive modulus were found to be 140.9 and 1201 kPa and porosity 77.5%. This study demonstrated that the low molecular weight chitosan produces composite scaffold with higher mechanical strength compared to the chitosan with high molecular weight. Further there is no significant

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change observed in porosity and cellular biocompatibility which is confirmed by in-vitro cell growth study.

29.P17
Polycaprolactone electrospun scaffolds incorporating chitosan micrograins for enhanced cell migration

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Scaffolds produced from a combination of materials of different origins, chemical nature and length scales are extensively studied in TERM due to the expected synergistic combination of their respective properties. In particular, electrospinning is often combined with other techniques in order to increase the pore size and interconnectivity between pores sufficiently large to allow cell infiltration in electrospun nanofiber mats. We produced and characterized membranes composed of alternating layers of the synthetic polyester polycaprolactone (PCL) and the natural polysaccharide chitosan (CS). The PCL layers consist of microfibers obtained by electrospinning and CS layers are made by scattering grains of CS over the PCL fibers. By this process, membranes with different PCL/CS ratios (from 1:0 to 1:1) as well as with different CS grain sizes (below 100 μm, from 100 to 200 μm and from 200 to 300 μm) were produced. The membranes were subjected to mechanical testing, contact angle measurement and determination of their porosity. Cell culture tests were carried out with epithelial cells MCF7-GFP. The results showed that the membranes have mechanical properties that allow their easy handling, with a low elastic modulus and tensile stress and a high elongation at break. All membranes exhibit high porosity and a hydrophobicity similar to that displayed by PCL. Cell adhesion, proliferation and infiltration was observed and confirmed using confocal and phase contrast microscopy.

29.P18
Nanocomposite microfibers based on alginate and PVA hydrogels with incorporated silver nanoparticles

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Biocompatible nanocomposite hydrogels such as alginate and poly(vinyl alcohol) (PVA) with incorporated silver nanoparticles (AgNPs) have advantageous potentials for biomedical applications such as antimicrobial wound dressings for deep, necrotic wounds and as soft tissue implants with prolonged sterility. Microfibers are especially suitable for efficient AgNPs delivery due to high surface to volume ratio and simple arrangement into different 3D structures. In this study, we have produced alginate microfibers (1.9%w/v, ~300 um in diameter) with incorporated, electrochemically synthesized AgNPs (1-3 mM). In addition, a novel gelation procedure based on extrusion followed by repeated cycles of freezing and thawing, was developed for production of microfibers formed from blends of PVA (5.6%w/v) and alginate with AgNPs (1.3% w/v, 1-2 mM AgNPs). The microfibers could be used in wet as well as in dried forms whereas the re-swelling ratio and AgNPs release depended on the swelling medium being significantly higher in saline solution as compared to distilled water.

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30. Hyaluronan and its Use in Tissue Engineering (in coop. Biodesign)

30.01
Keynote: Safe and efficient *in-vivo* gene gene transfer and silencing technologies using natural pathways

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Introduction: The advance in nucleic acids therapeutic had been outstanding in recent years, which have opened new possibilities in regenerative medicine to tackle many serious diseases. To make nucleic acids based therapy a reality, the fundamental problem of tissue specific cellular delivery has to be accomplished. Here we present a new approach to develop the next generation of delivery vectors.
Materials and methods: Hyaluronic acid were modified to complex DNA plasmids for luciferase transfection. Transfection: HCT 116 cells and mice where tranfected by adding HA-DNA complex. The transfection experiment with commercially available reagent polyethyleneimine (PEI) and plasmid alone were used as controls.
Results and discussion: Efficient tranfection was shown using the new non toxic and safe non viral vector. Using ECM components (such as hyaluronic acid or HA) that allow both *in vitro* and *in vivo* transfection of plasmid DNA in CD44 positive cells. The chemically modified ECM components (non-toxic) binds to nucleic acids and are taken in by cells by natural receptor mediated endocytosis. The amount transported via these routes naturally is around 10 gram per day to give a potential capacity far exceeding the need for transfection if it is targeted. By molecular association strategies (joining the bandwagon) it is now possible for nucleic acids to follow these pathways that utilise the highly efficient receptor mediated endocytosis- door opener into cells.

30.02
Cross-linked hyaluronic acid as a scaffold for the treatment of cartilage defects

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One important feature of biomaterials for cartilage regeneration is their influence on establishment and stabilization of a chondrocytic phenotype of embedded cells. We examined the effects of a porous three-dimensional scaffold of cross-linked hyaluronic acid on the expression and synthesis performance of human chondrocytes. Therefore, scaffolds were cultured with osteoarthritic chondrocytes for up to 3 weeks. Cell content within the scaffold was estimated by determination of the metabolic activity (XTT assay) and quantification of DNA (CyQuant cell proliferation assay). The expression of chondrocyte-specific genes was determined by real-time RT-PCR, and the synthesis of sulphated glycosaminoglycans (sGAG) was analysed biochemically. Cells showed homogenous distribution within the scaffold. Although DNA quantification indicated only partial loss of cells most probably due to matrix degradation, the metabolic activity within the scaffolds decreased dramatically. This might be attributed to a stop in cell proliferation combined with a switch of the cellular genetic program from cell division to differentiation towards a chondrogenic phenotype. Analysis of gene expression and sGAG synthesis substantiated this hypothesis as both chondrocyte specific gene expression and sGAG synthesis were increased and the differentiation index was clearly improved. To conclude, these results suggest that the investigated material has a chondroinductive effect on embedded cells.

30.03
Novel hyaluronic acid hydrogels for spinal cord repair

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Spinal cord injury and repair is one of the important focus areas in tissue regeneration. Mechanical trauma caused due to factors such as contusion, compression or involuntary stretching induce post-traumatic secondary tissue damage in many Spinal Cord Injury (SCI) patients. Therefore, there is a need for scaffolds that provide a conducive three-dimensionsal (3D) environment for injured cells to attach and grow. In this study we propose to synthesize 3D polymeric scaffolds in order to study the mechanical and adhesive properties & the nature of the interactions between hyaluronan-based (HY) biomaterials and cells and tissues both *in vitro* and *in vivo*. Here we have synthesized 3D HY-based hydrogels with robust mechanical and adhesive properties and demonstrate the use of this material for neuronal-related applications such as the treatment of SCI. Cell culture and survivability studies were done with NSC-34 cells. Live/Dead assay performed on the cells revealed significant differences in the staining of live cells and showed increased viability and proliferation. The number of live cells in the HY-based hydrogels with 0.1% collagen showed higher cell numbers compared with the other hydrogels. In this study we show that Injectable HY-based hydrogels with high elasticity, comparable to the mechanical properties of nervous tissue have been used in this study to study their biocompatibility and neuroprotective properties and they show better affinity for neuronal cells.

30.04
Thiol-ene clickable PEG based thermoresponsive hyperbranched copolymer for *in situ* crosslinking hybrid hydrogel

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In recent years, the *in situ* gelling hydrogels have attracted much attention because they can be injected directly into irregular cavities and provide a homogenous environment for cellular and molecular distribution. Thermoresponsive gels can be widely applied for the *in situ* gelling scaffold as the gel is easy to handle and the shape can be easily mould in the cavities. This physical thermal gel, however, is mechanically too weak for desired clinical applications. With the hypothesis of a thermoresponsive copolymer containing of vinyl functional groups can be also cross-linked with a cross-linker with thiol groups via thiol-ene

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Michael Addition, the objective of this study is to develop a PEG based hyperbranched copolymer containing a high level of vinyl groups which can be *in situ* cross-linking with a thiol modified Hyaluronan (HA-SH) biopolymer. This injectable and *in situ* crosslinking hybrid hydrogel system offers great promise as a new class of hybrid biomaterials for tissue engineering. PEGMEMA-MEO2MA-PEGDA copolymers with both thermoresponsive and chemical cross-linkable behavior were synthesized via in-situ DE-ATRP. The polymer solution could form a thermal gel at body temperature, while the chemical gelation occurred less than 10 min once mixing with thiolated HA. LIVE/DEAD® assay was employed with 3T3 and ADSCs after 1 week in culture, which was indicated that with very few dead cells, both cell types survived well in 3D hydrogels.

30.05
The release of rhBMP-2 from a bisphosphonate hydrogel

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Introduction: There are several disadvantages with the present carriers used in rhBMP-2 products including risk for immunological response, inefficient release and inferior handling properties. Our aim was to examine the release of rhBMP-2 from a bisphosphonate hyaluronan hydrogel (BP-HA) carrier with the hypothesis that it will cause a slower release.
Methods: Triplicates of hydrogels with 5 μg of rhBMP-2 and with (HA-BP) or without (HA) bisphosphonate were prepared, after which cell culture medium was added and refreshed at 1, 3, 6, 12, 24, 48, 72 h and day 6, 9, 12, 14. The extracts were analysed in enzyme-linked immunosorbent assay. After the study period the gels were degraded and analyzed by alkaline phosphatase assay on W20-17 stromal cells.
Results: The HA-BP hydrogel showed a gradual release with small amounts of rhBMP-2 at all time points but by the end of the study only 6% of rhBMP-2 had been released compared with 100% for the HA hydrogel. ALP assay showed that the retained rhBMP-2 was active.
Discussion & conclusions: Adding bisphosphonate caused a remarkable slower release for rhBMP-2 when added into a hydrogel. The retained rhBMP-2 was still intact in the biological functions and could induce ALP expression of stromal cells.

30.P01
Hyaluronic acid production from eggshell (membrane): optimization of isolation condition

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Hyaluroic acid (HA) - based scaffolds have been of great interest, owing to the important role HA plays in tissue development. In this work, the isolation performance of HA from eggshell (membrane) as an economical source was investigated in terms of extraction time (1, 2, 4 and 6 days), temperature (4, 9 and 14 °C) and extraction strategy (without pH control, with pH control at 3.5) using a full factorial experimental design with three times replications. A 350 ml contactor (id = 80 mm) consisted of a water jacket circulation and an on/off pH controller, was used for the extraction process and HA was extracted by acetic acid digestion at 200 rpm. The HA concentration in the crude extracts was determined by the carbazole assay. The obtained results and their F-test analysis of variance with 95% confidence interval showed that extraction strategy (F0 = 653.8), extraction time (F0 = 29.3), extraction time-temperature interaction (F0 = 18.21) and temperature (F0 = 11.82) had significant effect on the HA isolation. The maximum HA content of the crude extracts was obtained about 0.53% in the strategy of pH control, temperature of 9 °C and extraction time of 4 days, the value of which was three times higher than that in the strategy without pH control. The amount of HA isolated from eggshell (membrane) was comparable with the known source of rooster comb, showing the potential of eggshell (membrane) as an economical source for HA isolation and scaffold preparation in tissue engineering.

30.P02
Optimization of physical-chemical properties hyaluronic acid based hydrogels

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Hyaluronan (HA) is a naturally occuring glycosaminoglycan and one of the chief components of the extracellular matrix, articular cartilage or synovial fluid. One of promising applications of HA are hydrogel-based scaffolds for articular cartilage regeneration used in the field of tissue engineering. Thanks to the role of HA in cartilage formation, the HA scaffolds enhance cartilage reconstruction performed by chondrocytes or MSCs in comparison with e.g. fibrin or PEG hydrogels used so far. On the other hand, like the most other naturally derived polymers-based hydrogels, HA-based hydrogels lack mechanical properties to withstand physiological loads. In our previous work we found out that this problem can be partially eliminated by introduction of optimal alkyl based linker into the HA polymer structure. The results showed that the introduction of suitable alkyl linker into the HA derivative structure improves mechanical properties and resistance of HA hydrogel-based scaffolds. The presence of the linker in HA structure increases flexibility of HA polymer segments and variability of polymer microconformations. The secondary effect is the enhancement of crosslinking efficiency, which leads to better homogeneity of formed hydrogels. The aim of this study is a determination and optimization of these HA derivatives with the introduced alkyl linker from the point of view of chemical synthesis practicability and final mechanical properties of hydrogels based on these HA derivatives.

30.P03
Development of multifunctional hyaluronic acid-based carriers by 'click' chemistry

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Introduction: We have developed methods to simultaneously introduce chemoselective groups to hyaluronic acid (HA) that can be crosslinked and used for immobilization of various molecules. It is easy to switch the functionalities of obtained materials through orthogonal 'click' chemistries.
Methods: HA polymers were dually functionalized with hydrazide and thiol groups, or aldehyde groups. Different functional molecules such as bisphosphonate (BP) groups for mineralization or camptothecin (CPT) as anti-cancer drug can be linked to thiol groups. HA polymers were characterized by 1H, 31P NMR, and UV-Vis Spectroscopy. Characterizations of materials were performed using SEM, TEM, rheology, and colorimetric calcium assay.
Results and discussion: The functional polymers were successfully synthesized. They can form hydrogels within 1 min with HA-aldehyde polymer. BP groups showed high affinity to calcium ions and inorganic nanoparticle phases were observed within BP containing hybrid hydrogel. On the other hand, CPT-linked HA polymer via S-S bond can

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self-assembled into nanoparticles, and the release of the drug is triggered by the addition of reduce agents (e.g. dithiothreitol).
Conclusions: The use of orthogonal click modification opened a versatile way to prepare different kinds of HA based carriers with various functionalities by simple methods. The multifunctional HA polymers show high potentials to be used for drug delivery (pro-drug) or tissue regeneration (BP linked HA).

30.P04
Dynamic hyaluronic acid hydrogels for cardiac therapy are biocompatible and degradable

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Tissue-specific elasticity arises from developmental changes that occur in the environment over time, e.g. ~10-fold myocardial stiffening from E3 to E10 in the chick embryo. Recently, we showed that pre-cardiac mesodermal cells plated on a thiolated hyaluronic acid (HA) hydrogel engineered to mimic this time-dependent stiffening improves cardiomyocyte maturation compared to cells on static compliant matrices. Here we determined *in vivo* biocompatibility and cell-matrix interactions using subcutaneous and intramyocardial injections. Prior to assembly, HA hydrogels were injected into rats, removed over a post-injection time course, and subjected to histological, immunological, and mechanical analysis. Histological analysis shows minimal infiltration of host cells and capsule formation for subcutaneously injected rats, indicating a limited local immune response. Hematological analysis shows no significant systemic immune response was elicited in pre- versus post-injection animals compared to controls. Importantly, atomic force microscopy analysis of samples from subcutaneous injections demonstrates dynamically increasing hydrogel stiffness over time similar to that previously found *in vitro*. When injected intramyocardially, host cells begin to actively degrade HA within 1 week post-injection and lay down host matrix, nearly replacing the gel with host tissue by 1 month. Altogether, these data suggest HA is a viable source for therapeutic use in treatment of myocardial infarction.

30.P05
Both low and high molecular weight hyaluronan support neuronal progenitors *in vitro*

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Hyaluronan (HA) is an abundant component of the extracellular matrix in all tissues including the nervous system. However, an importance of HA for differentiation of neural progenitor cells (NPCs) is not well characterized. Particularly unknown are the effects of low molecular weight (MW) HA, which is suggested to posses different biological activity compared to high MW HA. Thus, here we tested the effects of external addition of various MW HA on the differentiation of the NPCs obtained from mouse embryonic stem cells through differentiation *in vitro* and NPCs isolated from mouse embryos. Both high MW HA 970 KDa and low MW HA 13, 33, 59, and 87 kDa HA prevented a decline in neuronal markers in cell culture during a time period of up to 6 days suggesting an increase in the fraction of NPCs in cell culture. Interestingly, in contrast, both low and high MW HA modified the NPCs morphology so that the cells lost their typical shape with long protrusions. Combined treatment by high and low MW HA showed an additional supportive effect on NPCs, both on neuronal markers and cell viability. To determine importance of endogenously produced HA 4-methylumbelliferone, an inhibitor of HA synthesis, was tested that showed decreased levels of neuronal markers in cell culture and decreased the NPCs viability. Interestingly, combined treatment by 4-MU together with HA of various MW completely annulled the effects of these exogenously added HA preparations.

30.P06
Smart design of stable hydrazone crosslinked extracellular matrix mimetic hydrogel for tissue engineering application

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Injectable hydrogels are important biomaterials with enormous applications. They are used for various biomedical applications such as diagnostics, 3D cell culture matrix, drug reservoir, encapsulation of bioactive compounds and growth factors, scaffold for tissue engineering etc. We here present our recent development in our efforts to develop hydrogel scaffolds with enhanced rigidity, stability, swelling characteristics. Hydrazone crosslinked gels are attractive due to its simplicity and versatility which could be formed by mixing appropriate aldehyde and hydrazide functionalized hyaluronan. By fine-tuning the electronic character around the hydrazone linkage, we succeeded in developing extremely stable hydrazone bond and utilized it for developing hyaluronan (HA) based synthetic extracellular matrix (ECM) hydrogel. Among the different hydrazides tested, we identified carbonyldihydrazide (CDH) as the best candidate to deliver stable hydrazone linkage. This stability is presumably due to extensive delocalization of the positive charge across neighboring amino groups of CDH. The hydrolytic stability imparted by this group was found to be several folds under acidic, basic and physiological pH when compared to other hydrazones. This tailored hydrogel with CDH also exhibited superior swelling and mechanical properties and enzymatic stability which makes it ideal for tissue engineering application.

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31. Bioinspiration and Biomimetics

31.01
Keynote: Native chemical ligation and related reactions for polymer hydrogel cross-linking

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We recently described the formation of polymer hydrogels by native chemical ligation (NCL), whereupon a thioester-containing polymer and a N terminal-cysteine modified polymer rapidly form rigid hydrogels under physiological conditions, without the use of toxic initiators and catalysts. This method has the benefit of being highly chemospecific, even in complex biological fluids. However, the thiol leaving group can have adverse biological effects, and the traditional NCL reaction proceeds rapidly only at alkaline pH. In this presentation we describe alternative leaving groups that improve the biological performance of the NCL hydrogel system, and facilitate its use under physiologic conditions. Furthermore, physical observations of the formed hydrogels reveal a secondary cross-linking reaction involving disulfide bond formation among regenerated free thiol groups. This secondary cross-linking has a significant effect on swelling and other physical properties of the hydrogel. Finally, we will report the results of our ongoing work involving the use of these hydrogels for drug delivery, medical sealing and wound healing.

31.02
Functionalisation of methacrylated gellan gum hydrogels by anti-angiogenic dendrons

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The regeneration of cartilage in the intervertebral disc nucleus pulposus and joints is impaired by the formation of fibrocartilage that is caused by the invasion of the tissue by blood vessels. Peptides have been identified by phage display technique which are able to bind VEGF thus inhibiting angiogenesis. The present works focusses on the synthesis of poly(epsilon-lysine) dendrons of three branching generations in which the molecular root of the dendron bears a di-phenylalanine sequence to promote hydrophobic interactions with material surfaces and the uppermost molecular branches are functionalised with the amino acid sequence WHLPFKC that is known to block VEGF. These biofunctionalised dendrons were entrapped in methacrylated Gellan Gum (GG-MA) hydrogels and tested for their ability to inhibit endothelial cell sprouting by both a 3D *in vitro* cell models and an in ovo model. The results show that when GG-MA is functionalised with the dendronised VEGF blockers, a regression of angiogenesis takes place around the hydrogel boundary. The in ovo study supports these findings as the GG-MA functionalised with the dendronised VEGF blockers did not elicit any acute inflammatory response, and decrease the number of converging macroscopic blood vessels as compared to positive controls. Moreover, the hydrogels prevented the infiltration of blood vessels, after 4 days of implantation.

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31.03
Fibronectin matrix mimetics accelerate repair of chronic wounds

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During tissue repair, fibronectin (FN) is converted from a soluble, inactive form into biologically-active extracellular matrix (ECM) fibrils through a cell-dependent process. ECM FN promotes numerous cell processes critical to tissue repair and regulates the assembly of other proteins into the matrix. To functionally mimic ECM FN, we developed a series of recombinant FN matrix mimetics by directly coupling the heparin-binding fragment of the first type III repeat of FN (FNIII1H) to various sequences from the integrin-binding domain (FNIII8-10). Two of these mimetics, GST/III1H, 8, 10 and GST/III1H, 8RGD, support cell adhesion, migration, proliferation, and ECM assembly to a similar or greater extent than full-length FN. Non-healing wounds show reduced levels of ECM FN. Thus, we asked whether FN matrix mimetics could act as ECM FN analogs to heal chronic wounds. Full-thickness, punch biopsy wounds were produced in genetically-diabetic mice and FN matrix mimetics were applied directly to the wounds. A significant enhancement of wound closure was observed by Day 10 in response to GST/III-1H,8RGD treatment (74.7 ± 5.4% closure vs 49.6 ± 6.4% closure in GST-treated controls). Two weeks after injury, granulation tissue thickness of GST/III1H,8,10-treated wounds was twice that of GST controls (565.5 ± 17.8 μm vs 272.7 ± 80.7 μm). These results indicate that treatment of chronic, diabetic wounds with recombinant FN matrix mimetics can accelerate tissue regeneration.

31.04
Transfer printing of tissue with anisotropic extracellular matrix assembly by using cell interactive and thermosensitive hydrogels

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The structure of tissue plays critical roles in its function, and thus many approaches have been developed to re-build artificial scaffolds with native tissue-like architecture in tissue engineering. However, these systems have often reported several problems associated with foreign body reactions or toxicity upon implantation. Alternatively, transfer printing of tissue using smart biomaterial substrate has been considered to generate tissue-like constructs. In this study, we developed thermosensitive hydrogels with cell-interactive peptide and micro-scaled anisotropic groove patterns via bio-inspired enzymatic polymerization as a substrate to harvest tissue. Transfer printing of tissue is based on tissue-like constructs cultured on thermosensitive hydrogels that can be simply transferred to any substrates by change in hydrogel size in response to temperature stimuli. We found that tissue constructs of assembled muscle cells with anisotropic patterns can be easily transferred to many types of substrates including glass, film and electrospun fiber, and then confirmed that transferred tissue like constructs maintained their structural characteristics. In addition, multi-layered tissue constructs were prepared for up to four layers by this transfer printing technique. Furthermore, tissue constructs were successfully trans-

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planted onto subcutaneous skin regardless of shape and size. Our system may hold potential to engineer tissue that can closely mimic native tissue architecture.

31.05
Sculpturing the complex lamellar architecture of annulus fibrosus tissue using chemically modified silk scaffold

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The study focused on development of unique custom-made silk fibrous scaffold having lamellar criss-cross fibre orientation in successive layers, using a novel winding machine based on Textile Technology. Silk fibroin fibre surface was modified by covalent crosslinking of chondroitin sulfate (CS). Attachment of CS with silk was characterized by ATR-FTIR and NMR. De-differentiated goat chondrocytes were cultured over only Silk and silk-CS scaffolds upto 6 weeks. Immunofluorescence studies demonstrated that cells followed fibre alignment. Engineered tissues were analyzed at regular interval by SEM, histological (H&E, Safranin-O, Picrosirius red staining), MTT, biochemical analysis (GAG and collagen estimation). To find contribution of orientation of ECM matrix on biomechanics, 6 weeks cultured constructs having fibre orientation angles 0, 30, 60 were subjected to mechanical characterization (tensile, compression, shear at a low strain rate) and compared with goat AF tissue. Mechanical properties are significantly higher in silk-CS constructs, compared to only silk constructs and closely resembled that of native goat AF tissue. Differential expression of ECM proteins on silk and silk-CS constructs was studied by MALDI-TOF/TOF. Cells seeded on Silk-CS constructs expressed proteins like laminin, perlecan, ankyrin, unlike cells on only silk scaffolds. Enhanced ECM assembly, differential expression and orientation resulted in augmented mechanical features of Silk-CS construct.

31.P01
Poly (L-lactide) nanofibers coated with bioactive glass nanoparticles enhance osteogenic differentiation of adipose tissue-derived stem cells

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A combination of stem cells and nanofibrous membranes has the potential of tissue-engineered scaffolds for regenerative medicine applications. In this study, poly (L-lactide) (PLLA) nanofibers were fabricated via electrospinning, coated with bioactive glass nanoparticles (BG) and characterized using SEM and tensile testing. The adipose tissue-derived stem cells were cultured on BG-coated nanofibers and their proliferation and osteogenic differentiation was compared to those cultured on uncoated PLLA and TCPS using MTT, alkaline phosphatase (ALP) activity, calcium content and gene expression assays. BG-coated scaffolds had a nanofibrous structure with a homogenous distribution of BG on the surface of PLLA fibers. Stem cells attached well to the surface of scaffolds and showed a flattened morphology. During osteogenic differentiation, ALP activity reached a maximum on day 14 in stem cells and showed a greater value in stem cells on BG-coated PLLA. A higher amount of calcium depositions were also observed on stem cells cultured on BG-coated PLLA compared to uncoated scaffolds. Bone-related genes such as Runx2, ALP and osteocalcin were expressed in higher values on BG-coated scaffolds. This study shows that PLLA nanofibrous scaffolds coated with BG support attachment and proliferation of stem cells and enhance their differentiation toward osteogenic lineage. The complex of stem cells and BG-coated nanofibrous scaffolds hold promising potential for tissue engineering applications.

31.P02
Cell differentiation on electrospun poly (ϵ-caprolactone) membranes modified with hydroxyapatite

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The present challenge for the progress of bone tissue engineering is to fabricate reproducible, bioresorbable scaffold, that mimic certain features of ECM. Our efforts have been focused on incorporation of bioactive inorganic nanoparticles within the polymer matrix reaping up the combinatory roles of bone-bioactivity of hydroxyapatite and degradability and shape-formability of polycaprolactone. The PCL membranes modified with HAp have been obtained by electrospinning. The complex structure of the membranes and their chemistry were characterized using SEM, FTIR, and WAXD. The mineralization and degradation processes of PCL/HAp membranes during 11 weeks of incubation in SBF were studied. The presence of HAp nanoparticles provides the nucleation sites for apatite growth in SBF and have influence on the faster degradation of PCL (as resulted from WAXD and weight loss study). In order to investigate the effects of biomaterials' on attachment, proliferation, and morphology of cells, NHOst cells were cultured on PCL and PCL/HAp membranes. The results of *in vitro* studies showed that cells attached well to both samples, better cells viability was observed in the case of modified PCL/HAp electrospun nanocomposites. Both samples evoked faster cell differentiation, therefore the cell proliferation on the control material was much higher than on investigated samples. *In vitro* biological evaluation showed that the presence of HAp offered higher activity of ALP and better mineralization.

31.P03
Investigation of layered bionic artificial articular cartilage/bone composite implants *in vitro* and *in vivo*

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Development of artificial articular cartilages with high biomechanical and biocompatible properties is a still significant challenge, especially the long-term fixation of artificial cartilage implants on ambient tissue. Inspired from the structural characteristics of human's mature articular cartilage, a kind of layered bionic artificial articular cartilage/bone composite implants were designed and prepared from polyvinyl pyrrolidine (PVP)/PVA blend hydrogel and bioglasses (BG)/PVA composites hydrogels. The composite implants were fabricated by assembling the layered bionic hydrogels and the allogeneic bones *in vitro*, which can be readily implanted to the defect and connecting with subchondral bone. The elastic modulus and friction coefficient, as well the changes of the structure and shear strength at the interfaces between artificial cartilage and bone in simulated body fluid were studied. A layer of crystalline bone-like hydroxyapatite carbonate (HCA) was formed at the interface between artificial cartilage and bone, and the shear

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strengths improved obviously. The macroscopic and histological observation of animal experiments displayed that the composites implant combined tightly with ambient tissues, and some bone-like tissue grew into the bottom of the implants from the base bone to form more deep-set binding. This study is supported by the Royal Society-NSFC international joint project (51111130207) and Beijing Municipal Sci. and Tech. Plan Project (Z111103066611005)

31.P04
Determination of motility of human pluripotent stem cells on various substrates

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The self-renewal of human embryonic and induced pluripotent stem cell (hESC/iPSC) require specific niches that have not yet been completely understood. In particular, the machinery underlying hESC/iPSCs' response to physicochemical cues provided by a substrate to which cells attach and spread remains largely elusive. We characterized the attachment and spread of hESC/iPSC on various types of substrates in response to physicochemical cues. The substrates that we tested include ECM and synthetic peptide coated flat or porous membrane substrates. The time-lapse imaging microscopy technique was used to quantify the trajectory and velocity of hESC/iPSC colony movement on these substrates. Many microspikes were observed from these colonies, driving the colonies to moving around vigorously on the TCP. The length of the micorspikes formed on peripheral of hESC colonies was approximately $10.91 \pm 3.96\ \mu$m in average, while it was $10.08 \pm 5.67\mu$m on iPSCs. Furthermore, about 70% more microspikes were observed on hESCs colonies, indicating high motility of hESCs, as compared to iPSCs. Furthermore, compared to the motility of hESC/iPSC colonies formed on Matrigel coated TCPs, cell colonies move much slower on synthetic peptide coated TCPs. Although hESC/iPSC proliferated in a similar rate, the motility of cell colonies was remarkably different. These studies suggested that the motility of cell colonies on substrates is adjusted by the physiological cues provided by the substrates.

31.P05
Engineered alkaline phosphatase with improved functionality immobilized on bone implant surfaces

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Titanium (Ti) implants are widely used to replace damaged or diseased bone because of their good mechanical properties and biocompatibility [1]. In order to obtain a rigid implant fixation in the surrounding bone tissue, Ti implant surfaces have been modified with the enzyme alkaline phosphatase (ALP). Since ALP increases the local inorganic phosphate concentration required for physiological mineralization of hard tissues, ALP coatings enabled enzyme-mediated mineralization onto implant surfaces [2]. This study aimed at developing advanced ALP coatings with improved functionality by enhancing the catalytic activity of ALP. A mutant ALP produced from a genetically engineered E.coli strain was shown to be 20 times more active than the wild-type (wt) ALP [3]. Both mutant and wt ALPs were immobilized on Ti to form functionally active coatings. The biological response was evaluated using both *in vitro* soaking and cell culture experiments. This study demonstrated that the engineered ALP was successfully deposited on Ti surfaces while maintaining its high activity; both wt and mutant ALP coatings accelerated mineralization as compared to non-coated Ti implants. Particularly, significantly more mineral was formed on the mutant ALP coatings. To summarize, these novel engineered ALP coatings hold promise for an early and strong anchoring of Ti implants in bone.[1] Stevens et al., SCIENCE, 2005.[2] de Jonge et al., ADV FUNCT MATER, 2009.[3] Xu et al., BIOCATAL BIOTRANSFOR, 2003.

31.P06
Mussel-inspired functionalization of metal surfaces with bioactive self-assembled peptide nanofibers

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Implant surfaces, e.g., cardiovascular stents and orthopedic supports, need to be functionalized with bioactive cues so that they could promote rapid regeneration of the native tissue surrounding them. For example, rapid endothelialization over the stent surface plays an indispensible role in preventing restenosis and in-stent thrombosis for the long-term treatment of the cardiovascular diseases. Likewise, successful osseointegration in dental and orthopedic implants highly depends on the proliferation and migration of mineral deposing cells on the implant site. In this work, we developed bioactive peptide nanofibers which were conjugated with bioactive peptide sequences, REDV (endothelial-specific adhesion motif), KRSR (osteoblast-specific adhesion motif), and mussel-inspired adhesive molecule, 3,4-dihydroxy-L-phenylalanine (Dopa). These nanofibers mimic the structure and function of the native tissue extracellular matrix and could be attached onto metal surfaces through Dopa-mediated adhesion. We analysed endothelial and smooth muscle cells on REDV/Dopa nanofiber-functionalized steel substrates and osteoblast and fibroblast cells on KRSR/Dopa nanofiber-functionalized TiAl6V4 substrates. *In vitro* results demonstrated that endothelial cells and osteoblasts could specifically adhered, spread, and proliferated on REDV/Dopa and KRSR/Dopa nanofibers, respectively. The growth of smooth muscle cells and fibroblasts, however, was inhibited on these bioselective nanofibers.

31.P07
Development of instructive scaffolds for tissue engineering using ureido-pyrimidinone polymers

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Supramolecular polymers are highly suitable as core-material in tissue engineering due to their molecular dynamics that resemble the plasticity of the native extracellular matrix (ECM). Functionalization of polymers and ECM-derived peptides with ureido-pyrimidinone (UPy) moieties enables the incorporation of instructive peptide motifs into a supramolecular scaffold. This allows the design of an intelligent biomaterial in which mechanical properties and biological cues can be tuned towards its specific requirements as a biomaterial via a 'mix-and-match'-principle. The current study explores the biomaterial properties of two polycaprolactone (PCL)-based polymers: bifunctional UPy-modified PCL (PCLdiUPy) and multifunctional chain-extended UPy-PCL (CE-UPy-PCL). To prevent protein adsorption and random cell adhesion both polymers were mixed with UPy-poly(ethylene glycol) (PEGdiUPy) that renders the surface antifouling. Whereas 30% PEGdiUPy is needed to add antifouling properties to PCLdiUPy, 10% is sufficient to prevent adhesion of 3T3 fibroblasts to CE-UPy-PCL surfaces. Next, surfaces coated with the UPy-functionalized RGD-motif were shown to promote cell adhesion and introduction of this fibronectin-derived pep-

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tide reactivates PCLdiUPy/PEGdiUPy films. These results show that PEGdiUPy can be used to obtain antifouling properties in UPy-materials and that UPy-functionalization of ECM-derived peptides allows the incorporation of functional biological cues in a synthetic scaffold.

31.P08
Novel methodology based on biomimetic superhydrophobic substrates to immobilize cells in hydrogel spheres for tissue engineering applications

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The low retention/integration of injected cells by host structures represents an important challenge in cell based therapies for regenerative medicine purposes. Cell immobilization in hydrogels for target cell delivery has been developed to circumvent this issue. However, the existing immobilization methodologies sometimes have several steps under wet conditions and present some drawbacks, including poor encapsulation efficiency and the use of harmful conditions for cells or other fragile molecules, such as proteins or growth factors. In order to surpass these problems mesenchymal stem cells isolated from rats (rMSCs) bone marrow and fibronectin (FN) were immobilized in alginate beads to mimic extracellular matrix environment using an innovative approach involving the jellification of the liquid precursor droplets onto superhydrophobic surfaces. The alginate drops with cells and FN hardened very fast, at room temperature, into hydrogels spheres in an isolated environment which avoided the loss of FN and any contamination or exchange of molecules with other liquid phase. The process for particle fabrication employed allowed a very high efficiency on FN encapsulation and also the mild conditions prevented the loss of cell viability. Encapsulated rMSCs remained viable and were slowly released from the beads during more than 20 days.

31.P09
Continuous functionally graded materials (cFGMs) for TE

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Biological structures are not uniform but possess spatially distributed functions and properties, or functional gradients. To ensure functional, mechanical and structural integration, a tissue engineered (TE) scaffold has to reproduce these functional gradients. However the fabrication of functionally graded materials is challenging and usually an experimental trial-and-error approach is used. In this work we present a controlled method for the fabrication of cFGMs using the gravitational sedimentation of discrete solid particles within a primary fluid phase. To have an overall control over particle distribution, a time-varying dynamic viscosity solution (i.e. thermo-sensitive) was used as fluid phase. Computational fluid dynamic models were developed to have a fine control over particle distribution. Biomimetic osteochondral cFGMs scaffolds were fabricated using hydroxyapatite (HA) and gelatin. Glutaraldehyde was used to covalently bind gelatin-HA graded scaffolds. Mechanical properties were measured and correlated as a function of HA volume fraction. SEM-EDX analysis was used to further characterise HA content and its distribution within gelatin-HA cFGMs. Finally gelatin-HA cFGMs scaffold were seeded using periosteum derived progenitor cells, to investigate how the HA gradient modulates cell response. This approach represents an innovative yet simple tool for the fabrication of tailored cFGMs with biologically and physiologically relevant gradients for TE applications.

31.P10
Multifunctional peptide nanofiber scaffolds for neural differentiation

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Extracellular matrix (ECM) is composed of various fibrous proteins and proteoglycans providing mechanical support and cues for cell adhesion, migration, proliferation and other cellular functions. ECM show great variation between tissues according to varying needs of cells of different tissues. ECM is also highly modular, decorated by a variety of molecules, even the inorganic ones, to keep functionality of specific tissues. For tissues of high mechanical strength ECM is highly collagenous besides being mineralized while for softer tissues with high water content, it is full of hyaluronic acid which acts as a reservoir of water. Such a high modularity in ECM is highly inspiring for regenerative studies which aim to repair damaged tissues. By considering native tissue structure including the abundance of specific ECM components and their relation to the requirements of resident cells, it is possible to design synthetic materials that mimics the natural environment of cells. We used peptide nanofiber scaffolds with bioactivities incorporated according to requirements of neural cells and stem cells for neural differentiation. By decorating the bioactive part of the peptide molecules with different epitopes derived from neural ECM, we were able to induce differentiation and neurite outgrowth of different cells. Encapsulating conductive molecules in neurite inducer peptide nanofibers allowed electrical stimulation of neural cells on peptide nanofibers yielding longer neurites.

31.P11
Characterization of enzymatic crosslinked hydroxyapatite/collagen nanocomposite for bone tissue engineering

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The regeneration of damaged or diseased skeletal tissues remains a significant clinical challenge. Although small bone fractures are capable of self-repair after trauma, large defects or diseased (i.e., osteoporotic) tissues fail to heal properly. In this study, a novel biomimetic bone matrix with inorganic (hydroxyapatite, HA) and organic (collagen, Col) compositions were developed as major components of nanocomposite. Three-dimensional porous HA/Col scaffold was fabricated by freeze-drying method. The physicochemical and mechanical properties of HA/Col scaffold have been investigated after enzymatic cross-linking with microbial transglutaminase (mTGase). The results showed that the crosslinked HA/Col scaffold could provide human mesenchymal stem cells (hMSCs) well adhesion, proliferation and growth. The nov-

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elty of this work is (i) to develop a novel biomimetic HA/Col nanocomposite scaffold, and (ii) to use natural biologically-derived molecules as cross-linking agent instead of synthetic chemical agents, which has been long-term regarded with cytotoxicity concerns. In summary, enzymatic cross-linking HA/Col scaffolds possess more superior properties and potential compared to conventional collagen or HA matrices as a novel bone substituent for bone tissue regeneration.

31.P12
Effect of nanofiber scaffolds on organelle morphology in human bone marrow stromal cells

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It is widely acknowledged that stem cell fates can be controlled by biomolecules and recent work suggests that physical cues, such as scaffold architecture, can also influence stem cell functions. Scaffolds with a nanofibrous structure are effective in promoting stem cell differentiation because they provide a physiologically relevant 3D microenvironment that mimic native extracellular matrix (ECM). Recent work suggests that nanofiber scaffolds are effective because they drive stem cells into a morphology that induces differentiation. However, it is not known how changes in cell morphology can influence cell fate. We hypothesize that the shape and function of subcellular organelles are modulated by changes in cell shape. Herein, we cultured human bone marrow stromal cells (hBMSCs) on poly-ϵ-caprolactone (PCL) flat 2D films and in 3D PCL nanofiber scaffolds and then measured the shape of organelles. The shape of actin, nucleus, peroxisomes and mitochondria organelles were imaged by confocal microscopy and analyzed using ImageJ software. Multiple differences in the area, aspect ratio and sub-cellular locations of these organelles were observed between hBMSCs culture on 2D films versus 3D nanofiber scaffolds. These results support the hypothesis that material morphology guides stem cell function by driving cells into shapes that alter the structure and function of sub-cellular organelles such as the actin, peroxisomes and mitochondria.

31.P13
Platelet lysates scaffolds prepared by supercritical fluid technology as autologous templates for cartilage regeneration

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The tissue engineering strategy proposed in this work regards the development of a novel autologous scaffold based on platelet lysates (PLs) with the ultimate goal of promoting the regeneration of an orthopaedic osteochondral interface. PLs are a high concentration of platelets in a small volume of plasma that, when activated, release several growth factors (GFs). Most of current PLs-based hydrogels present several limitations, specifically the lack of stability, the constant shrinking in culture and the need of activation with animal-derived thrombin. This study represents a major breakthrough as it demonstrates that a stable scaffold can be prepared only from PLs, thus acting simultaneously as a template for cell colonization and as multiple GF release system. The PL scaffolds, crosslinked with genipin were prepared by supercritical fluid assisted phase inversion at 100 bar and 40 °C. The morphological properties of the scaffolds were assessed and *in vitro* GF release profile was studied by micro BCA and ELISA assays.Scaffolds were seeded with human adipose-derived stem cells (hASCs) and cultured *in vitro* up to 28 days. Cell viability and proliferation were assessed as well as histology and immunohistochemistry. Results showed the deposition of cartilage extracellular matrix and the expression of chondrogenic gene makers, demonstrating the feasibility of the constructs to simultaneously provide architectural support and biological cues to promote chondrogenic differentiation.

31.P14
Directing MSC fate in 3D through cell inert and adhesive block copolymer domains

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Novel materials design for the tissue engineering often requires both chemical and physical cues on the nano- and micro- scales. To date, physicochemical properties directing mesenchymal stem cell (MSC) fate have been well studied in 2D, however understanding MSC differentiation in 3D warrants investigation. Here, we report the synthesis of highly porous matrices that are surface functionalised in 3D, with cell adhesive and inert chemistries using amphiphilic block copolymers as surfactants in a high internal phase emulsion (HIPE) templating method. Foam morphology and surface functionality were characterised by SEM, XPS and contact angle measurements while the presence block copolymer surface domains were demonstrated by chemical force spectroscopy mapping. Protein adsorption and clustering was found to be composition dependent which in turn determined the adhesion and spreading of human embryonic derived- mesoderm progenitors (hES-MP) and human bone marrow derived mesenchymal stem cells (hBMSC) cultured on these 3D scaffolds. In the absence of soluble induction factors, stem cell fate was composition and cell source dependent with the hES-MP and hBMSCs exhibiting osteo- and adipogenic differentiation respectively. These data show the importance of mimicking the heterogeneities of native extracellular matrix to control MSC fate using the HIPE process, which provides a platform for 3D matrices in tissue engineering and regenerative medicine applications.

31.P15
3D culture of mesenchymal stem cells on nanofiber PCL and PS meshes

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In the field of tissue engineering, scaffolds should mimic the extra cellular matrix (ECM) to provide cells with the best 3D environment for their attachment, growth and differentiation. We have developed the jet-spraying method to produce nanometer sized polymer meshes that mimic the structure of collagen fibers. Two types of polymers, polycaprolactone (PCL) and polystyrene (PS), were processed, characterized by using scanning electron microscopy and inserted into 12-well plates. After gamma sterilization, human bone marrow mesenchymal stem cells (hMSC, 1.106 per 2 cm^2) were seeded and cultured onto the PCL and PS meshes. The high permeability of these scaffolds allowed the proliferation, migration and osteoblastic differentiation of hMSC. The growth of cells was corroborated by Alamar Blue assay while cell colonization inside the meshes was observed by confocal microscopy and histology of cryosections. Culturing of hMSC into these 3D microenvironments allowed more physiological conditions than conventional 2D cultures on standard dishes. This method can be applied to a variety of polymers producing complex structures for a wide range of tissue engineering applications.

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31.P16
Engineering biomimetic hydrogels for induced recruitment of mesenchymal stem cells *in vitro* and *in vivo*

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Treatment of bone defects with growth factors, such as bone morphogenic protein (BMP), requires doses that greatly exceed physiological levels. Thus, making treatment expensive as well as increasing the risk of side effects. One of the limiting factors to successful regeneration is the sufficient availability of responsive mesenchymal stem cells (MSCs), which in the healing wound proliferate and then differentiate into osteogenic cells. The recruitment of MSCs to the natural wound site is guided by soluble and matrix bound bioactive molecules, such as growth factors. Fostering bone regeneration and healing of clinically relevant non-unions by treatment with MSC mobilizing factors could therefore be a highly attractive approach. Here, we present a modular designed PEG-based platform, which allows for studying MSC recruitment and differentiation in a 3D *in vitro* setup. In order to screen for MSC recruiting factors, matrix immobilization strategies allowing for production of arrays of biomolecule-presenting PEG-matrices were developed. Selected biomolecules were incorporated in cell instructive matrices and *in vivo* recruitment of mouse MSCs was analyzed in a bone healing model. We believe that these biomimetic hydrogels used in concert with engineered biomolecules will lead to novel insight in the complex mechanisms underlying MSC recruitment, thus facilitating the fabrication of next generation smart implants for bone healing.This work was supported by SNF grant CR3213_1254

31.P17
Strontium incorporation into calcium-phosphate ceramic coatings

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Replacing biological growth factors by inorganic compounds such as $Mg2^{+}$, $Sr2^{+}$, F^{-}, etc., which are either present in bone or known to have an effect on bone growth and remodeling is a promising approach for improving the biological performance of bone graft substitutes. In the present study, $Sr2^{+}$, in varying concentrations, was incorporated into different calcium phosphate coatings on Ti surfaces using a biomimetic coating method. Varying $Sr2^{+}$ concentrations were successfully incorporated into both carbonated apatite and octacalcium phosphate coating. It was shown that the presence of $Sr2^{+}$ had a significant dose-dependent effect on crystal morphology of both coating types. In addition, an effect on proliferation and differentiation of murine osteoblastic MC3T3-E1 cells was observed.
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31.P18
Intelligent bio-materials for tissue regeneration and nanomedicine

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During the last decade, in the orthopaedic field the well-established approach for curing diseased bone parts, based on replacement with inert substitutes, has progressively given way to new regenerative approaches, based on the use of bioactive and biomimetic devices. The present work illustrates how bio-mineralization can be used to guide the development of biomaterials for bone and osteochondral regeneration. The reproduction of biomimetic conditions of bone synthesis allows to obtain hybrid constructs where the mineral phase is nucleated upon guidance by the chemical features and physical confinement imposed by the polymeric matrix, so that the mineral phase has physical, chemical and ultra-structural resemblance with mineral bone. Besides, the possibility to vary the degree of mineralization allows to obtain multi-layer graded devices able to regenerate the different districts of the articular region. Finally, pinning on the recent development of intrinsic superparamagnetism exhibited by hydroxyapatite nanopowders upon crystallographically and chemically controlled doping with Fe(II)/Fe(III) ions, it will be illustrated how bio-hybrid bone-like devices with intrinsic magnetic properties can be obtained, to increasingly assist the osteogenic and angiogenic capacity of biologically inspired bone and osteochondral scaffolds, through magnetically-driven release of specific growth factors.

31.P19
Creation of a tissue development model in an artificial biomimetic niche microenvironment

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Regenerative cellular therapies usually depend on implementation of the *in vivo* niche conditions to *in vitro* settings for controlling cell proliferation and differentiation. The stem cell niche defines stem cells together with the accompanying cells, extracellular components and cues of the microenvironment. Here, an artificial biomimetic niche microenvironment was developed and applied to the chondrogenesis event as an approach for tissue engineering. Basically, the system was composed of a hyaluronic acid(Hya)-collagen(Col) matrice containing bone marrow mesenchymal stem cells(BM-MSCs) which was triggered into the chondrogenic phenotype by using hyaluronidase(Hyase) and TGF-β1. Hya-Col scaffold was fabricated via lyophilisation and characterised using SEM and FTIR. BM-MSCs were isolated from rat femurs, cultures established and then the cells were seeded onto the Hya-Col scaffold. The culture medium was supplemented with TGF-β1 and Hyase to ensure the controlled digestion of Hya and the formation of the neocartilage for 1-month duration. Constructs retrieved at time points were evaluated using MTT,GAG and Hya assays, and then by histology/IHC and SEM. Findings demonstrated that the artificial niche microenvironment supported controlled formation of the neocartilage tissue expressing specific hyaline cartilage proteins (i.e.typeIIcollagen and aggrecan) and transformed the phenotypic and morphologic modulation (i.e.differentiated isogen cell groups in lacuna) of chondrogenesis.

31.P20
Property-based screening and characterization of cell-selective adhesion peptides using clustering analysis

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The long-term efficacy of vascular implants critically depends on the regeneration efficacy of the disturbed tissue surrounding the implant. Late regeneration can cause life-threatening side effects such as thrombosis and neointimal hyperplasia. To reduce such risks, there are two

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essential but counter-opposed conditions that must be satisfied by tissue engineering technology. One is the acceleration of the monolayer formation of endothelial cells (ECs) by rapid adhesion, and the other is the inhibition of the invasion of smooth muscle cells (SMCs) into the intima. For such acceleration, we have been proposing short synthetic cell-selective tripeptides as scaffold for implant coating. These cell-selective peptides provide selective adhesion of ECs or SMCs mainly by their physicochemical properties. We here report the comprehensive screening strategy to screen such peptides by the combination of in silico clustering analysis and the cell-assay method on SPOT peptide array. By the application of clustering approach, we found some commonly observed physicochemical property that can regulate the cell-selectivity.

31.P21
Cell-instructive matrices for healing and engineering of fetal membranes

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Iatrogenic preterm prelabour rupture of fetal membranes (iPPROM) significantly raises the risk of preterm labor and consequently contributes to fetal morbidity and mortality. Naturally occurring materials have shown to have insufficient properties for sealing ruptured membranes to prolong pregnancy and none of them has achieved clinical breakthrough. To overcome these limitations, we aim to engineer fully defined, cell-instructive matrices for sealing and healing of ruptured membranes. By using a growth factor presenting, modular designed poly(ethylene glycol) (PEG) -based hydrogel platform, a series of growth factors stimulating the 3D migration of human amnion mesenchymal cells (hAMC) or epithelial cells (hAEC) were determined. Additionally, some of these factors could stimulate proliferation and formation of interconnected cellular networks. Furthermore, using the PEG hydrogel platform, amnion mimicking constructs consisting of hAMC and hAEC could be successfully grown for several weeks and displayed morphology similar to native amnion.This study provides evidence that hAMC and hAEC in response to appropriate molecular cues can be mobilized and expanded in 3D in fully synthetic ECM mimicking matrices. Amnion analogs could be used to develop cell-instructive plugging materials, tailored to promote the healing capacity of fetal membranes by local induction of cell mobilization, proliferation, and matrix formation *in vivo*.

31.P22
Gradients of platelet lysates as guiding mechanism for angiogenesis

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Native tissues are regulated by gradients of bioactive factors and cell densities. *In vitro* generation of platforms with embedded biochemical cues to mimic the heterogeneous spatial cellular microenvironment may be helpful not only for understanding the biological phenomena, but also for allowing the development of tissue-mimetic graded constructs. The effect of specific concentrations of Platelet Lysates (PLs) over cell attachment and differentiation has been previously evaluated but conclusive knowledge regarding the effective dosage is still lacking. Thus, the aim of this study is to generate a gradient of PLs embedded in a photocrosslinkable hydrogel of methacrylated gellan gum (MeGG) in order to correlate the effect of lysates concentration with cell adhesion, proliferation and expression of angiogenic markers. A cross-gradient of PLs and MeGG was generated by flow convection and stabilized by photopolymerization. Human adipose stem cells (hASCs) were cultured onto the hydrogel gradient up to 28 days. Cells cultured onto the engineered platform showed to preferentially attach onto the PLs-rich regions and to be absent in the regions where no PLs were present. Histological and immunohistochemical characterization showed that encapsulated hASCs express angiogenic markers preferentially in the regions with higher concentration of PLs, emphasizing the potential of designing gradients of PLs within hydrogel microenvironments to stimulate neovascularization.

31.P23
Development of marine-based nanocomposite scaffolds for biomedical applications

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Despite the increasing attention that marine organisms are receiving, many of those are not efficiently exploited and subproducts with valuable compounds are being discarded. Two examples of those subproducts are the endoskeleton of squid, from which β-chitin and consecutively chitosan can be obtained; and fish-bones, as a source for the production of nano-hydroxyapatite. In this work, inspired in the nanocomposite structure of human bone, marine-based nanocomposite scaffolds composed by chitosan and nano-hydroxyapatite (nHA) were developed using particle aggregation methodology. Chitosan was obtained from endoskeleton of giant squid Dosidicus Gigas while fish hydroxyapatite nanoparticles were synthesized from fish-bones by pulsed laser in deionized water. An innovative methodology was used based on the agglomeration of prefabricated microspheres of chitosan/nHA, generally based on the random packing of microspheres with further aggregation by physical or thermal means to create a marine nanocomposite (CHA) .The morphological analysis of the developed nanocomposites revealed a low porosity structure, but with high interconnectivity, for all produced scaffolds. Furthermore, the nanocomposite scaffolds were characterized in terms of their mechanical properties, bioactivity, crystallinity and biological behavior. The obtained results highlight that the chitosan/nHA-based marine nanocomposite can be a good candidate for biomedical applications, namely on bone regeneration.

31.P24
Inkjet printing of conductive substances for stymulation of mineralization in bone cell culture

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The aim of the experiment was to develop cell patterns stimulated by the presence of conductive polymers on the surface of electrospun scaffolds. Polymer solutions for electrospinning were prepared from polycaprolactone (PCL), which was dissolved in a solvent mixture consisting of chloroform and methanol. Electrospinning was carried out using custom-made apparatus consisting of a power supply, syringe and electrodes. The solution were placed in a 10 ml plastic syringe with a stainless-steel blunt needle of 0.7 mm in diameter. The injection rate was 1.5 ml/h. The solutions were spun at a working distance of 20 cm with a driving force of 30 kV. Conductive polymers were deposited on the surface of electrospun membranes using piezoelectric material printer DMP-2831. Applied system enables for precise deposition of 10 pl drops of customized printing solution. NHOst cells (at a density of 20 000 cells per well) were seeded onto polymer discs to evaluate cell viability and morphology after 3 and 7 days of cultivation. Cell viability

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was determined using ATP bioluminescence technique (ViaLight® Assay, Lonza). Cell functionality was assessed by alkaline phosphatase (ALP) activity assay, and mineralizaton by OsteoImage mineralization test (Lonza). Combination of electrospun, biodegradable scaffolds with conductive polymers allows to stimulate cellular functions, including attachment, proliferation, and differentiation.

31.P25
Novel mesh labeled with an MSC affinity peptide for detecting MSC *in vivo*

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Mesenchymal stem cell (MSC) is a cell that candidate in regenerative medicine and tissue engineering (TE). a significant barrier for MSC-based therapies is the inability to target MSCs in tissues. a major concern about MSCs has been mobilization and transfer of these cells to damaged sites . In this study, we recognized a peptide sequence with some amino acids by phage display technology that has a high specific sympathy to bone marrow-derived MSCs. following investigation suggested that the peptide could capable interact speci?cally with MSCs without any type specificity. Subsequently, these peptide was label with polycaprolactone electrospun meshes to make an 'MSC-homing device' for the conscription of MSCs both *in vitro* and *in vivo*. This complex were implanted in defect site of rat knee joints, accompany with a microfracture procedure to mobilize the endogenous MSCs. After 1 week staining by immunofluorescence demonstrated that the cells grown PCL meshes acquiesced a high positive rate for specific MSC surface markers (CD44, CD90, and CD105) compared with other complexes. The proportion of inflammatory marker (CD68) in this novel mesh was much lower than that other meshes.this result cnfirm that it would be more biocompatible. This peptide has a high particular affinity to MSCs.conjugating this peptide with PCL significantly increased the MSC recruitment of PCL *in vivo*.

31.P26
Glutamic acid grafted nanofibers as a biomimetic template for mineralization and osteogenic differentiation of mesenchymal stem cells

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Glutamic acid (Glu) sequences associated with the proteins in bone ECM play a major role in initiating mineralization on collagen fibers. Here, we use a biomimetic approach and a layer-by-layer assembly to produce apatite-coated nanofiber thin sheets. The objective of this work was to investigate the effect of grafted Glu sequences on the formation of apatite crystals on nanofibers and the extent of mineralization of marrow stromal cells. Glu sequence was attached to short PLGA by the reaction of acrylate with sulfhydryl group of cysteine (Glu-PLGA). Nanofibers surface functionalized with Glu were fabricated by electrospinning. The fibers were incubated in simulated body fluid (SFB) to deposit apatite crystals. Next, the apatite-coated nanofibers were seeded with mesenchymal stem cells (MSCs, isolated from rat bone marrow) and cultured in osteogenic media. At each time point, samples were analysed by biochemical, immunocytochemical, and mRNA analysis. Apatite nanocrystals grew on Glu-grafted fibers but not on untreated fibers. The modulus of the Glu-grafted fiber mesh increased from 300 MPa to nearly a GPa after incubation in SBF. Mineralized Glu-grafted fibers had significantly higher ALPase, mineralization, and significantly expression of osteogenic markers. The biomimetic layer-by-layer assembly of mineralized Glu-grafted nanofibers is a promising approach to producing synthetic bone grafts with mechanical and biochemical properties comparable to that of natural bone.

31.P27
bFGF-immobilization on heparinized thermoresponsive cell culture substrate for enhancing cell proliferation

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We developed heparinized thermoresponsive cell culture substrates for enhancing cell proliferation. Growth factors such as basic fibroblast growth factor (bFGF) and vessel endothelial cell growth factor (VEGF) were immobilized via affinity interactions with the heparinized thermoresponsive substrate without deactivation. Here, we investigated the adhesion and growth of mouse fibroblast cells (NIH3T3) on the bFGF-immobilized substrate. Heparin was covalently immobilized onto poly(N-isopropylacrylamide-co-2-carboxyisopropylacrylamide) (poly(IPAAm-co-CIPAAm))-grafted TCPS by condensing reaction. bFGF was immobilized on heparinized poly(IPAAm-co-CIPAAm)-grafted TCPS with incubation in bFGF solution (1000, 500, 100 and 10 ng/cm^2) at 37 °C for 24 h (bFGF/Heparinized-C1). Enhanced proliferation and growth of NIH3T3 were observed on the bFGF/Heparinized-C1 surface compared with physisorbed bFGF on PIPAAm surface. It would appear that immobilized bFGF via heparin retained their high activity, while the activity of physisorbed bFGF was decreased probably due to denaturation. Although 120 h-incubation was required for the fabrication of cell sheets on PIPAAm-grafted surfaces, the incubation time for reaching confluence became shorten to 72 h on the bFGF/Heparinized-C1 surface. Moreover, the cultured NIH3T3s were recovered as a single sheet by decreasing the temperature to 20 °C. Consequently, this cell culture surface would be useful for rapid fabrication of cell sheets.

31.P28
Diblock copolymer foams with adhesive nano-domains promote stem cell differentiation

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Focal adhesions are signal transducers from extracellular matrix (ECM) to the cell and vice versa. Most biomaterials are uniformly adhesive, but that does not match heterogeneous adhesivity in native ECM. Mixed copolymer vesicles undergo interface-confined phase separation; by using diblock copolymer mixtures of non-adhesive and adhesive components, i.e. polyethylene oxide (PEO)-polystyrene (PS) and polyacrylic acid (PAA)-PS, respectively, we determined if foam structures made in a high internal phase emulsion would have surfaces composed of adhesive and non-adhesive domains just as with ECM. Incorporation scaled with composition but neither foam morphology nor surface roughness dramatically changed. However, surface phase separation did occur; nano-domains were found when either copolymer fraction was ≤25%. Size and spacing was similar to the heterogeneous adhesivity of native ECM. Protein attached to PAA-PS produced nano-domains with a distribution that also mimicked native ECM. Two mesenchymal stem cell sources cultured on the foams were adherent on and expressed the most robust vinculin-containing adhesions on 25% PAA foams. qPCR microarray data indicates that these two cell sources undergo both nano-domain-dependent and – independent differentiation depending on the lineage to which the cell commits. Thus foam phase separation can create adhesive nano-domains mimicking native ECM, induce stem cells to differentiate, and should be used in future regenerative strategies.

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31.P29
The *in vitro* micro-environment interface: macromolecular crowding meets cell-sheet tissue engineering

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Introduction: Advancements in cell biology and polymer chemistry enabled the development of scaffold-free substitutes for tissue engineering applications; a technology termed Tissue Engineering by Self Assembly. Despite efficacious *in vitro* and *in vivo* results to-date, very few products have been commercialised due to prolonged culture time required to develop an implantable device. It has been recently demonstrated that macromolecular crowding (MMC) enhances deposition of extracellular matrix. Herein, the influence of crowding molecules on matrix deposition and potential of this technology in Tissue Engineering by Self Assembly was investigated.
Methods: Human primary fibroblasts were cultured under MMC (e.g. dextran sulphate; polysodium 4-styrene sulfonate; Ficoll™70 & 400; carrageenan & sepharose) and various fetal bovine serum (FBS) concentrations (0–10%). Results: SDS-PAGE/densitometry demonstrated that MMC significantly increase collagen I deposition ($p < 0.0001$) at all tested FBS%. Fibroblasts deposited maximum collagen I in two days with 0.5%FBS. Immunocytochemistry confirmed enhanced deposition of collagen I and its co-localisation with fibronectin in presence of MMC. N-isopropylacrylamide based heat responsive polymer facilitated detachment of intact cell-matrix sheet.
Conclusions: All in all, these data indicate that MMC facilitates the production of rich in ECM cell-sheets. An extensive proteomic study is underway to comprehend the expression and deposition of proteins.

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32. Biomaterials for Regenerative Medicine (in coop. ESB)

32.01
Keynote: Self-assembling bioactive polymer systems for tissue engineering. Modulation of angiogenesis

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The design and development of 'Polymer Drugs' are one of the most attractive fields of advanced systems for new therapeutic applications of bioactive compounds. In one biomimetic approach, these systems are based on the preparation of polymeric chains with specific hydrophobic or hydrophilic character by the reversible linking of bioactive compounds to macromolecular systems, by means of reaction of specific functional groups present in the macromolecule or by copolymerization of functionalized bioactive compounds or drugs. This second approach allows very interesting designs by selecting polymerization mechanisms, composition and microstructure. A new family of acrylic polymers bearing sequences of acrylic derivatives of 5-aminonaphthalen sulphonic acid will be presented as systems that are able to interact with growth factors (FGF and VEGF) and therefore present a very interesting inhibition of angiogenic processes at relatively low dose and very low toxicity. The behavior of these systems has been tested in vitro using a 3D model for cell cultures, as well as *in vivo* in an animal model with good correlation of results. Financial support from projects MAT2010-18155, and CIBER-BBN is acknowledged.

32.02
Biomaterials technology of regenerative medicine for patients

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Regeneration therapy is a new therapeutic trial based on the natural-healing potential of body itself to induce tissues regeneration. To this end, it is necessary to create a local environment of cells to promote their proliferation and differentiation for tissue regeneration. If a cell scaffold or a bioactive molecule is supplied to the right place at the right time period or concentration, the body system initiates to physiologically function, resulting in the natural induction of cell-based tissue regeneration. The functions of bioactive molecules with *in vivo* instability can be augmented with drug delivery system (DDS) technology. Biodegradable hydrogels achieved the controlled release of bioactive molecules to experimentally and clinically succeed in the cell-induced tissue regeneration. This release and/or cell scaffold technologies can be combined with cell transplantation to significantly enhance the therapeutic efficacy in tissue regeneration. The biomaterials technology of regenerative medicine is also applicable to the basic researches of stem cells biology. The further development of stem cells biology will be effective in enhancing the therapeutic efficacy of cell-based tissue regeneration. In this paper, several applications of DDS and cell scaffold technologies to the tissue regeneration therapy as well as the basic research of stem cells are introduced to emphasize clinical significance of biomaterials technologies in tissue regenerative therapy.

32.03
Osteopromotive scaffolds for bone tissue engineering: a solvent-free manufacturing process based on extrusion and supercritical fluid processing

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Bone tissue engineering is one of the most promising technologies challenging traditional bone grafting. Human adipose stem cells (hASCs) present an attractive alternative for the regeneration of bone. However, a suitable biomaterial scaffold that supports the cell attachment, proliferation and differentiation towards bone-forming cells, is required to deliver hASCs to the bone defect site. By combining the viscoelastic properties of bioabsorbable polymers to the bioactive properties of calcium phosphate ceramics, the temporary environment for hASCs can be tailored to enhance the cell proliferation and differentiation. We present here a composite technology based on extrusion and supercritical fluid processing for developing biodegradable and osteopromotive scaffolds. This method enables us to compound bioactive ceramics into biodegradable polymer matrixes in large quantities and to produce porous structures. Additionally, the manufacturing method is totally solvent-free and easy to scale up. Pore size, interconnectivity and structure can be tailored with process parameters and the choice of materials. The preliminary results gained using poly(L-lactide-co-ϵ-caprolactone) (comonomer ratio 70/30) and β-tricalcium phosphate (β-TCP) as the composite scaffold materials show good mechanical properties and they support the attachment of hASCs and stimulate their proliferation. They have the potential to fulfil the criteria for an ideal scaffold for bone tissue engineering.

32.04
Sugar-responsive water-solubilization of bone morphogenetic protein-2-incorporating gelatin hydrogel microspheres in cell aggregates

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The objective of this study is to design a culture substrate, which not only functions initially as a cell scaffold for cell aggregation, but also can be removed from cell aggregates constructed without any cytotoxicity. As such a substrate, bone morphogenetic protein (BMP)-2-incorporating gelatin hydrogel microspheres with the property of sugar-responsive water-solubilization were prepared. Briefly, an aqueous mixture of m-aminophenylboronic acid-introduced gelatin and poly(vinyl alcohol) was emulsified and solidified in olive oil. Then, an aqueous solution of BMP-2 was impregnated into the resulting microspheres. When placed in a culture media with sorbitol of a sugar, BMP-2 was released from the BMP-2-incorporating microspheres by their water-solubilization. However, the microspheres were stable and no BMP-2 release was observed without sorbitol. These results indicate that sorbitol disrupts the crosslinking of the microspheres, leading to the water-solubilization and the subsequent BMP-2 release. When mesenchymal stem cells were co-cultured with the microspheres, stable cell aggre-

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gates containing the microspheres were formed. Upon adding sorbitol in the culture media, the microspheres disappeared in the cell aggregate. No cytotoxicity was observed after the sorbitol addition. These results indicate that the present hydrogel system of sugar-responsive water-solubilization is a promising culture substrate to allow cells to form a living tissue-like structure of 3-dimension.

32.05
Keynote: Structural impact of textile based implants for regenerative medicine

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Introduction: Body tissues react specifically not only to implant materials but also to structural dimensions, e.g. to fibers and to pore diameter in fabrics resp as well as to the surface and orientation of fibers.
Materials and methods: Mostly for tissue engineering nonwoven with fiber diameters ranging from 0.1 to 20 μm and more are used. Precisely oriented fibers could be fabricated with fiber diameters of 1 μm or higher. These were oriented unidirectional as bundles, bi-directional by weaving and braiding, or forming meshes by knitting. Additionally surface structures like grooves or surface roughness were used to stimulate selected cell types.
Results: 2D cell organizations, e.g. the endothelium, need small pores by small fibers where the cells were able to form many focal adhesion points. Movement between fibers stimulated the cells. 3D cell types require larger pores and thus larger fibers to stabilize the scaffold. Chondrocytes did not keep their typical round shaped phenotype on fibers >> 20 μm, but dedifferentiate to fibroblasts. Yet hepatocytes were found to organize best in large (>100 μm) caverns of a PU melt blown web. Nerve regeneration and oriented outgrowth of axons was supported by grooved fibers. These fibers were bundled and introduced into a capillary membrane. Ligament fibroblasts needed a considerable cyclic strain to differentiate. By crimping the fibers they allowed a moderate straining on the fibroblasts.

32.P01
Unfouling PEG-based substrates for cancer stem cells

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Cancer stem cells (CSCs) are becoming an appealing research area, leading to a revision of the conventional chemotherapic approach. To maintain their 'stemness' and preclude their differentiation to the bulk tumor, 'non-adherent' conditions for *in vitro* test of CSCs are requested. The goal of this study was to design suitable PEG-based matrices and explore their potentiality as non-adherent substrates. PEG-diacrylate was cross-linked by UV-A and chemical structure of XXPEG matrices was analyzed by FTIR. Swelling and stability were evaluated at 37 °C in DMEM. Rheological analyses were performed on samples swollen in DMEM. The *in vitro* adhesion of HT1080 and HCT116 human tumor cell lines was evaluated by Giemsa staining 2 weeks after seeding onto XXPEG matrices (TCPS as control). The formation of the cell 'spheres' and their morphology was monitored by OM. Crosslinking was confirmed by the disappearance of double bond vibrational absorptions. XXPEG matrices proved to be stable in DMEM up to 15 days of incubation. The increase in storage modulus and the decrease in loss modulus by increasing the test frequency indicated a major contribution of the elastic component at higher test frequencies. Cell staining highlighted that both cell lines did not adhere to XXPEG matrices and formed colonies (clones) with spherical and regular morphology, while they were adherent to TCPS. These results demonstrate the potentiality of XXPEG as substrates for the *in vitro* study of CSCs.

32.P02
Piezoelectric polymers for tissue regeneration: neural and bone tissues approaches

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Bone and neural tissues respond to electrical stimulation, with bone growth and neurite outgrowth being enhanced upon the application of direct electrical stimulation. Superficial charges on biomaterials are also known to influence biomolecules, affecting protein adhesion and bone mineralization, among other biological processes. Poly (L-lactic) acid (PLLA) is a synthetic semicrystalline biocompatible polymer known in the biomedical field for its applications such as screws or sutures, approved by the Food and Drug Administration. PLLA is also piezoelectric, a property that has still to be evaluated in the field, although electric field generated by mechanical deformation is expected to induce higher tissue regeneration. This study aims to assess the viability of cells from bone and neural tissue on poled piezoelectric PLLA substrates and the effect of polarization on the cells' response. PLLA films were prepared by solvent casting method and poled using a corona discharge (10 kV, 10 μA for 30 min at 100 °C and during posterior cooling). Two sets of samples, negatively and positively poled, were then cultured with different cell lines (MG63 osteoblast-like, human bone marrow, and neuroblastoma SH-SY5Y cells) and cells viability, adhesion, and morphology were monitored. Results are discussed in terms of the effect of polarization on cells behavior, and how negatively poled PLLA surface generally enhance cells bioactivity, when compared to a positively poled sample.

32.P03
Engineered cell sheets using thermo-reversible hydrogel

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Cell sheet (CS) technology allows to obtain sheets of interconnected cells in contact with their extracellular matrix (ECM) with potential application in many fields of medicine including regeneration of oral tissues. Thermo-reversible behavior of methylcellulose (MC) gels is related to increases in temperature and in salt concentration in the MC aqueous solutions. This phase-transition make it a promising functional hydrogel for biomedical applications. In the present study we assessed the fibroblasts CS formation using a thermoreversible MC hydrogel. Hydrogel was prepared by dissolving 8% w/v MC in a 0.05 M Na2SO4 solution, at 4 °C and then heating it at 37 °C prior to use. Fibroblast (NIH-3T3), previously transduced with Green Fluorescent Protein, were seeded (50 000/cm^2) on hydrogel and cultured until confluent; afterwards, cells sheet was detached by cooling down temperature (30 min, 4 °C). Cells sheets were characterized by immunofluorescence (IF) staining and MTT assay. In-vitro adhesion to a new surface was characterized. Moreover, the CSs were subcutaneously implanted into SCID mice and histomorphometrically evaluated.CSs were successfully collected by cooling down temperature. IF staining confirmed cells monolayer aggregation and ECM integrity. CS adhesion to a new sur-

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face was confirmed and proliferation was observed after 24 h. CSs survived and grew *in vivo*. This technique seems promising in tissue engineering of various tissues including periodontal ligament.

32.P04
Human Elastin-Like Polypeptides (HELPs) - a novel thermoresponsive material for cell culture applications

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Elastin-like polypeptides (ELPs) have a distinct thermal behaviour, known as inverse temperature phase transition. Below a critical temperature the polypeptides are present as aqueous solution, above this temperature they aggregate. Their smart properties make them attractive for the development of biomaterials to support cells in tissue engineering (TE). Recently, a family of ELPs based on the human tropoelastin primary structure has been developed. In this study, we aimed to test the novel HELP macromolecules for their ability to support growth of both transformed cell lines (murine C2C12 cells) and primary cells (human adipose-derived stem cells (hASCs)). C2C12 cells and hASCs attached and proliferated on HELP-based coatings. ASCs were clearly more sensitive regarding attachment and growth in culture. Biopolymers comprising hydrophobic and cross-linking domains gave the best results. Interestingly, these coatings support the differentiation of C2C12 cells into the myogenic lineage as could be demonstrated using qRT-PCR. The growth of both cell types could be supported by the coating in a concentration-dependent manner. In summary, the thermoresponsive HELPs presented in this study are a suitable material for the coating of surfaces to allow cell adhesion and growth for TE applications.

32.P05
Growth and differentiation of human osteoblast-like MG 63 cells on composite with micro/nano-sized hydroxyapatite

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Bone prostheses are usually manufactured from metals, ceramics, less from polymers, but their properties are not ideal. We developed a fibrous composite material composed of polyamide fabric reinforcement, polydimethylsiloxane, and nano/microparticles of hydroxyapatite (nHA, μHA), and evaluated its ability to support the attachment, growth and differentation of human osteoblast-like MG 63 cells *in vitro*. MG 63 cells were seeded in a density of 8450 cells/cm^2 on the composites containing 0, 2, 5, 10, 15, 20, and 25 vol% of nHA or μHA. Cell population densities were evaluated on day 1, 3, and 7. Cells were stained for vinculin, talin, osteocalcin and osteopontin, and concentrations of these proteins, as well as of beta-actin and ICAM-1, were measured by ELISA. All samples supported the cell attachment with high cell viability. The μHA samples enhanced proliferation compared to nHA, but only up to 15 vol%; higher concentrations of μHA were toxic. High concentrations of osteocalcin were found in cells on materials with higher concentrations of μHA, while high concentrations of osteopontin were in cells on samples with low concentrations of nHA. On all samples, the cells developed focal adhesion plaques containing talin and vinculin. Thus, all tested composites with nHA and composites with lower concentrations of μHA seem to be promising materials for bone prostheses. Supported by the Grant Agency of the Czech Republic (grant No. 106/09/1000).

32.P06
Strontium substituted bioactive glasses stimulate osteogenic differentiation of hMSCs *in vitro*

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Bioactive glasses (BG) are biocompatible and have been used as bone graft substitutes for decades. BG dissolution ions stimulate osteoblast differentiation *in situ*. Strontium (Sr) stimulates osteoblast differentiation and has been used to treat osteoporosis in the form of Sr ranelate. We combined the osteoinductive properties of Sr with the osteoconductive properties of BG and tested its effects on bone marrow mesenchymal stem cells(hMSCs).SiO2-P2O5-Na2O-CaO-SrO BG was substituted with 0 and 10 mol% of Ca with Sr and produced by a melt-quench route and referred to as SR0-SR10. BG dissolution ion enriched media(BGDM) was prepared as described before. Alkaline Phosphatase(ALP) activity of cultured hMSCs was measured from cell lysates using p-nitrophenyl phosphate(pNPP) as a substrate and normalized to cell number against an alamarBlue® cell proliferation assay.ALP activity per cell is an early marker for osteogenic differentiation. ALP activity of hMSCs cultured with Sr substituted BGDM was higher compared to cultures treated with BGDM and control media following 3 weeks in culture. Therefore, Sr substitution into BG promoted osteogenic differentiation of hMSCs *in vitro*.It has been shown that Sr promotes osteogenic differentiation in hMSCs *in vitro*. Here we report that Sr substitution into BG enhanced the osteoinductive properties of BG stimulating osteogenic differentiation of hMSCs *in vitro* and thus may be of great potential for bone tissue engineering applications.

32.P07
Influence of surface modification on cell response: case study for ocular applications

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In the present work, various biofunctionalization strategies were elaborated to covalently immobilize gelatin onto polyimide. The implant materials are anticipated to be applied for ocular applications. Three strategies were compared: (i) 2-Aminoethyl methacrylate (AEMA) was coupled onto PI after applying an Ar plasma. Next, the AEMA amines were linked to EDC-activated gelatin, (ii) introduction of vinyl groups on PI using aminopropylmethacrylamide and subsequent coupling with methacrylamide-modified gelatin and (iii) introduction of reactive esters on PI using the succinimidyl ester of 4-azido-2,3,5,6-tetra-fluorobenzoic acid (AFB) and subsequent coupling with the primary amines of gelatin. (iv) deposition of a bio-inspired primer layer onto PI, followed by gelatin immobilisation. The polymer sheets developed were characterized in depth using a series of analysis techniques including among other static contact angle measurements (SCA), atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and radiolabelling experiments. In a final part, the *in vitro* cell-interactive properties of the various polymer sheets developed will be screened and the results will be correlated with the applied modification strategy on PI. The authors would like to acknowledge the Research Foundation – Flanders (Belgium) and the EU for financial support (FP6 project PolExGene, http://www.polexgene.eu).

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32.P08
The effect of chemical composition, pore size and mechanical properties of macroporous hydrogels on behavior of MG63 osteogenic cells

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In the area of synthetic bone graft materials, there is a great need to engineer multi-phase materials with a structure and composition approaching to that of bone. In this study, the effects of charge, pore size and mechanical properties of the polymer matrix on MG63 cells were monitored. The model non-degradable HEMA (2-hydroxyethyl methacrylate)-based macroporous hydrogels were composed of HEMA (no charge) and its copolymers with ethoxyethyl methacrylate (no charge), sodium methacrylate (negative charge), methacryloyloxyethyltrimethylammonium chloride (positive charge). The sample morphology was characterized by SEM and the mechanical properties of swollen samples by dynamic mechanical spectroscopy in shear geometry. The total DNA content, doubling time and growth curves were determined based on results of PicoGreen assay on day 7, 14, and 21. On day 7, the highest cell densities were observed on non-charged samples, whereas very low densities were seen on positively-charged samples. After 21 days, the DNA content/cm^2 was the same on all scaffolds and was significantly higher than that on the glass. On negatively-charged samples, the MG63 cells grew in clusters. Thus, the samples without any charge seem to be a possible alternative as the matrix for engineering of composite materials combining the advantages of nanofibres (e.g. collagen), supporting macroporous gels as well as bioactive inorganic components (e.g. HAP). This work was supported by grant GACR No.106/09/1000

32.P09
Comparison of chitosan-coated, collagen-coated, and uncoated polypropylene mesh in a rat model for abdominal wall reconstruction

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An ideal surgical mesh for hernia repair would provide a substrate for tissue ingrowth resulting in mechanical and contractile properties similar to native tissues without significant fibrosis. The goal of this study was to determine if chitosan coating encourages incorporation and lessens fibroplasia observed following implantation of polypropylene mesh (PPM) *in vivo*. This study compared chitosan-coated PPM(Ch-PPM) with two commercially available meshes in a rat model: PPM, and collagen-coated PPM(Col-PPM). Adult rats were subjected to 1.2 × 1.2 cm excisions of the external and internal oblique layers of the abdominal wall while the underlying layers remained intact. The defects were repaired with one of three test articles: Ch-PPM; PPM, or Col-PPM. Prior to euthanasia at 2, 4, and 12 weeks, the contractile properties of the remodeled tissue and surrounding native tissue were determined *in situ* and compared with the native tissue (control). Uniaxial testing and immunolabeling of histology samples were used to find tissue strength and the degree of fibroplasia. The contractile and mechanical properties of the remodeled tissue using Ch-PPM were significantly closer to properties of native tissue than PPM and Col-PPM at 2, 4 and 12 wks. The tetanic and twitch forces of Ch-PPM increased over time whereas with Col-PPM, they decreased. Histological analysis showed rats implanted with Ch-PPM and Col-PPM have significantly more myoblast growth with fewer foreign-body giant cells at 2 weeks.

32.P10
Immobilization of neurotrophin GDNF on electrospun scaffolds promotes the survival and integration of transplanted neural stem cells

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Given the brain's limited capacity for repair, new and innovative approaches are required to promote regeneration. Whilst examples of neural transplantation have been demonstrated, major limitations in the field include poor cell survival and integration. This in part is due to the non-conducive environment of the adult brain – failing to provide adequate chemical and physical support for new neurons. Here we examine the capacity of electrospun PCL scaffolds combined with the immobilization of the neurotrophin, GDNF, to improve graft survival and integration. Immobilization of GDNF was confirmed using ELISA and immunohistochemistry, prior to and at 28 days post-implantation, demonstrating long-term delivery of the trophin. *In vitro*, PCL enriched for neural stem cells and astrocytes in primary cortical cultures, compared to conventional 2-dimentional cultureware. Subsequent implantation of cells, cells + PCL or cells + PCL-GDNF demonstrated that PCL-GDNF was capable of supporting grafts, as demonstrated by the increased penetration of cells and neurites into the material. In conclusion, the study demonstrated that modified electrospun scaffolds can support grafted neurons - promoting survival, penetration/integration and encouraging neurite growth. These findings could have implications for improving cell replacement therapy for the treatment of neurodegenerative diseases. This study was supported by Bethlehem Griffith Research Foundation.

32.P11
Studies on shear stress-dependent cell detachment from temperature-responsive cell culture surfaces by using microfluidic devices

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A new approach to quantitatively estimate the strength of cell-material interaction has proposed by using a microfluidic system, which was made of poly(dimethylsiloxane) chip bonding on a temperature-responsive cell culture surface consisted of poly(N-isopropylacrylamide) (PIPAAm) grafted tissue culture polystyrene (TCPS) (PIPAAm-TCPS), having five parallel test channels for cell culture. This construction allows concurrent generating five different shear forces to be applied to cells in each microchannel by varying the resistance of each channel and simultaneously giving on identical cell incubation condition to each test channel. NIH/3T3 mouse fibroblast cells (MFCs) were adhered and spread on PIPAAm-TCPS in each channel at 37 °C. Reducing temperature to 20 °C and starting a flow, cells was peeled off from substrate by shear forces applied to cells in microchannels. Shear stress dependent cell detachment process was evaluated with different shear stress. MFCs in microchannel received with strongest shear stress were found to be detached from the substrate more quickly than those in other microchannels. An intrinsic detachment rate constant for cell detachment was obtained through studying the effect of shear stress on cell detachment times. The proposed device and quantitative analysis could be used to assess the interaction between cells and PIPAAm layer with a potential application to design a cell-sheet culture surface for tissue engineering.

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32.P12
Cell behavior on nanofiber scaffold with different geometry

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Control of growth and differentiation of mesenchymal stem cell (MSC) is highly desired for their application of regenerative medicine. To develop an efficient method to control cell responses, we focused on anisotropic structures of extracellular matrix (ECM). It is well known that cells are oriented along with ECM *in vivo*. Therefore, mimicking the structures would lead to the development of novel biomaterials for regenerative medicine, such as an effective repair of wounds. To mimic ECM structures, we have employed aligned nanofiber scaffolds fabricated by an electrospinning method. Scaffolds with different geometry were fabricated by a rotating collector with different rotation speed. We have examined the extension, the proliferation and the differentiation of human MSCs cultured on them. As a result, cells cultured on the aligned fibers showed less elongation than cells on the random fibers, and also showed more alignment along with fibers. No significant relationship was observed between cell spreading and cell growth, but the proliferation was inhibited on the random fibers. In addition, we found that the random fibers also showed an inhibitory effect of adipoinduction of MSCs by immunohistochemical analysis and gene expression level. These results imply that the different geometry would lead to the different cell response. Therefore, the nanofiber scaffolds can be applicable for the differentiation of MSCs in a 3D-culture condition by regulating their geometry.

32.P13
Synthetic polymer coatings for improved adhesion of human cells

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Previously, we showed that addition of primary alkyl amines, which are lysine mimics, provides significant improvements in cell adhesion and proliferation on swollen hydrogels. With these results in mind we set about designing coatings that could be used to add both primary amine and carboxylic acid functionality to other scaffolds.We chose poly(butyl methacrylate) as a base polymer and then produced : telechelic oligomers; highly branched polymers or linear polymers with pendant or chain end carboxylic acid or amine functionality. We describe the synthesis of these novel coating materials. In contrast to our previous results on hydrogels none of the alkyl amine functional polymers provided useful surfaces for the adhesion of primary human dermal fibroblasts or human primary osteoblasts. However, the carboxylic acid functional materials provided excellent surfaces provided the polymers had Mn above 2000 g/mol The effect of polymer architecture (linear telechelic, linear pendant or highly branched) on cell adhesion was significant and we were able to provide an optimised highly branched polymer that consistently had the best performance in comparative tests. Importantly, these results showed differences in the performance between polymer coatings, with different architectures. Also comparison to our earlier work shows key differences between hydrogels with amine or carboxylic acid functionality and these non-swollen coatings that present the same functionality.

32.P14
Development of label-free cell separating system based on the functional surface with thermoresponsive polymer brush

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Cell separation technologies become increasingly important in the fields of regeneration medicine. Recently, fluorescence-activated cell sorting (FACS) and magnetic cell sorting (MACS) are widely used as precise cell separation methods. However, these cell separation methods require the modification of cell surfaces with fluorescent antibody or magnetic particles. Thus, a cell separation method that requires no modification on the surface of cell is preferable for utilizing separated cells for transplantation. In this study, a new cell separation system using thermoresponsive polymer brush has been developed for cell separation without modification on cell surfaces. Poly(N-isopropylacrylamide)(PIPAAm) brush with various brush lengths were modified on glass substrate through surface-initiated atom transfer radical polymerization (ATRP). Temperature-dependent adhesion and detachment behaviors of four different human cells, fibroblasts (NHDF), endothelial cells (HUVEC), myoblasts (HSMM), smooth muscle cells (SMC), on the prepared surface were observed at 37 °C and 20 °C, respectively. Using the surface with moderate brush length of PIPAAm (Mn: 12 800), HUVEC and HSMM were separated, due to the different detachment properties of these cells from the thermoresponsive surface. These results indicated that different types of cells could be separated with proper length of PIPAAm brush, and this method would be useful as a new cell separation method.

32.P15
Charged nanomatrices as efficient platforms for modulating cell adhesion and shape

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In this report, we describe the design and manipulation of charged nanomatrices and their applications as efficient platforms for modulating cell behaviors. Using electrospraying technology and well designed biomaterials, poly(ε-caprolactone) (PCL) and polyethylenimine (PEI), the negatively charged PCL nanomatrix (nPCL nanomatrix) and the positively charged PCL nanomatrix (pPCL nanomatrix) were fabricated. Our results showed that the pPCL nanomatrix promoted adhesion of NIH 3T3 fibroblast cells more than the nPCL nanomatrix. Detachment of NIH 3T3 fibroblast cells on the pPCL nanomatrix by trypsin-ethylenediamine tetra acetic acid (EDTA) was about two times faster than on the nPCL nanomatrix. The pPCL nanomatrix had NIH 3T3 fibroblast cells with a relatively spherical shape, whereas NIH 3T3 fibroblast cells on the nPCL nanomatrix had an aligned narrow shape. It was also found that charged nanomatrices influenced cross-sectional cell shape. The cross-sectional cell shape on the pPCL nanomatrix was extremely flattened, whereas the cross-sectional cell shape was relatively round on the nPCL nanomatrix and some of the adhered cells floated. We also

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showed that the surface of the nPCL and the pPCL nanomatrices adsorbed the different serum proteins, respectively. These data collectively demonstrated a combination of factors including nanoscale structure, electrostatic forces, and absorption of biomolecules on charged substrates affected cell responses such as adhesion and shape.

32.P16
Phagocytosis of adsorber-microparticles by THP-1 derived macrophages

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Adsorbent microparticles of the Microsphere-based Detoxification System (MDS) are used for extracorporeal blood purification in liver disease and sepsis. Here, we intended to clarify if microparticles escaped from MDS into the patient's blood stream could be phagocytosed by macrophages. Monocytic THP-1 cells were treated with 50 ng/ml phorbol myristate acetate (PMA) to induce macrophage phenotype. Different concentrations of 3 μm-, 5 μm- and 10 μm-Polystyrol-Divenylbenzol-Adsorber (PDA) microparticles were added, cells were incubated for various durations (4–48 h) and were observed after HE-staining by light microscopy. To discriminate between internalized particles and cell-surface adhered particles, FITC-labeled adsorbent-microparticles in combination with an extracellular fluorescence quenching agent (trypan blue) were used for the identification of particle internalization by fluorescence microscopy and flow cytometry. Fluorescence microscopy revealed the phagocytosis of PDA microparticles by THP-1 derived macrophages: 69% of the macrophages incorporated 3 μm-particles, 57% 5 μm-particles and 12% 10 μm-particles. Flow cytometric analysis indicated high fluorescence intensities at 530 nm in contrast to 670 nm. The use of trypan blue inversed the fluorescence characteristics, thus indicating phagocytosis of PDA microparticles.By the use of fluorescence microscopy it was shown that PDA microparticles size-dependently get phagocytosed by THP-1-derived macrophages.

32.P17
Lymphocyte separation columns using thermoresposnive polymer brush grafted glass beads

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Recently, a strong demand for cell separation technologies has been increased due to various applications in biotechnology and biomedical fields. In this study, we prepared poly(N-isopropylacrylamide) (PIPAAm), thermoresponsive polymer, brush grafted glass beads through surface-initiated ATRP as novel cell separation matrices. The prepared beads were packed into poly(vinyl chloride) tubing fitted with nylon mesh column supports. Rat lymphocyte suspension, from the mesenteric lymph nodes of Wistar male rats, was passed through the column at 37 °C for 3.5 min at a flow rate of 0.4 ml/min using an infusion pump, and lymphocyte retention was estimated. Then, HBSS was passed through the column at 5 °C for 3.5 min at a flow rate of 0.4 ml/min, and the lymphocyte recovery rate was estimated. Lymphocyte retention at 37 °C decreased and recovery at 5 °C increased with increasing PIPAAm brush length, because the hydrophilicty increased with grafted PIPAAm brush length. Retention of B cells decreased with increasing the grafted amount of PIPAAm, while T cells percentage scarcely decreased. Thus, B cell contents in lymphocyte was adjustable by flowing them into PIPAAm brush modified glass beads packed column and changing the column temperature, and the cell retention properties of PIPAAm brush would be useful as effective cell separator.

32.P18
Selective osteoblastic citotoxicity induced by nanostructured ZnO in PU /ZnO composites

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Nanosized particles of certain materials have recently demonstrated to be highly beneficial to human health, acting either as tissue regeneration promoters or as strategic cell destroyers. Zinc oxide (ZnO) nanoparticles can produce no cytotoxicity on certain cells types, whereas inducing apoptosis in others. This selective cytotoxicity makes ZnO nanoparticles interesting potential therapeutic agents for the treatment of several diseases, such as cancer. However, a deeper understanding is still necessary on ZnO toxic effects and on its interactions with biological systems. In this work nanostructured ZnO particles, either synthesized by chemical precipitation or commercially available, were incorporated (2 and 50%wt) into a polyurethane (PU) matrix to obtain composites that were characterized, submitted to *in vitro* acellular bioactivity tests and to cell viability assays using MC3T3 cells. Composites loaded with 50%wt ZnO were more bioactive than PU alone or 2%wt PU/ZnO. However, cell viability was higher for the 2%wt PU/ZnO formulations, while the toxicity effects of the 50%wt PU/ZnO composites (with both commercial and precipitated ZnO) were similar to those induced by the ZnO particles alone. These differential responses are discussed in terms of the Zn^{2+} released to the environment. Given that PU/ZnO composites bioactivity and cytotoxicity may be strategically controlled by manipulating ZnO content, this opens a wide variety of medical therapeutic applications.

32.P19
Nanostructured multicomponent coatings via layer-by-layer technique for biomedical applications

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As surface properties of biomaterials influence their initial interaction with cells, surface modifications play a central role in material design. In this work, the layer-by-layer (LbL) technique was used to perform multilayered coating, through alternately deposition of polyelectrolytes, to modify cardiovascular devices and wound healing dressings. Poly(L-lactic) acid (PLLA) films were aminolized by incubation in 1,6-hexamethylendiamine/1 propanol solution. Stainless steel (SS) plates were soaked in (3-Aminopropyl)triethoxysilane (APTES) solution in 1/1 (v/v) water/ethanol. The pre-functionalized substrates were coated via LbL using heparin (HE) as polyanion and chitosan (CH) or poly(diallyldimethylammonium chloride) (PDDA) as polycation. The multilayered films were characterized for their physico-chemical properties and *in vitro* cell response. Successful functionalisation of PLLA and SS substrates with HE/CH and HE/PDDA coatings was confirmed by surface characterization techniques: XPS, UV-Vis, AFM and contact angle analyses. HE/CH coatings showed antibacterial properties. Whereas HE/PDDA coatings inhibited platelet adhesion and activation. In conclusion, LbL coatings were developed: (i) with antibacterial properties for wound healing dressings and (ii) with anticoagulation properties for cardiovascular devices. NANOSTENT (Regional Project,

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32.P20
Enzyme mediated, oxidative crosslinking of multifunctional, branched PEG: Characterization, particle formation and stem cell encapsulation

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The successful use of stem cells within the regenerative medicine field relies heavily on the generation of functional synthetic support systems that provide the necessary biochemical cues for cell attachment, survival and fate specification. While there has been a vast amount of research into this area to date, a tunable, scalable and defined high throughput approach is yet to be developed. In answer to this, we have generated hydroxy phenol functionalised branched PEG hydrogels, cross-linked via an enzyme mediated, oxidative process. The use of the branched PEG as a hydrogel material has the distinct advantage over other materials as it is biocompatible and bioinert, while also having the ability to be functionalised with specific peptide sequences enabling specific cell-gel interactions. ATR-FTIR and 1H NMR was used to confirm and quantify the degree of hydroxy phenol functionalisation. The rate of gelation and modulus of the hydrogel is shown to be tunable by variation to the enzyme/initiator ratio, as confirmed using oscillatory rheometry. The polymer concentration and degree of hydroxy phenol substitution were also shown to affect the resulting hydrogel modulus. A high throughput approach to the encapsulation of human mesenchymal stem cells (hMSCs) has been developed where the effect of particle size and frequency, cross-linking agent concentration and cell feed density has been investigated for the optimisation of functional cell-gel spheroids.

32.P21
Physico-chemical and enzymatic properties of PVGLIG and PVGLIG-alginate hydrogels

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ECM-like matrices based on peptide-modified hydrogels are being increasingly explored not only for tissue engineering applications but also as 3D cell culture models. We previously reported that bi-functionalization of alginate hydrogels with cell-adhesion (RGD) and protease-sensitive (PVGLIG) domains provides a dynamic environment to 3D-cultured mesenchymal stem cells (MSC), promoting their interaction with the matrix and other cells. Here, the system was further characterized at different levels. The effect of peptide-grafting on hydrogel's viscoelastic properties was analysed by DMA. PVGLIG hydrolysis by matrix metalloproteinases (MMP) was evaluated using a FRET-peptide. MMP expression during MSC differentiation was analysed by zymography and RT-PCR. DMA showed that PVGLIG could be strategically inserted on the low molecular weight (MW) fraction of hydrogels with a binary composition of low-high MW, without altering their mechanical properties. PVGLIG was a good substrate to MMP9, MMP2, MMP14 and MMP13, but not to MMP1 and MMP8. During osteogenic differentiation, MMP2 was still expressed, albeit at lower amounts compared to basal conditions, while the opposite was observed for MMP14. The cocktail of cell-secreted proteases present on the conditioned medium of MSC cultures hydrolysed the FRET-PVGLIG but not the scrambled control peptide.

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32.P22
Polyhydroxybutyrate and polyethylene glycol composites as biomaterials for biomedical applications

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Polyhydroxyalkanoates (PHAs) are hydrophobic biopolyesters which are naturally produced by-products of a range of bacteria. The most common type of PHAs is Polyhydroxybutyrates (PHB) which is biocompatible, biodegradable and FDA approved polymers, and has been used as biomaterials for implantation devices. Recent studies have suggested combining PHB with polyethylene glycol (PEG) through blending or 'BioPEGylation', in order to promote cell responses and improving PHB material properties. This study will examine the effects of blending and microbial hybridisation on the material properties and biocompatibility of PHB. This study found that increasing the PEG loading led to an improvement in PHB's ability to uptake water and the hydrophilic characteristics of the films. The mechanical strength and flexibility of PHB/PEG (80/20) films were found to have improved significantly when compared to PHB. The ability for PHB in blended and hybrid films to crystallise was found to have reduced significantly. This study also investigated the biocompatibility of PHB/PEG and hybrid films by examining on cell attachment, proliferation and the cell cycle of neural associated olfactory ensheathing cells (OECs). Our positive results show that blending PHB with PEG or 'BioPEGylation' is one way to improve degradation rate, overall biocompatibility and material properties of PHB. Therefore, make it a potential use for neural tissue engineering strategies in regenerative medicinal applications.

32.P23
Sustained oxygen release from PLGA microspheres

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One of the leading causes of cell death in large cell-scaffold complexes is a lack of oxygen. The aim of this research project was to develop a material with slow oxygen release that can be used as a side kick for bone scaffold materials. By incorporation of calcium peroxide into poly-lactic-co-glycolic acid (PLGA) microspheres, any contact with water will release oxygen through degradation of the peroxide. This system will keep cells alive so that vascular networks can be developed after which a natural sustained oxygen supply can be achieved. Human mesenchymal stem cells (hMSCs) were isolated with a Ficoll density gradient method from reaming debris that was collected during total hip replacement. Microspheres were produced in an oil-in-oil (o/o) solvent evaporation method out of a 10% PLGA (w/v) and 5% CaO_2 (w/w) solution. hMSCs were grown on PLGA/CaO_2 films and in the presence of PLGA/CaO2 microspheres. Microspheres were produced that showed a reproducible oxygen release for up to 100 h. hMSCs were seeded on PLGA/CaO_2 films, but adherence to the material did not occur. The presence of microspheres was well tolerated by hMSCs grown *in vitro*. If high amounts of microspheres were added to tissue culture wells, cell death occurred due to the toxic levels of H_2O_2, a negative product of the reaction of CaO_2 and water. A sustained oxygen

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release system has been created that can serve as a side kick in large cell-scaffold constructs and that is well tolerated by hMSCs.

32.P24
Facile preparation of macroporous Resomer®/ Gelatin/Chitosan cryogel: Physical-chemical, mechanical and biological properties

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Porosity plays a significant role in the overall function of tissue engineering scaffolds. In particular, pore characteristics such as pore size and interconnectivity are critical in hydrogel properties. Enhancing porosity and pore interconnectivity have been shown to enhance nutrient diffusion and waste exchange, while decreasing the mechanical properties of hydrogels. Optimizing technology scaffold fabrication is essential for tissue engineering(TE) applications. The aim of this study was to propose a novel technique scaffold preparation. Porous hydrogels chitosan/gelatin/ Resomer® particles were prepared using a double emulsion process succeeding by freeze-drying method followed by a chemical treatment and photocrosslinking (UV radiation). Morphology hydrogels showed high porosity observed by ESEM. Chemical interactions were demonstrated by FTIR and thermal characterization (DSC). Mechanical properties in cryogels showed a unimodal behavior (41 MPa ± 10) while in hydrogels showed bimodal behavior (148 KPa ± 24 and 254 KPa ± 27 Young modulus). Moreover, cell viability and cell adhesion were tested by Live/Dead kit, ESEM and TEM. Biopsy samples of articular cartilage chondrocytes were obtained from intercondylar area of male with a post-traumatic defect of anterior cruciate ligament. The developed process can be easily used to generate porous hydrogels to further promote cell proliferation and may be of use for a broad range of TE applications.

32.P25
Enhanced mechanical property and pH neutralization effect of PLLA films blended with OLA-grafted magnesium hydroxide nanoparticles

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Biodegradable polymers, such as poly(lactide) (PLA), poly(glycolide) (PGA), and poly(caprolactone) (PCL), have been widely used in biomedical applications. However, their degradation by-products have been concerns because they are a source of inflammatory reaction in the body. In addition, relatively weak mechanical property is another shortcoming. In this study, we synthesized oligolactide (OLA)-grafted magnesium hydroxide nanoparticles and investigated the neutralization effect on acidic pH caused by biodegradation as well as the improvement of mechanical properties in the nanocomposite with PLLA. OLA-grafted magnesium hydroxide nanoparticles was characterized by FTIR and TGA. With increasing the portion of nanoparticles, PLLA matrices showed enhanced mechanical properties, which increased tensile strength. This means that grafted OLA enhanced the interfacial interaction between PLLA matrix and the nanoparticles. Moreover, the pH change was tested in deionized water at 37 °C for 8 weeks. OLA-grafted magnesium hydroxide nanoparticles effectively neutralized acidic environment that was caused by hydrolytic degradation of PLLA film. Therefore, this study demonstrated that OLA-grafted magnesium hydroxide nanoparticles effectively not only improved mechanical properties of PLLA matrix but also showed pH neutralization effect, which could be very promising for biomedical applications such as biodegradable stents and prostheses.

32.P26
Response of marrow stromal cells to encapsulation in inert hydrolytically degradable polyethylene glycol hydrogels

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PEG hydrogels are inert and non-immunogenic and have been used extensively as a matrix to investigate the effect of microenvironment on cell function. However, their use for *in vivo* applications is severely limited by their non-degradability. The objective of this work was to investigate the response of marrow stromal cells (MSCs) to encapsulation in degradable star poly(ethylene glycol-co-lactide) acrylate (SPELA) hydrogels. The SPELA macromonomer, MSCs, Ac-GRGD, and rhBMP-2 were mixed and crosslinked by UV polymerization and cultured in osteogenic media for 21 days. At each time point, samples were used for determination of extent of mineralization and mRNA analysis. SPELA hydrogels with compressive moduli as high as 500 kPa were synthesized. In 4 weeks, the SPELA hydrogels with 2 and 4 lactides per macromonomer arm had 35% and 90% mass loss, respectively, compared to <5% mas loss for non-degradable PEG hydrogel. When MSCs were encapsulated, ALPase activity, osteognic markers Dlx5 and Runx2 peaked at day 7. The peak intensity was significantly higher with the addition of osteoinductive factor rhBMP-2. The expression of osteopontin and osteocalcin increased with time, but the increase was greater with rhBMP-2. The extent of mineralization increased with time, with those treated with rhBMP-2 having slightly higher extent of mineralization. The SPELA hydrogel is attractive as inert, degradable, remodelable, and injectable matrix for cell delivery in TE applications.

32.P27
Preparation and characterization of nano-hydroxyapatite/silicone rubber composites for potential use as soft-tissue implants

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Introduction: Silicone rubber (SR) has been widely used clinically as implant material characterized by heat and chemical stability and physiological inertness. This material does not unite organically with tissues, due to its bio-inertness, leading in some cases to inflammation and foreign-body reaction after implantation. In order to overcome the above drawbacks of SR, Hydroxyapatite (HA) can be used as an additive. ExperimentalSR/HA nanocomposites, with 5–20 phr loadings, were prepared using the sonication technique and subsequently characterized by X-ray diffraction (XRD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), tensile and tear tests. Also cell cultures of primary fibroblasts has been performed on SR hybrids in order to asses biomaterial biocompatibility (MTT test). Scanning Electron Microscopy (SEM) analysis has been performed after 48 h of culture on both HA/SR nanocomposites and pristine elastomer.
Results: The incorporation of nano-HA into silicone rubber does not affect the thermal transitions of polymer, improves the thermal stability in inert atmosphere and the mechanical performance of the derived

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nanocomposites. From SEM analysis it is obvious that the reinforced materials present an improved surface for cells attachment and distribution. The above suggest that the studied hybrids would display improved biocompatibility and bioactivity when used in soft-tissue implants applications.

32.P28
Study of adsorbed proteins on nanofibrous substrates

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Protein adsorption to substrates is important in the mediation of cell behaviour and can be used to reveal underlying cell-substrate interaction. Here, we investigate adsorption of serum proteins onto polycaprolactone (PCL) nanofibres using fluorimetry and TOF-SIMS to determine variation of proteins adsorbed from pure protein solutions and from stem cell conditioned media. PCL was electrospun in two different orientations (aligned or random) onto glass coverslips. Substrates were incubated in a range of single protein containing solutions (laminin, collagen, fibronectin) and conditioned media. After incubation protein/media supernatant was collected and protein concentration quantified using NanoOrange fluorimetric assay. Substrates were analysed using TOF-SIMS and Principle component analysis (PCA). PCA analysis of TOF-SIMS data revealed significant spectral differences when comparing adsorbed protein layers on control glass coverslips to nanofibre substrates. Data suggested that protein-substrate interactions gave rise to differences in adsorbed proteins from conditioned media, with quantification also showing that chemistry and topography variation in surface alters the concentrations and types of absorbed proteins on the substrates. Substrate chemistry and topography influences protein adsorption. TOF-SIMS analysis demonstrates the ability to identify adsorbed proteins on synthetic substrates from media which is relatively undefined.

32.P29
Characterization of poly(N-isopropylacrylamide) modified polydimethylsiloxane surfaces as new type of temperature-responsive cell culture surfaces

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It is known that conventional temperature-responsive cell culture surfaces (TRCS) showed nano-level polymer thickness dependency on cell adhesion properties as well as hydrophobicity of TRCS. To recover targeted cells sheet from TRCS, the polymer thickness and the polymer graft density was adjusted for preparation of TRCS appropriate for the targeted cells culture by using different monomer concentration. However, such adjustment may be sometimes difficult because of precise construction of nono-orderd polymer layer on the TRCS, and several trials needed to be carried out to obtain appropriate TRCS. Convenient adjustment of the polymer thickness and density is desired for preparation of TRCS for culturing the targeted cells. To achieve the adjustment conveniently, we tried to utilize mechanical stretching for TRCS. Namely, new type of TRCS was prepared by modifying stretchable polydimethylsiloxane (PDMS) as base substrate with poly(N-isopropylacrylamide) (PIPAAm-PDMS) on the basis of the concept that the grafted PIPAAm thickness as well as the polymer density can be decreased by mechanically extending the PIPAAm-PDMS. In this presentation, to demonstrate the concept, we compared PIPAAm-PDMS surface properties such as wettability, the polymer graft density and cell adhesion behaviour before and after an induction of mechanical extension. The results possibly suggest mechanical stretching easily adjust the polymer thickness and density of the PIPAAm-PDMS surfaces.

32.P30
Corrosion stability of silver-doped hydroxyapatite/lignin coatings in simulated body fluid

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The composite coatings containing biopolymers and bioactive ceramic materials, such as hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, HAP), on titanium may have excellent properties like biocompatibility, osteoconductivity and mechanical strength. But, bacterial infection of hard tissue implants is still the main problem that can be prevented by using the antimicrobial coatings containing silver. In this study, the chosen concentration in silver-doped hydroxyapatite/lignin (HAP/Lig) coating was 0.5 wt. % Ag, with different lignin concentrations. The Ag/HAP/Lig coatings fabricated by electrophoretic deposition have been investigated by various characterization techniques. The X-ray photoelectron spectroscopy (XPS) analysis showed that the atomic Ca/P ratio was 1.63 ± 1 and remains almost the same after sintering. The cytotoxicity and antimicrobial activity of Ag/HAP/Lig coatings were investigated by MTT test and antimicrobial test in suspension, respectively. The evaluation of corrosion stability of non-sintered and sintered Ag/HAP/Lig coatings with 1 wt. % Lig concentration was investigated in simulated body fluid (SBF) at 37 °C by electrochemical techniques: open circuit potential–time measurements and electrochemical impedance spectroscopy (EIS). It was shown that sintered Ag/HAP/Lig coatings exhibited good corrosion stability in SBF solution, while non-sintered coatings showed significantly lower stability.

32.P31
Comparison of nanoscale hydroxyapatite produced by wet precipitation and sol-gel methods

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Hydroxyapatite (HA) has been used extensively in dental and orthopaedic applications, because of its excellent biocompatibility and osteoconductivity. Nanoscale HA is expected to have even greater bioactivity when compared to current microcrystalline materials due to the high surface area: volume ratio and biomimetic nature of nanoscale HA. However, preparation of nanoscale HA is a technically greater challenge than the production of conventional macroscale materials. The aim of this study was to compare wet precipitation and sol-gel methods to produce nanoscale HA. The wet precipitation method involved the titration of a phosphorus solution into a calcium solution using a peristaltic pump. Factors such as chemical precursors, pH, synthesis temperature and the presence of ethanol were investigated. Similarly, the sol-gel method comprised of the titration of a calcium nitrate solution into a triethyl phosphite solution. The pH and sintering temperatures were varied to investigate their effect on the purity and size of the HA particles. X-ray Diffraction (XRD) was used to confirm the formation of HA, while Transmission Electron Microscopy (TEM) was applied to determine particle size and morphology. It was concluded that both

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methods were suitable for production of HA, although wet precipitation might be superior for commercial adoption.

32.P32
Fibrous protein adsorption on the bioglass surface studied by XPS and FTIR spectroscopy

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The proteins adsorption on the scaffold surface leads to changes in their conformation and will further influence the adhesion, migration and growth of cells. The aim of this study was to monitor the attachment on bioglass powders and the conformational changes of two fibrous protein, fibrinogen (bovine plasma) and collagen (calf skin type III). Samples of SiO_2-CaO-K_2O-P_2O_5 bioactive glass system were prepared by sol-gel method. After aging at room temperature under atmospheric conditions, the dried gel was annealed at 300 °C in air for 30 min. The obtained powder samples were immersed in fibrinogen/collagen solution for 24 h at a constant temperature of 37 °C. The relative concentration of C, N and O determined from XPS survey spectra analysis after immersion in protein solutions points out the protein attachment on the surface of the bioactive glass particles. Comparative analysis of FT-IR spectra recorded from native protein and biocomposite samples, after immersion in protein solution, shows changes of the secondary structure in amide I absorbtion band. The content of the α-helix, β-sheet, β-turns and random structures was estimated by applying a deconvolution procedure to the large absorption amide I band by using a Gaussian line function, after determining the number of components and their peak positions by second derivative analysis. K.M. author wishes to thank for the financial support provided by Contract POSDRU/89/1.5/S/60189.

32.P33
Protein adsorption onto bioactive glasses – conformational changes and quantitative adsorption analysis

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One important aspect that has to be considered in investigating the biocompatibility of a biomaterial is its response in terms of interactions with proteins. However, protein behavior after adsorption and details concerning the amount of the protein attached are relatively few elucidated. Most of the techniques that are frequently used to gain such information exhibit serious limitations and are not easily adaptable to proteins adsorbed on solid surfaces. The goal of this work is to use EPR spectroscopy for investigating adsorption of horse hemoglobin and BSA on Bioglass 45S5 functionalized with glutaraldehyde (GA). Native cysteines from position β-93 in horse hemoglobin and position 34 in BSA were spin-labeled with Iodoacetamide spin label. Cw EPR spectra of the proteins in buffer solution were recorded to assess the dynamics of the attached spin labels and the amount of the protein bound on the bioglass. The obtained results were correlated with the results obtained by XPS spectroscopy and SEM images. The distance between the spin labels was measured at 50 K, by using the DEER pulsed EPR technique. The results suggest that GA positively influences the protein binding stability and induces polymerization of hemoglobin. The research was accomplished in the framework of PNII Idei PCCE-312 /2008 project granted by the Romanian National University Research Council. EV author wishes to thank for the financial support provided through the Contract POSDRU/89/1.5/S/60189.

32.P34
Studies on bioactive glasses loaded with vitamin C

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Since L-ascorbic acid, also known as vitamin C, is one of the essential nutrients playing an important role in the synthesis of bone matrix collagen, the aim of our study was to entrap vitamin C in bioactive glasses with possible further application in bone tissue regeneration [1]. A bioactive glass composition of SiO2-CaO-P2O5 system was synthesized via acid catalyzed sol-gel route and it was loaded with vitamin C in two ways. The first approach was to mix the glass sample in the sol stage with a vitamin C solution. The second loading way was by immersing wet gel, dried gel and thermally treated samples in vitamin C solution. The samples were analyzed before and after loading, to see the influence of ascorbic acid uptake on the structural, morphological and bioactive properties of the glass matrix, using XRD, FTIR, SEM and *in vitro* tests in SBF. The capability of protein adsorption on these samples was investigated by means of XPS and FTIR spectroscopy.[1] S.J. Padayatty, A. Katz, Y. Wang, P. Eck, O. Kwon, J.-H. Lee, S. Chen, C. Corpe, A. Dutta, S.K. Dutta, M. Levine, J. Am. Coll. Nutr. 22 (2003) 18-35.
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32.P35
The risk of water soluble and bio-stable polymers in regenerative medicine

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Water soluble and biostable polymers distribute and accumulate within organisms, despite that knowledge they are frequently suggested for newly devised strategies in regenerative medicine. Indeed carefully evaluated save applications in medicine are known for polyvinylpyrrolidone (PVP), polyacrylic acid, polyvinyl alcohol and others. It is tenuous to use such polymers for drug delivery, cell culture or for any material such as wound dresses or structural support elements, where the polymers can eventually be released into circulation. Associated risks are not obvious due to lack of classical toxicological properties, yet long term consequences are documented. A novel approach to fluorescently label PVP allows observation of pinocytosis into blood cells and FACS analysis. Whole zebra fish imaging revealed time dependent systemic distribution. A comparison between clearance of small molecular weight substances, biostable and biodegradable polymers is presented. Polymer circulation, pinocytosis by blood cells and possible entry routes and accumulation into organs was studied. Missing links between those steps were revealed by using a much improved optical labeling strategy over conventional radio nucleotide studies in that field. Pinocytosis into cells that are involved in tissue regeneration and repair indicate possi-

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ble explanation for reported organ failure in the past. Patients suffering from polymer accumulation symptoms nowadays might benefit from this much refined picture.

32.P36
Advantages of composite-scaffolds prepared by freeze drying for bone engineering

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Scaffolds in tissue engineering are used for providing a temporary 3D environment that promotes cell adhesion and proliferation, temporary mechanical support and guiding for new tissue growth. Scaffolds based on polymers and ceramics may provide with excellent material properties such as tunable biodegradability rates and tailored mechanical properties.Preparation of a porous scaffold consisting of a polymeric matrix of polyvinyl pyrrolidone (PVP) with polyethylene glycol dimethacrylate (PEGDMA) and hydroxyapatite has been undertaken using a freeze drying technique. NMR spectra confirmed the polymerization reaction. TGA shows a weight loss between 316 and 480 °C which corresponds to the decomposition and volatilization of the polymer component on heating and indicate a real ceramic content of 10 and 25%. FTIR was used to study the incorporation of both VP and PEGMA and OHAP in the system and DMA studies indicates an increase of elasticity in the sample with 25% of OHAp. The ceramic component dispersion is homogenous and that the reinforcement is based on phase aggregation. Pore content and pore interconnection can be modulated between 20 and 100 μm by controlling the freezing rate.The combination of the hydrophilic character together with the porosity features, the mechanical properties and non-toxicity of the composite system makes this approach promising for bone tissue scaffolding.

32.P37
Synthesis of biodegradable poly(e-caprolactone)-organosiloxane nanohybrids with charged functional groups for bone tissue engineering applications

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Poly(ϵ-caprolactone)-organosiloxane nanohybrids with charged functional groups were synthesized by sol-gel method with the capacity to conjugate biomolecules for the potential application as a bone tissue engineering scaffold. These nanohybrids contained positively charged amine groups were synthesized by end-capping α,ω-hydroxyl poly(ϵ-caprolactone) with (3-isocyanatopropyl)triethoxysilane followed by sol-gel reaction with (3-aminopropyl)triethoxysilane. This resulted in a nanohybrid composed of poly(ϵ-caprolactone) segments connected by siloxane linkages containing positively charged amine groups. The successive succinlylation of amine groups created negatively charged carboxylate groups. The zeta potentials of the two nanohybrids with amine and carboxylate groups were about +3.1 mV and -5.9 mV, respectively. AFM determined an increase in surface roughness from about 19.1 nm to 37.4 nm caused by introducing carboxylate groups onto amine groups. Conjugation of an GGGGRGDASSK peptide onto the carboxylated groups noticeably improved the proliferation and differentiation activities of osteoblast-like cells. These results suggest that these novel nanohybrids possess an encouraging potential to be used as a tissue engineering scaffolding material due to their high capacity for conjugating bioactive molecules.

32.P38
Low temperature hydroxyapatite/gelatine robocasted scaffolds for bone tissue engineering

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The fabrication of bioactive scaffolds featuring controlled structures from the nano up to the macro scale represents one of the most challenging issues of bone tissue engineering (BTE). However, most current scaffold fabrication techniques do not allow an accurate control on such a wide dimensional range. The aim of this work is to exploit the low-temperature self-setting ability of calcium phosphate cements (CPC) to fabricate robocasted scaffolds that combine the intrinsic CPC nano/micro porosity with the controlled macroporosity introduced by robocasting. The rheological properties of an α-tricalcium phosphate/gelatine slurry with two granulometries were optimized for robocasting. After that, three-dimensional scaffolds were fabricated using a rapid prototyping machine (NScrypt, USA) with tapered tips of 0.84 mm of inner diameter. The scaffolds were characterized by N2 adsorption, field emission SEM and X-ray diffraction. Coarse or fine powders led to plate- or needle- like calcium deficient hydroxyapatite nanocrystals respectively, exhibiting specific surface areas between 13 and 40 m^2/g. The liquid to powder ratio allowed tuning the microporosity of the strands between 35 and 55% whereas robocasting enabled controlling the macroporosity and architecture of the scaffold. This accurate control of the porosity at different scales represents a step forward in the development of bioactive, osteoconductive and potentially resorbable scaffolds for BTE.

32.P39
Macroporous hydrogels to recruit osteogenic signals for bone regeneration

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Hydrogels have been largely studied to mimic basic functions of the extracellular matrix of mineralized tissues. Major lacks in bone-substituting use concerned the inability to calcify inert hydrogels for promoting cell differentiation into osteogenic way. However, their peculiar transport properties can be successfully used to create reservoir systems which properly recruit environmental signals to cells. Here, we introduce an intriguing approach to obtain Poly(ethylenglycole)diacrilate (PEGDA) by the combination of UV photopolymerization and salt leaching technique. This versatile strategy allows creating an interconnected macropore network – ca.200 μm in pore size - requirement for an optimal cell hosting, while peculiar swelling properties of scaffold trabeculae, which well-fit a Fick-like diffusion, assure an optimal supply of signals able to variously trigger *'in vitro'* cell activities. Accordingly, we investigated the ability of macroporous hydrogels to induce hMSCs differentiation in osteogenic ways. hMSC preliminary showed a good affinity, not evidencing any citotoxicity until 6 days of culture. A copious deposition of calcium deposits has been detected by Alizarin Red since 7 days in osteopromotive medium with an increasing amount of mineral phases up to 21 days. These results have also been corroborated by EDX spectra which revealed the presence a Magnesium peak (ca. 6% of Ca or P elements) so confirming the formation of mineral phases with a bone-like composition.

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32.P40
Biologically active copolymer hydrogels

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Previous work from this group has shown antibacterial activity of diblock and triblock synthetic biocompatible polymers [1]. Since such copolymers have potential biomedical applications, the aim of the current study was to study the relationship between copolymer composition and architecture so as to elucidate their likely antibacterial mechanism. More specifically, the antibacterial activity of various different monomers/copolymers of poly(2-(methacryloyloxy) ethylphosphorylcholine) [PMPC], poly(2-hydroxypropyl methacrylate) [PHPMA] and poly(glycerol monomethacrylate [PGMA] towards Staphylococcus aureus will be examined. Block copolymers were synthesized using either atom transfer radical polymerization [ATRP] or reversible addition-fragmentation [RAFT] polymerization and characterized via 1H NMR, GPC and rheology. Anti-bacterial activity was assessed using several well- known assays, including direct contact, adhesion, minimum inhibitory concentration [MIC] and minimal bactericidal concentration [MBC]. We have also prepared fluorescently-labeled copolymers using a rhodamine 6G-based ATRP initiator to view their interactions with cells and bacteria. The combination of antimicrobial activity with mammalian cell biocompatibility is unusual and suggests potential biomedical applications for these materials. 1. Bertal, K., et al., Antimicrobial activity of novel biocompatible wound dressings based on triblock copolymer hydrogels. Journal of Materials Science, 2009

32.P41
Optimising gas foaming method to obtain highly porous gelatin scaffolds

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An ideal tissue engineering scaffold should have a high porosity and inter-connected porous structure. Gas foaming is effective technique in obtaining such structures. In this study, porous gelatin scaffolds were prepared through gas foaming. Acetic acid and sodium hydrogen carbonate were used to produce CO_2 gas within gelatin solution. As result of gas production, gelatin solution turns into foam. Prepared foam crosslinked using Glutaraldehyde and freeze-dried for 24 h before testing. The impact of gelatin Bloom Index on the final product is studied via compressive mechanical testing, SEM analysis, and water absorption. As of any other foaming system, viscosity and rheology of gelatin solution are pivotal in obtaining an optimal porous structure, thus the influence of viscosity changes as a function of solution temperature on the prepared scaffolds macro-structure was also studied. It was shown that at the same temperature, gelatin samples with different bloom indices show different solution viscosities. An ideal viscosity for obtaining optimum scaffold macro-structure is suggested. It was verified that the gelatin bloom index has an impact on scaffold porous micro-texture, mechanical, and physical properties. Preparing porous scaffolds from gelatin with a higher bloom index allows the final structure to show an improved compressive strength, more desirable porous microstructure with better interconnectivity among pores, and a better water absorption capability.

32.P42
Mechanical properties and cell viability of lactide and caprolactone based 3-dimensional scaffolds filled with inorganic bioactive particles

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The understanding of the mechanical properties of the materials is of vital importance in the design of 3-D porous scaffolds for tissue engineering applications. In this work, the mechanical behavior of porous poly(ϵ-caprolactone) (PCL), poly(L-lactide) (PLLA) and poly(L-lactide/ϵ-caprolactone) (PLCL) was investigated and compared to their non-porous counterparts. The presence of pores reduced dramatically the mechanical properties of the structures; as an illustration, the Young moduli of PCL, PLLA and PLCL were reduced respectively, from 183.70 to 0.95, 2263.44 to 16.21 and 5.72 to 1.00 MPa. The addition of bioglass or hidroxiapatite particles improved noticeably the stiffness of the porous PLLA. On the contrary, porous PCL and PLCL suffered a high reduction in elongation at break when adding these particles with little improvement in their stiffness, probably because of the incompatibility between inorganic particles and polymer matrix. Finally, we also investigated the attachment, proliferation and growth *in vitro* of adipose derived mesenchymal stem cells (ASCs). The MTT assay showed that the employed materials did not present any cytotoxic effect for ASCs and were valid substrates for cell adhesion. We conclude that the scaffolds fabricated, combined with ASCs, have a promising future in tissue-engineering applications because of the abundance of this tissue in the human body, the easiness in obtaining high number of cells and the multilineage differentiation of ASCs.

32.P43
Hydroxyapatite nanoparticles modified by lauroyl chloride: a very efficient method for mineral dispersion in PLLA scaffolds

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Scaffolds for cell growth aiming at bone repair applications were prepared by electrospinning of poly(L-lactide) (PLLA) containing hydroxyapatite nanoparticles (HAP-Np). HAP-Np were modified with lauroyl chloride yielding grafted HAPLC, attested by FTIR, TG/MS and solid 31P MAS-NMR. Different contents of HAPLC, HAP grafted with L-lactide (PLLA-g-HAP) and HAP-Np were dispersed in electrospun PLLA matrix. TEM and SEM were employed to access the morphology and the dispersion of these nanoparticles in the fibers. The mechanical properties were investigated in PLLA cast films containing different amounts of HAPLC, PLLA-g-HAP and HAP-Np, while MTT assay was carried out to monitor fibroblast NIH–3T3 cell growth. Lauroyl chloride grafting allowed a much higher and better dispersion compared to PLLA-g-HAP and HAP-Np, as demonstrated by TEM analysis. Inclusion of up to 30% HAPLC in PLLA cast films exhibited higher strength properties compared with PLLA-g-HAP and neat HAP-Np. MTT assays and SEM revealed the same cell growth for PLLA fibers containing 5 wt% of HAPLC, PLLA-g-HAP and HAP-Np, however the PLLA fibers with 30 wt% of HAPLC presented best cell adhesion and proliferation.

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32.P44
Bioactive and biodegradable three dimensional fibrous scaffolds for orthopaedic applications

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In this study, electrospinning technique was used to prepare a tridimensional nanostructured triphasic (polyhydroxybutyrate-polyhydroxyvalerate/nano-hydroxyapatite/silk fibroin) fibrous composite. While electrospinning can produce small diameter fibre structures, with high surface area and tailored porosity, thus supporting cell migration and differentiation, the natural polymeric phases incorporated into the matrix provide a biodegradable, biocompatible and bioactive platform for cell growth and bone tissue remodelling. The study shows that simultaneous electrospinning of natural polymers and ceramic phases, such as nanohydroxyapatite is possible, with the formation of smooth and continuous fibrous membranes, and finally a tridimensional porous structure. The constructs were created either by manipulating the bidimensional membranes previously collected on Al plate collector or via layered hydrospinning, where fibres were spun onto a water bath instead of a collection plate. At specific time intervals one thin fibrous layer was removed from the water surface on a glass, eventually creating a hydrospun scaffold composed of many fibrous layers.Degradation, conductivity and pH measurements evidenced that both the pure polymer and composite samples have a low degradation rate (<1% weight loss, pH between 6.8 and 7.4 after 31 days of degradation), both in membrane and tridimensional forms.

32.P45
The effect of solvent systems on structural properties of electrospun PCL-gelatin nanofibrous composite scaffolds

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Introduction: As the effect of scaffold structure on cell behavior is approved by different studies, ability of preparation of scaffolds with different morphology for tailoring different requirements of various cells is an aim of researchers. One of the important factors affecting the nanofibers morphology is solvent system. Polycaprolactone- gelatin composite electrospun scaffolds, the successful complex in scaffolding for soft tissue engineering, can be fabricated using different solvent systems.
Materials and methods: In the present study we investigate the effect of different solvent systems (formic acid: glacial acetic acid, TFE:formic acid and TFE:glacial acetic acid) on the nanofiber formation and morphology of polycaprolactone- gelatin composite electrospun scaffold using SEM micrographs.
Results and conclusion: The results suggest that using a mixture of formic acid and glacial acetic acid has higher productivity compared to using a trifluorothanol -based solvent system. Also, it will be shown that using a mixture of formic acid and glacial acetic acid for dissolving polymers leads to smaller fibers comparing to using TFE-based solvents. It might be due to higher electrical conductivity of formic acid and acetic acid. Therefore, it can be concluded that nanofibers with different diameter size can be fabricated only by changing the solvent.

32.P46
Modification of fibrous PLGA scaffold or PLGA micro-particles with tripolyphosphate nanoparticles

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The current study was designed to quickly neutralize the acidic degradation products of PLGA fibrous scaffolds or micro-particles by incorporating tripolyphosphate (TPP) nanoparticles. A homogeneous mixture of PLGA and TPP was first obtained by water-in-oil emulsion-dispersion followed by freeze-drying. The dried blend was either melt-spun to yield fibers which were processed into scaffolds or underwent second emulsion to obtain micro-particles. The resulting scaffolds or micro-particles were immersed into PBS to verify the degradation properties. The pH of the saline was monitored. The amount of TPP was optimized to improve the acidic degradation of PLGA. Cellular compatibility of the modified and pristine scaffolds was evaluated using rabbit adipose-derived stem cells (rASCs). It was shown that TPP particles within the PLGA fibers were roughly 100 nm in diameter and mainly located inside fibers instead of on the superficial layer. For the fibrous scaffolds, the acidic degradation of PT-16 and PT-64 (PT-X is termed when the monomer molar ratio of TPP to PLGA was 1:X) was significantly improved as the pH values of their respective solutions were maintained in a well neutralized state during the degradation. For PLGA micro-particles, the PT-64 sample has the improved acidic degradation property over pristine PLGA. The cellular compatibility of PT-64 was also improved. Hence, the method developed could be an effective strategy to adjust the acidic degradation of PLGA.

32.P47
Electrospun poly(L-lactide)/poly(ϵ-caprolactone) blend nanofibrous scaffold: characterization and biocompatibility with human adipose-derived stem cells

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The essence of tissue engineering is the fabrication of autologous cells or induced stem cells in naturally derived or synthetic scaffolds to form specific tissue types. Polymers are thought as an appealing source of scaffold owing to the diversity of their physicochemical properties and can be electrospun into nano-size to mimic natural structure. Poly (L-lactic acid) (PLLA) and poly (ϵ-caprolactone) (PCL) are both excellent aliphatic polyester with almost 'opposite' characteristics. The controlling combination of PLLA and PCL provides varying properties and makes diverse application. Compared with the copolymers of same components, PLLA/PCL blend demonstrates potential in regenerative medicine as a simple, efficient and scalable approach. In this study, we electrospun PLLA/PCL blend of different weight ratios into nanofibrous scaffold (NFS) and their properties were detected including morphology, porosity, degradation, ATR-FTIR analysis, stress-stain assay, and inflammatory reaction. To explore the biocompatibility of the NFS we synthesized, human adipose-derived stem cells (hASCs) were used to evaluate proliferation, attachment, viability and multi-lineage differentiation. In conclusion, PLLA/PCL blend fibers prepared by electrospinning with three weight ratios supported hASCs well and the NFS of 1/1 weight ratio showed better properties and cellular responses in all assessments, implying it a biomimetic scaffolds for tissue engineering.

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32.P48
Non-linearly elastic nanobiomaterials for soft tissue engineering

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Mechanical dissimilarity between synthetic biomaterials and host tissues is one of the reasons for graft failure of biomaterials. The stress-strain curves of elastomers are linear at low strains, whereas those of biological tissues are non-linear. We have successfully developed non-linearly elastic nanofibrous materials from poly(polyol sebacate) (PPS), using core/shell electrospinning. The non-linearly elastic, soft nanofibrous materials can closely resemble any biological soft tissues in terms of mechanical properties and thus have a potential of wide applications in soft tissue engineering. The newly developed nanofibrous matrices were evaluated *in vitro* and ex vivo using mouse stem cells for the treatments of heart disease and Hirschsprung disease. Without any pre-conditioning treatments, the materials demonstrated excellent cytocompatibility in in-vitro evaluation using embryonic stem cells (ESC)-derived cardiomyocytes and neural crest stem cells (NCSC). The former provided beating cells surviving for 1 month until interrupted, the latter provided neurons in ganglion-like groups with connecting nerve fibres. More importantly, the mouse NCSC on the biomaterial matrix can, when the matrix is attached ex-vivo onto the surface of aneural colon, depart from the matrix carrier and relocate to the interior of the colonic wall in a layer where they would be expected to reside in normally innervated colon tissue. *In vivo* trials using a rat model are undergoing.

32.P49
New building blocks for thermoplastic polyurethane elastomers for soft tissue engineering

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Thermoplastic polyurethanes (TPUs) are particularly interesting in soft tissue engineering because of their good biocompatibility, elastomeric properties and the amenability to be processed from melt and solution. These benefits have already been exploited in producing narrow diameter artificial blood vessels with very good compliance. We have studied a series of ester based TPUs with good mechanical properties and slow rates of biodegradation. Controlling the rate of degradation to correlate with regrowth of native tissue has good promise in cardiovascular regenerative medicine and other areas of tissue engineering. Existing TPUs have been improved by replacing the aliphatic diisocyanate monomers with alicyclic diisocyanates and by the use of acid free chain extenders or softblocks. Alicyclic TPUs are expected to have comparable mechanical performance to commercially available aromatic TPUs with reduced toxicity from amine degradation products. In comparison to esters, acid free TPUs should provide slower degradability. Further it is expected that resulting material will undergo surface- instead of bulk erosion to maintain good mechanical properties. Bulk mechanical properties are measured and biodegradation is simulated in-vitro at elevated temperatures with and without enzymes. Possible application areas for these improved biodegradable TPUs include drug delivery systems, disposable medical tubing, textiles and show great promise in all areas of soft tissue engineering.

32.P50
The potential of human hair keratins as a biomaterial for tissue engineering and regenerative medicine (TERM) applications

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The potential of using keratin as a biomaterial has been discussed for a few decades. However it has largely been overlooked in comparison with clinically established extracellular matrix components such as collagen, fibrin and hyaluronic acid. Nonetheless keratin as a biomaterial has gained popularity in recent years, evident from the increase in the number of related publications. Primarily, keratin is an attractive natural biomaterial because (i) it is readily extracted from human hair or animal hooves, feathers etc., which are all abundant; (ii) it contains cell adhesion motifs (LDV) and; (iii) it is easily processed into various templates. In this report, we present our data on the extraction and characterisation of keratins from human hair and demonstrate the feasibility of fabricating keratin hydrogels, porous foams and fibrous meshes. Cell culture studies using mouse and human fibroblasts were conducted in keratin hydrogels to evaluate cell compatibility *in vitro*. Hydrogels and porous foams were implanted subcutaneously into black six mice to evaluate *in vivo* host response. Our results show that keratin hydrogels were capable of supporting fibroblast growth and development. After 7 days' implantation *in vivo*, keratin hydrogels and freeze-dried foams were found to support cell infiltration without significant host tissue reaction. In conclusion, we showed that keratin can be processed into various templates, with the potential to be used in TERM applications.

32.P51
A novel method to produce electrospun scaffolds with tailored geometries

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Introduction: Electrospinning is a technique widely used to fabricate fibrous scaffolds for tissue engineering applications. Experimental parameters can be adjusted to influence the morphological aspects of the scaffold. More recently the ability of collector plate design to effect fibre patterning during the electrospinning process has been investigated. In this study, we show the ability of patterned collectors, to generate electrospun scaffolds with tailored geometries, that influence cell adherence and migration.
Methods: Patterned collectors with different geometries (including hexagonal and sinusoidal) were designed and manufactured on a rapid prototyping system. Poly(lactide-co-glycolide) was electrospun onto the patterned collectors and the generated fibrous scaffold geometries were visualised by scanning electron microscopy. Electrospun PLGA disks (0.5 mm) were cut from the patterned scaffolds and seeded with mammalian cells. The seeded scaffolds were cultured *in vitro* for up to 4 days and imaged using a fluorescence microscope.
Results and conclusions: Resin based collectors with different geometries were successfully fabricated using rapid-prototyping. Electrospun scaffolds with patterns that resembled the designed collector geometries were produced. The patterning of the fibres were found to directly effect cell adhesion and migration, illustrating that this method can be used to produce scaffolds with directional cues to influence cell behaviour.

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32.P52
Chemically functionalised poly (ethylene) glycol monolayers as scaffolds for primary hepatocytes: cell targeting with galactosamine

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A scaffold capable of maintaining cultures of viable, metabolically active hepatocytes could provide a suitable system for assessment of new drug compounds; it is, however, difficult to maintain primary cultures. Synthetic materials with tuneable chemical properties offer a way to selectively target cell surface receptors which promote adhesion and function. Hepatocytes are capable of binding terminal galactose molecules via the asialoglycoprotein receptor (ASGPr); integrating the galactosamine molecule into biocompatible materials may provide a suitable scaffold for primary hepatocytes. Rat hepatocytes were cultured on poly (ethylene glycol) (PEG) coated glass surfaces, chemically modified to contain galactosamine, for up to 10 days. Viability and function was determined using a combination of live/dead fluorescent staining, crystal violet staining and immunostaining for hepatocyte nuclear factor (HNF-4-α), gap junctions (Connexin 32) and tight junctions (ZO-1). Cells cultured on galactosamine modified surfaces begin to show the development of spheroid structures after 4 days, however, cell numbers are greatly reduced. Immunostaining indicates that galactosamine surfaces maintain the cell phenotype for up to 10 days; staining positive for all three markers. By specifically targeting the ASGPr we have induced desirable changes in morphology and maintained the cell phenotype.

32.P53
Thermosensitive poly-caprolactone scaffolds for 3D myogenic and osteogenic differentiation of C2C12 cells

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For tissue engineering (TE) applications, the natural process of regeneration is imitated by using bioresorbable scaffolds that support cellular attachment, migration and proliferation, along with cells capable of differentiation upon exposure to inductive factors. Based on the idea of combining a fully degradable polymer (Poly(ϵ-caprolactone) with a thermoresponsive polymer (polyethylene glycol methacrylate) a scaffold was developed, which liquefies at 4 °C and solidifies at 37 °C. The mouse C2C12 cell line has the potential to differentiate towards myocyte as well as osteocyte lineage. In this study, the novel thermoresponsive material and C2C12 cells were combined to generate a myogenic or osteogenic TE construct, depending on the differentiation medium. The construct can be expanded under 3D conditions *in vitro*. The constructs were cultured in media supporting the myogenic or osteogenic differentiation of C2C12 cells for up to 7 days. First, biomaterial seeding for C2C12 cells was optimized and their attachment, survival, distribution and persistence within the 3D material were characterized. 3D differentiation of the cells was examined using qRT-PCR.C2C12 cells attached to the polymers were viable and evenly distributed in all scaffolds. Further, the thermoresponsive scaffold presented in this study was able to support myogenic as well as osteogenic differentiation of C2C12 cells.

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33. Regeneration and Inflammation - Biocompatibility Issues

33.01
Keynote: Orchestration of tissue repair by substance-P through timely control of inflammation and recruitment of reparative stem cells from the bone marrow

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Tissue injury may create a specific microenvironment, which brings up the systemic participation of reparative stem cells in the repair process. Previously, we identified a new role of substance-P (SP) as an injury-inducible messenger to mobilize bone marrow stromal cells, home to the injured tissue, and be engaged in the tissue repair. In addition to SP's BMSC mobilizer function, SP also mobilize endothelial precursor cells (EPC), which was distinct from that of G-CSF. In contrast to the strong inflammatory response of G-CSF, SP did not accompany inflammatory response in the injury model but suppressed the injury-stimulated inflammation response, which came earlier than its BMSC and EPC mobilization effect. We explored SP's dual positive roles in the orchestration of the tissue repair in a variety of the animal models such as the spinal cord injury, radiation-induced BM injury, and gastrointestinal injury. At the early stage of wound healing, anti-inflammatory effect of SP seems to play positive roles in the reduction of inflammation-induced secondary cell death, in the creation of favorable microenvironment for the engraftment of incoming stem cells, later, and finally in the facilitation of tissue repair.

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33.02
Macrophage phenotypes in the foreign body reaction in rats

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Implantation of biomaterials into the body elicits a material-dependent inflammatory response called the foreign body reaction (FBR). During this inflammatory response, macrophages play a pivotal role. They orchestrate the pro-inflammatory microenvironment in and around the biomaterials by secreting cytokines, chemokines and growth factors. Furthermore they are able to regulate degradation of the biomaterial by secreting proteolytic enzymes or by phagocytosis. We hypothesize that macrophages present in different microenvironments in the FBR have different phenotypes. To investigate this hypothesis we developed and validated immunohistochemical phenotype-specific markers for rat macrophages *in vitro*, and applied them in our standard model of the FBR. Bone marrow-derived macrophages were cultured and stimulated with IFNγ and LPS, IL4 and IL13, or IL4 and dexamethasone. After stimulation with IFNγ and LPS, M1-specific genes were upregulated. M2-specific genes were upregulated after stimulation with IL4 and dexamethasone, but only slightly after stimulation with IL4 and IL13. Western blot and IHC data revealed that on protein level, iNOS and CD206 are specifically expressed by M1 and M2 respectively. Using these markers on samples of the FBR, we found that a proportion of the macrophages display an M2 phenotype, whereas the M1-phenotype was not detected. Our data suggest that many macrophages in our model of the FBR do not fit into the classical 'M1 or M2' dichotomy.

33.03
In vivo characterisation of the inflammatory kinetics of biologic and synthetic abdominal wall repair biomaterials

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In vivo biomaterials are interrogated by the inflammatory response, with the goal of returning compromised tissue to normal homeostasis. In this study abdominal wall repair biomaterials were implanted in rat models to conclude their *in vivo* inflammatory dynamics.Materials: Synthetics; polypropylene (PP), polyester terephthalate (PET) & polyglycolic acid (PGA) varying in polymer composition & fibre conformation. Biologics: human & porcine dermis & small intestinal submucosa (SIS) varying in decellularisation & cross linking chemistry. Implants delivered into 6 week old, male wistar rats. SC, each animal received four implants adjacent to dorso-lumbar musculature. IP; 2×3 cm full thickness of abdominal wall excised & replaced using a 3.5×2.5 cm prostesis secured inlay using 6–10 resorbable PGA sutures. *In vivo* synthetics demonstrated more extensive cell colonisation than biologics. However, the synthetic materials all displayed similar inflammatory properties despite their polymer composition & weave. Biologic materials showed more substantial differences in host response as a function of tissue origin & fabrication chemistry. SIS was considerably more inflammatory than dermis, characterised by an extensive & prolonged neutrophil response. Additionally SDS used in decellularisation was also shown to heighten the inflammatory nature of dermal materials. Cross linking using HDMI did not modify host response.

33.04
Local macrophage polarization and tissue remodeling following bilateral implantation of ECM scaffold materials in the abdominal wall

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Polarization of macrophages towards an M1 (pro-inflammatory) or an M2 (anti-inflammatory, regulatory) phenotype has been shown to be an important modulator of disease and tissue remodeling outcomes. The present study investigated the local macrophage response following implantation of autologous tissue (AT), non-crosslinked extracellular matrix (NC-ECM) scaffolds, or crosslinked ECM (X-ECM) scaffolds in the abdominal wall of a rat. Implantation of NC-ECM resulted in constructive remodeling, while implantation of X-ECM or AT resulted in encapsulation or scar tissue formation, respectively. The macrophage population interacting with the NC-ECM was shown to possess more M2-like characteristics than the AT or X-ECM implant groups as early as 7 days post-implantation. No differences in remodeling or macrophage phenotype were observed when a second material was implanted adjacent to the first in the same animal. *In vitro* testing showed that M2 macrophages promote chemotaxis of a cell populations known to par-

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ticipate in the constructive remodeling of skeletal muscle tissue. Additional work suggests that local differences in macrophage polarization may be due, in part, to distinct surface ligands present upon scaffold materials at implant as well as matricryptic peptides which are released during scaffold remodeling. These results suggest that tissue remodeling outcomes are highly dependent upon local host macrophage-biomaterial interactions at the site of implantation.

33.05
Inflammatory reaction of macrophages to a contaminated environment depends on the biomaterial

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Macrophages are key immune cells in the foreign body reaction to a biomaterial after implantation. In a contaminated environment, integration of the biomaterial in the body is delayed. To examine this reaction in more detail, we investigated the inflammatory reaction of macrophages interacting with different biomaterials to a contaminated environment simulated *in vitro*. Monocytes isolated from the blood of healthy donors with Ficoll separation and magnetic cell sorting were seeded on Permacol™, Parietex™ Composite, polyethylene terephthalate (PET) and polypropylene (PP) meshes. After seeding, monocytes were cultured in X-vivo 15 with or without LPS and IFNγ for 3 days. Monocytes were analysed for protein production of TNFα, an inflammatory marker and CCL18, a wound healing marker. Protein production was corrected for DNA. In the presence of LPS and IFNγ, monocytes on Parietex™ Composite produced 2.4 times more TNFα than without the inflammatory stimulus; on Permacol™, 4.8 times more TNFα; on PET mesh, 6.8 times more; on PP mesh, 4.9 times more. On none of the seeded biomaterials, monocytes produced CCL18. The reaction of macrophages to LPS and IFNγ depends on the type of biomaterial they interact with. This indicates: (i) monocytes under these conditions acquire a more inflammatory phenotype, which confirms results from *in vivo* experiments; (ii) this *in vitro* system may be used to discriminate and predict the behavior of new biomaterials.

33.06
The resolution of inflammation during the regeneration of biological scaffolds by human tissue

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Implantation of natural biological scaffolds into animals has shown recellularisation of the material by host cells and formation of organised tissue. The biological process of tissue integration and factors that determine outcome are poorly understood. The aim of this study was to investigate the interactions at the interface of a natural biomaterial and human stromal tissue to gain insight into the early cellular events entrained following implantation.A novel organotypic culture system was developed in which decellularised porcine bladder matrix was maintained in close apposition to fresh, surgically-excised human stromal tissue. Histological examination of the tissue composites showed an ($p < 0.05$) increase in cells expressing the macrophage scavenger receptor, CD163, at the biological scaffold-tissue interface over 11 days. Primary human monocyte-derived macrophages treated with the glucocorticoid receptor (GR) agonist, dexamethasone or the anti-inflammatory cytokine IL-10 showed increased expression of CD163. Immunolabelling for GR in the construct showed intense nuclear GR expression in the cells at the biological scaffold-tissue interface throughout the culture period. The presence of $CD163^+$ macrophages at a tissue-biological scaffold interface infers an inflammation-resolving response which may be regulated by local factors in the wound environment. Understanding these interactions will reveal new directions for enhancing biomaterial integration into human tissue.

33.P01
In vitro investigation of cytotoxicity and antimicrobial activity of silver/alginate nanocomposite microbeads

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Biomaterials are often used as carriers or matrixes in different medical applications, which usually demand the use of multifunctional materials meeting the requirements of their purpose without being harmful to the surrounding tissue. Here, silver/alginate nanocomposite microbeads were obtained using electrochemical synthesis of silver nanoparticles in alginate solution at constant current density of 50 mA/cm^{-2}, followed by electrostatic extrusion of synthesized Ag/alginate colloid solution. *In vitro* determination of Ag/alginate microbeads cytotoxicity against immunocompetent peripheral blood mononuclear cells (PBMC) was performed by MTT test. In order to investigate the antimicrobial activity against E. coli and S. aureus using the test in suspension, Ag/alginate microbeads were synthesized so to have Ag concentration equal to that without cytotoxic effects against PBMC. The presence of Ag/alginate microbeads having 1 mM AgNO3 in the initial solution for synthesis and less decreased the PBMC survival up to 59.04 ± 35.45%. Higher concentrations of nanosilver in Ag/alginate microbeads induced pronounced decrease in PBMC survival. Ag/alginate microbeads obtained from initial Ag/alginate colloid solution with 1 mM AgNO3 significantly decreased the bacterial colonies count of E. coli and S. aureus comparing to the control sample for 2 and 3 orders of magnitude, respectively. This work has shown potentials for biomedical applications of Ag/alginate nanocomposite microbeads.

33.P02
Biocompatibility and haemocompatibility of self-assembling peptides

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A deepening of understanding of molecular self-assembly and the ability to easily control material properties and add functional groups makes self-assembling peptides appealing materials for tissue engineering applications. The aim of this work was to explore the biocompatibility of self-assembling peptides to develop anti-thrombogenic coatings and to develop design characteristics to tailor peptides to specific applications. A range of peptides developed at the University of Leeds, homo-polypeptides and peptides from the literature were used to represent different design characteristics in order to develop design criteria for biocompatibility and haemocompatibility. Biocompatibility was assessed using ISO standard cytotoxicity tests and tests to determine effects on complement activation. Peptides were assessed for thrombogenicity using the Chandler loop model with sheep blood and haemolytic activity was also assessed using sheep blood. These results showed

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the potential of some peptides to function in contact with blood with little detrimental effect. It was possible to identify design characteristics based on peptide charge and size that affected both biocompatibility and haemocompatibility. Whilst simplistic in nature this study has demonstrated a range of potential applications for self-assembling peptides not only in tissue engineering but in other biomedical applications.

33.P03
Comprehensive immunological assessment of xenogeneic collagens and elastins - a prerequisite for novel scaffold design

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Immunogenicity of extracellular matrix proteins (ECMp) used in biomimetic scaffolds might determine scaffold acceptance. In this study, innate and adaptive immune responses were examined in detail for collagen I and elastin. Porcine, bovine or human collagen I and elastin were co-cultured with human PBMC or purified T or B cells. Proliferation and surface marker expression was analyzed by flow cytometry. Supernatants of all cultures were screened for their cytokine profile. Additionally, maturation of immature dendritic cells (DC) by ECMp was tested. Complement activation was measured by C5a release.We could show that bovine elastin inhibited the proliferation of T cells, whereas porcine elastin induced a slight proliferation response when compared to the control. All other proteins tested did not alter either proliferation or $CD25^+$ T cell activation relative to the control. Focusing on B cells, all ECMp analyzed did not induce proliferation, differentiation or enhanced IL-6 secretion. Additionally, xenogeneic and human collagen I or elastin did not result in the maturation of immature DC measured as upregulation of CD83 and TNFα secretion. The release of C5a was also not induced by the ECMp. Porcine and bovine collagen I and bovine elastin have low immunogenicity and should be favoured as constituents for the design of novel scaffolds to avoid undesired immune and inflammatory responses.

33.P04
A non-thrombogenic, anti-biofilm surface for medical devices

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Indwelling and implanted medical devices are associated with high rates of thrombosis and infection due to biofilm formation. These complications are extremely detrimental to patient outcomes, often fatal, and represent an enormous economic burden on the healthcare system. Our lab has developed Slippery Liquid Infused Porous Surfaces (SLIPS) recently published in Nature (Wong et al. 2011). This surface repels liquids, cells and pathogens for use as a non-thrombogenic or non-fouling material for a wide range of medical devices. SLIPS repelled whole human blood compared to commercially available medical grade materials. Fibrinogen binding and polymerization was greatly reduced on SLIPS over 2 h compared to 316 l stainless steel and polysulfone. Whole human blood was pumped in a SLIPS loop for 1 h at a flow rate of 1.5 l/h without coagulation or macroscopic thrombus formation. Furthermore, SLIPS tubing exhibits reduced Pseudomonas biofilm formation by 70% over 3 days of culture at 15 ml/h. Current indwelling and implanted medical devices rely on anticoagulant or antiplatelet medication to prevent thrombosis. An additional detrimental complication is bacterial colonization and biofilm formation. Adequate treatment of implant related infections often requires antibiotics and implant removal causing prolonged hospitalization. SLIPS technology could combat these two major complications simultaneously, eliminating the need for extensive medication and reducing patient risks.

33.P05
Nanofibers as scaffolds for stem cells and immunosuppressive drug in regenerative medicine

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Introduction: Cell therapy based on allogeneic stem cell transfer requires a suppression of deleterious immune reaction against foreign cells. To overcome this problem, we prepared nanofibers loaded with an immunosuppressive drug cyclosporin A (CsA) and which can simultaneously serve as a scaffold for stem cell transfer.
Methods: Nanofibers were prepared by original needleless electrospun technology from polymer poly-L(lactid) or polyamid 6/12, loaded with 10 weight % of CsA. The kinetics of CsA release from nanofibers was determined *in vitro* and *in vivo*. The growth of mouse mesenchymal stem cells and limbal stem cells on CsA-free and CsA-loaded nanofibers was characterized *in vitro*. CsA-free and CsA-loaded nanofibers alone or stem cell-seeded were transferred on wounded skin or damaged corneal epithelium and the healing process was monitored *in vivo* and *in vitro*.
Results: CsA had an optimal kinetic of release from nanofibers *in vitro* and *in vivo*. Stem cells grew comparably on CsA-free and CsA-loaded nanofibers. Covering of the imflammatory sides with CsA-loaded nanofibers simultaneously seeded with stem cells inhibited a local imflammatory reaction and supported healing process.
Conclusion: CsA-loaded electrospun nanofibers can be used as scaffolds cell transfer and for simultaneous local immunosupression in cell-based therapy and regenerative medicine.

33.P06
Platelet lysate increases the expression of factors related to inflammation and the activation of NF-kB in mouse MSC *in vitro*

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Using an ectopic bone formation model in which MSC were implanted in association with suitable ceramic scaffolds *in vivo*, we previously demonstrated that bFGF selected mouse MSC are able to activate host responses and to trigger tissue regeneration. Our *in vivo* studies supported the idea that at injury site MSC secrete a wide spectrum of bioactive molecoles establishing a regenerative microenvironment that sustains tissue repair. In order to gain understanding in the MSC molecular pattern of expression affected by the injury milieu, we attempted to reproduce *in vitro* the stimula that act on MSC cells at the damaged site and we compared the expression of factors and the pathways activated in bFGF selected MSC cultured for 24 h with or without platelet lysate (PL) in the presence or absence of the inflammatory stimulus IL-1. In murine Bone Marrow derived MSC an increased expression of IL-6 was observed together with a strong increase of NF-kB in IL-1 treated cells stimulated with PL .We also observed that, in PL or IL-1 treated

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MSC, COX-2 and mPGE2 synthase were induced and that the expression was overinduced in cells stimulated with PL in inflammatory condition. PGE2 production was also increased indicating an activity of the induced COX-2 and mPGE2 synthase. These results indicate that PL induces in cultured mouse MSC an early pro-inflammatory response involving NF-kB activation, IL-6 secretion, COX-2 induction and PGE2 production.

33.P07
Hemocompatibility study of a bacterial cellulose/polyvinyl alcohol nanocomposite

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Cardiovascular disease is among the leading causes of death in the world. Grafts are usually used to treat these diseases by redirecting blood flow around occluded vessels. We previously showed bacterial cellulose (BC) is a suitable artificial alternative to commonly used autologous grafts. We found that the addition of polyvinyl alcohol (PVA) improves the mechanical properties of BC. For cardiovascular applications, hemocompatibility needs to be characterized. Here, we characterize the blood/material interaction of a BC/PVA nanocomposite to assure its hemocompatibility. Healthy donors' blood was placed in contact with BC, BC/PVA and ePTFE and parameters related to the hemocompatibility (whole blood coagulation time, plasma recalcification profiles, Factor XII activation, hemolysis and platelet activation and adhesion) determined. The results demonstrated good hemocompatibility of BC and BC/PVA when compared to ePTFE. BC and to a greater extent BC/PVA are shown to induce minimal activation of the coagulation cascade and therefore minimal thrombogenic activity. Overall, our data consistently shows that the addition of PVA further improves on the previously reported good hemocompatibility of BC. The results are on par with the industry standard ePTFE and therefore demonstrate that BC/PVA has potential application as a graft material. Work funded by FCT project PTDC/EBB-EBI/112170/2009. AFL and JPS are supported by FCT grants SFRH/BD/66094/2009 and SFRH/BPD/64958/2009.

33.P08
The behavior of fibroblasts is influenced by the paracrine signaling of M1 or M2 polarized macrophages

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Macrophages and fibroblasts are two major players in tissue repair and fibrosis. Despite the relevance of macrophages and fibroblasts in tissue homeostasis, remarkably little is known whether macrophages are able to influence the properties of fibroblasts. Here we investigated the role of paracrine factors secreted by human M1 (classically activated) and M2 (alternatively activated) macrophages on human dermal fibroblasts. Dermal fibroblasts stimulated with paracrine factors from M1 macrophages secreted inflammatory cytokines and upregulated matrix metalloproteinases. This indicates that factors produced by M1 macrophages induce a pro-inflammatory fibroblast and that these fibroblasts are able to degrade collagens. Dermal fibroblasts that are stimulated with paracrine factors secreted by M2 macrophages did not show specific myofibroblast markers but induced collagen production and proliferation of the fibroblasts. When dermal fibroblasts were stimulated with factors secreted by M1 macrophages followed by paracrine factors secreted by M2 macrophages, the induced pro-inflammatory fibroblast down-regulated the inflammatory markers and MMPs and upregulated their collagen production. In conclusion, paracrine factors of different polarized macrophages induced two phenotypes of dermal fibroblasts and the dermal fibroblasts show a dynamic plasticity, which can be influenced by the microenvironment.

33.P09
Physicochemical characterizations of drug-eluting PLGA nanofiber membranes and *in vivo* evaluation of their efficiency as anti-adhesion barriers

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Epigallocatechin-3-O-gallate (EGCG), a main polyphenolic component of green tea, has a wide range of pharmacological activities including antioxidant, anti-proliferative, anti-inflammatory, and anti-thrombotic effects. In this study, nanofiber poly(lactic-co-glycolic acid) (PLGA) membranes eluting EGCG were prepared via electrospinning and then characterized to evaluate their application potential to tissue-adhesion barriers. EGCG-eluting PLGA (E-PLGA) nanofiber membranes were electrospun from admixture of PLGA and EGCG. The average diameter of E-PLGA nanofibers was approximately 400 nm which was similar to that of pure PLGA fibers, but a new surface bonding was formed in hybrid nanofibers which made the surface of the fiber coarse. EGCG moderately increased the hydrophilicity of PLGA and uniformly dispersed into E-PLGA membranes showing sustained release patterns by controlled diffusion and PLGA degradation. The attachment and proliferation of L-929 fibroblastic cells were significantly suppressed on E-PLGA membranes. The anti-adhesion efficiency of E-PLGA nanofiber membranes was evaluated on the intraperitoneal adhesion model in rat. After 2 weeks of surgical treatment, tissue adhesion score of E-PLGA nanofiber membranes was noticeably lower than that of non-treated and similar with other commercialized tissue-adhesion barriers. In conclusion, the E-PLGA membranes may be exploited to craft strategies for the prevention of post-surgical adhesion formation.

33.P10
Matricryptic peptides as determinants of macrophage phenotype

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Several different phenotypes of macrophages with functionally distinct, often opposing, roles in wound repair have been identified. There are M1 macrophages which migrate toward and phagocytose pathogens and cellular debris and are considered to be pro-inflammatory in nature. There are also M2 macrophages with multiple subtypes whose roles are more tissue reconstructive and have anti-inflammatory effects. The cell signaling that triggers polarization into pro-inflammatory versus anti-inflammatory macrophages is complex and not completely understood. Recent studies have shown that biologic scaffolds composed of extracellular matrix, when implanted *in vivo*, elicit a phenotype shift toward M2 macrophages. This shift is associated with integration of the scaffold with host tissues and minimized scarring and fibrosis. In this *in vitro* study we attempt to identify the specific cues in ECM that guide macrophage polarization. Human-derived monocytes were cultured in the presence of four unique cryptic peptides derived from parent molecules within the ECM. The resulting populations of macrophages were classified into M1 or M2 using cell

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surface markers, Western blot protein analysis, transcription factor assays, and evaluation of secretory products. Determinants of macrophage polarization have the potential to prospectively direct the host response to a variety of biomaterials.

33.P11
Adhesion and differentiation of cells on dynamic hydrophilic surfaces

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Living cells are exposed to a variety of environmental conditions while being tightly associated with the diverse extracellular matrices (ECMs) in the body. They proliferate or differentiate into functioning cells with sensing the physical and biological properties of the ECMs. These phenomena must occur at the interface of the artificial biomaterials and a variety of the cells such as platelets, leucocytes, various somatic cells, or stem cells. Recently the elastic feature of the biomaterials is attracting great attention in the stem cell differentiation lineage. We are here focusing on the dynamic surface which possesses the mobile substrates under the aqueous condition. Surfaces with different molecular architectures were prepared by poly(ethylene glycol) (PEG) and polyrotaxanes (PRX) containing α-cyclodextrin (α-CD). PEG or PRX were immobilized on the material substrate via single and double bonding resulting in graft-type or loop-type architectures. Human platelets and endothelial cells adhered on loop surfaces with larger interaction than the graft surfaces. In addition, not only the adherent number but also the cell activation was greatly affected by their mobile feature. Moreover the mobile surface surprisingly improved the cardiac differentiation of a stem cell with self beating function. The relationship between these bioactivities and the mobile feature defined as a novel 'Mobile factor' will be discussed.

33.P12
Universal reference systems for evaluating three-dimensional cell cultures on candidate scaffolds for bone tissue engineering

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A fast development of various candidate scaffolds with complex structures raises a need for universal reference systems for preliminary *in vitro* evaluation of their potency in bone tissue engineering. Due to the high number of new scaffolds, comparison of only few in one experimental setting does not facilitate an objective selection for further studies. The aim was to establish reference systems for cell cultures on candidate scaffolds of various forms. The systems should be applicable for proper selection of the most appreciated scaffolds for bone regeneration both in dynamic and static culture conditions *in vitro*. For the purpose two control scaffolds were produced - highly-porous alumina and microfibrous polystyrene scaffolds. Human bone derived cell (HBDCs) or MG-63 cells were cultured on the scaffolds in static and dynamic (Spinner Basket) conditions. Undisturbed cell growth within the both 3D structures was shown by quantitative (DNA content, XTT assay) and qualitative assays (fluorescent microscopy). Osteoblastic phenotype was maintained on the scaffolds, which was confirmed by alkaline phosphatase activity, osteocalcin and collagen type I synthesis and expression of the bone matrix proteins (real-time PCR). Cell cultures on several ceramic and polymer candidate scaffolds in porous or fibrous forms have already been compared by us with those of the proposed reference systems. Comparable results enabled an objective selection of the proper scaffolds for further studies

33.P13
Recruitment of macrophages and formation of foreign body giant cells following 3D porous chitosan scaffolds implantation

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The implantation of a biomaterial causes injury that will lead to the onset of acute inflammation. Inflammation is a prerequisite for efficient tissue repair, but successful repair after injury requires resolution of the inflammatory response. The persistence of an inflammatory stimulus causes chronic inflammation, a hallmark of the non-healing wound. Macrophages are suggested to be the ones that orchestrate the inflammatory and repair phase of tissue healing around implants. The subcutaneous inflammatory air-pouch model was used to analyze the inflammatory reaction to 3D porous chitosan (Ch) scaffolds with different degrees of acetylation (DA) with a particular focus on macrophages. The scaffolds were implanted for different time points up to 10 weeks. The presence of inflammatory cells particularly macrophages and the formation of foreign body giant cells within the scaffolds was studied by histology and immunohistochemistry. The biological response to Ch s was influenced by the DA. Our data shows that Ch with higher DA induce a more intense inflammatory response with high numbers of recruited inflammatory cells when compared with Ch with lower DA. In contrast, high numbers of foreign body giant cells were found on Ch scaffolds with the lower DA. Work financed by FEDER funds through the Programa Operacional Factores de Competitividade – COMPETE and Portuguese funds through FCT – Fundação para a Ciência e Tecnologia in the framework of project PTDC/SAU-BMA/113030.

33.P14
Characterization of tissue after implantation of biomaterials covered by stem cells in subcutis of experimental animals

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Introduction: Mesenchymal stem cells (MSC) influence the tissue regeneration. Still there are unclear questions about the MSC/biomaterial raised tissue changes around the implant. Our aim was detection of soft tissue changes in subcutis of experimental animals after covered by MSC different type biomaterial.
Material and methods: Material was obtained from six rabbit spine subcutis 8 weeks after the hydroxyapatite (HAp) granuli and tablets seeded by MSC implantation. The MSC were obtained from the same rabbit. The right side of each animal was implantation place, but the left side was a control. Tissues were processed for detection of TNFalfa, NFkBp105, Hsp70, FGF1R, VEGF and apoptosis. Results demonstrated perivascular inflammation only in controls. Numerous connective tissue cells of MSC/biomaterial side showed NFkBp105 while this factor decreased in controls. Number of Hsp70-cells was smaller in the experi-

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mental side. Apoptosis more affected experimental side connective tissue and blood vessels. TNFalfa was absent, but number of FGF1R immunoreactive structures was similar in control and around MSC/biomaterial. Number of VEGF positive endothelial cells varied in MSC/biomaterial side, but showed stable numerous numbers in controls.
Conclusions: Soft tissue around MSC/biomaterial implants are characterized by decreased cellular stress, selective apoptosis and stimulation of cellular proliferation. Type of HAp material doesn't play a role in expression of tissue factors.

33.P15
Characterization of biocompatibility after different biomaterial implantation in bone of experimental animals

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Introduction: Reactogenicity is inherent property of a biomaterial to induce a local reaction after implantation. Bioactivity characterises a specific local reaction induced by biomaterial. We researched on specific biocompatibility changes in bone of experimental animals in case of different biomaterial implantation.
Material and methods: Material was obtained from six rabbits in lower jaw and right and left tibia 3 months after implantation of HAp, HAp covered by polimethylmetacrylate cement (PCL), commercial BiometBone Cement R, ABC-G3A PMMA, Ca phosphate cement (pH6, pH7) implants. Control was obtained from animals without any biomaterial implantation. Tissues were proceeded for detection of BMP2/4, osteoprotegerin (OPG), osteopontin (OP), osteocalcin (OC), and defensin 2. Results demonstrated abundant expression of all factors in bones of HAp implantation. Bone after HAp plus PCL implantation showed notable decrease of all factors, except OP. BiometBone Cement R raised decrease of BMP2/4 and defensin expression while OPG and bone proteins have elevated. ABC-G3A PMMA implants increased only bone proteins. Ca phosphate cement (pH6) increased only OPG-containing structures. Ca phosphate cement (pH7) elevated BMP2/4 positive structures.
Conclusions: The best biocompatibility is between jaw tissue and noncovered Hap. For other bioceramic materials that differ from the HAp characteristic is selective stimulation of distinct molecular events in bone after their implantation.

33.P16
Decellularized and sterilized xenogeneic tissues inhibit complement and neutrophil activation: *In vitro* investigation using a novel blood-compatibility screening system

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Aims: This study aims to develop a novel in-vitro blood-compatibility screening system which can maintain blood conditions, and to assess complement and neutrophil activation to decellularized and sterilized xenogeneic tissue.
Methods: We developed an airless chamber to incubate blood with tissue. Supply of O_2 and elimination of CO_2 were realized by using a gas-preamble silicone tube. The blood-filled chamber was rotated at 40 rpm in a 37 °C box. Heparinized and glucose-supplemented venous blood of healthy volunteers was used. This study was approved by the ethical committee of Waseda University. Complement and neutrophil activation to fresh bovine pericardium rinsed with antibiotics and antimycotics, decellularized and ethylene-oxide-gas sterilized pericardium, and glutaraldehyde-treated bovine pericardium (commercial product) were compared after 5 h' incubation ($n = 6$).
Results: Blood pH and O_2 concentration were successfully maintained within physiological ranges by regulating CO_2 concentration outside the chamber at 2%. ELISA assay of C3a and lactoferrin showed that complement and neutrophil activation of decellularized and sterilized bovine pericardium were markedly suppressed as compared with those of the fresh pericardium and comparable to those of glutaraldehyde-treated pericardium.
Conclusions: The in-vitro blood-compatibility screening tests demonstrated that decellularization and sterilization of bovine pericardium inhibited complement and neutrophil activation.

33.P17
Remeseothelialization after application of barrier materials for peritoneal adhesion prevention – a scanning electron microscopical study with insights for tissue engineering solutions

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Peritoneal adhesions remain a relevant clinical problem. Mesothelial cells play a decisive role in healing either with or without adhesion formation. In this study the quality and quantity of remesothelialization after application of five commercial adhesion barriers in an animal model was semiquantitatively analysed using scanning electron microscopy (SEM). After serosal wounding Wistar rats were either treated with Adept®, Intercoat®, Spraygel®, Seprafilm® and SupraSealel® or remained untreated. After 14 days the treated areas were explanted and morphological analysed by SEM. The surface morphology, the amount and morphology of covering cells were analysed by evaluating 100 random high power fields of x500 in each specimen. The barrier groups distinguished in quantitiy and quality of the cells colonizing the surface. The highest amount of flat mature mesothelial cells correlates with the highest antiadhesive effect in the SupraSeal®-group. The mesothelial cells showed either normal or activated phenotype in dependence on the underlying biomaterial. These experiments showed a useful insight of SEM-analyses for the performance of barrier materials. With respect to the anti-adhesive efficiency and biocompatibility, rapid remesotheliazation is of crucial importance. These findings open innovative aspects for potential Tissue Engineering approaches in adhesion prevention.

33.P18
Cytotoxicity of poly(propylene fumarate) per ISO 10993-5

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The clinical success of any biomaterial is significantly based on its lack of cytotoxicity. Poly(propylene fumarate) (PPF) is a biodegradable polymer that has been well characterized for use in bone tissue engineering; however, there has not been any extensive studies on PPF cytotoxicity. This work evaluated the cytotoxicity of PPF per ISO Standard 10993-5, though direct cell culture, indirect cell culture and cell culture with PPF extractant. PPF was synthesized and made into films as previously described. Cell (hMSCs, fibroblasts, primary osteoblasts and cMSCs) viability was evaluated by cell count, with trypan blue, and live/dead fluorescent imaging, with ethidium homodimer and calcein AM. Sol fraction was also determined to ensure that cytotoxicity results were based on a fully crosslinked film. Cell viability after incuba-

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tion with the extractant media was found to be statistically similar ($p < 0.05$) between PPF (99% ± 4%), the negative control (high density polyethylene) (89% ± 9%) and blank media (91% ± 9%) compared to the positive control (0% ± 0%). Fluorescent and microscopic imaging of the cells showed no change from normal morphology in the PPF, HDPE or blank media samples. Results indicate that hMSC population when directly or indirectly cultured with PPF remains viable. This study highlights the importance of evaluating cytotoxicity during biomaterial development using approved standards such as ISO 10993-5.

33.P19
ECM hydrogels as coating materials to modulate the host response to synthetic mesh

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Non-degradable synthetic surgical mesh materials are commonly used for ventral hernia repair. Though most synthetic meshes provide superior mechanical support, they are associated with complications, including recurrence, infection, and a foreign body inflammatory reaction. Biologic scaffolds isolated from decellularized tissues are typically fully degraded *in vivo* and support site appropriate constructive tissue remodeling. Therefore, an ECM coating on a mesh material may logically improve biocompatibility. In the present study, different ECM coatings derived from porcine dermal ECM (D-ECM) and urinary bladder (UBM) were evaluated for their ability to modify the host response to polypropylene mesh. The coated and uncoated meshes were implanted in a partial thickness rat abdominal wall defect for 3–120 days. Host cells rapidly infiltrated the ECM coatings and UBM coatings degraded more quickly than the D-ECM coatings. All ECM coatings decreased the inflammatory response to mesh fibers compared to the uncoated mesh between days 3–35. By 120 days, the associated interfiber connective tissue type differed between ECM coated and uncoated polypropylene implants. ECM coated mesh showed a greater amount of adipose tissue than the uncoated polypropylene mesh, which was almost entirely fibrous in nature. We conclude that an ECM hydrogel coating can modify the host response to a polypropylene mesh and change the downstream remodeling response.

33.P20
Understanding protein adsorption hysteresis for the immobilization of proteins on scaffolds for tissue engineering

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Proteins can be immobilized on surfaces either covalently or non-covalently. Non-covalent protein adsorption is measured as an adsorption isotherm, in which the surface concentration of the protein, {protein}s e.g. in $\mu g/cm^2$, increases as a function of the equilibrium protein concentration, [protein]eq in mol/l. Conversely, decreasing a finite surface concentration, {protein}s, as a function of decreasing protein concentrations, [protein]eq, results in a desorption isotherm. If the desorption isotherm does not retrace the adsorption isotherm, a loop is formed, which is called adsorption hysteresis [1]. This is a result of a thermodynamically irreversible process and the generation of entropy <delta iS> e.g. by a conformational change of the protein on the surface. As a consequence of protein adsorption hysteresis the parameters derived during the adsorption process are no longer valid after adsorption but are then governed by the parameters of the desorption process - often unnoticed. From the hysteresis data of BMP-2 and Fibrinogen [2] on quartz glass and titanium it will be shown that the binding constants (Ka) increase by magnitudes in passing from adsorption to desorption, finalizing at 10^9 - $10^{\wedge 13}$ M^{-1}, with apparent molar Gibbs free energies of the irreversible process <delta iG> at -9 to -40 kJ Mol^{-1}. [1] Hlady, V., Buijs, J., & Jennissen, H.P. (1999) Methods Enzymol., 309, 402–429. [2] Dohle, D.-S., Pasa, S.D., & Jennissen, H.P. (2010) Bionanomaterials, 11 (S1).

33.P21
Localization and characterization of enteric neural progenitor cells

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Introduction: Human myenteric plexus regeneration in the enteric nervous system is a proven fact, but it is still not known the process of regeneration of these nervous elements (neurons and glia). What are the cells that act as neural progenitors in this system? It has been recently reported that inside the ganglia, cells divisions only take place in glial cells. (Azan G et al 2011; Heanue TA et al 2010) Hypothesis: Currently, there are two hypotheses about the localization of these cells. Firstly, it is proposed that progenitor cells are located inside the ganglia. These cells would come from glial cells. On the other hand it is suggested that stem cells would be located outside the ganglia.
Results: Using proliferation cell markers (BrdU, Ki-67), we have found that dividing cells are located around the ganglias. To characterize these cells, we have proceeded to culture isolated gut cells from adult healthy mice, with and without mucosa. In the present study the different cell morphologies have been evaluated and also the specific responses of these progenitor cells to specific antibodies. We have studied the expression of Nestin, GFAP, Neu N and actin protein in the isolated cells. It suggested to discuss this population behaviour by a FACS study of neural progenitors specific markers as c-Kit, p75, Nestin and GFAP.

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34. Multi-scale and Hierarchical Scaffolds and Self Assembly Systems (in coop. Expertissues)

34.01
Keynote: Multi-scale scaffolds for tissue engineering

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A critical issue in the general area of Advanced Therapies is the need for temporary scaffolds to regenerate tissue defects. The scaffolds should be specifically designed to create environments that promote tissue development and not merely to support the maintenance of communities of cells. To achieve that goal, highly functional scaffolds may combine specific morphologies at different scales and dimensions capturing characteristic features and functionalities of the extra-cellular matrix. Many biomaterials have been proposed to produce scaffolds. We have a particular interest in developing systems based in biodegradable polymers. Those demanding applications require a combination of mechanical properties, processability, cell-friendly surfaces and tunable biodegradability that are specific for the application. Electrospinning is a versatile technique used to produce synthetic polymeric ultrafine fibers. It enables the production of non-woven meshes with fiber diameters in the nanometer range with high surface area-to-volume ratio and high porosity. Those meshes can mimic some aspects of the extracellular matrix of human tissues and be used as scaffolds for tissue engineering applications. We developed such structures to obtain the release of bioactive agents intended to direct and control stem cell differentiation. This talk will review our latest developments for bone and cartilage tissue engineering of scaffolds having microstructures at different scales and dimensions.

34.02
Magnetic 3D assembly of microgels for tissue engineering and regenerative medicine

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Bottom-up tissue engineering methods aim to generate complex tissue structures by assembling building blocks, such as cell encapsulating microscale hydrogels (i.e., microgels). Recently, assembly of microgels into complex 3D constructs has been performed via emerging non-invasive fields, e.g., acoustics, leveraging biomimetic properties of composite materials through micro and nanoscale technologies. Platforms driven by magnetics have been employed in several applications including direct cellular manipulation, sorting cells, 3D cell culture, local hyperthermia therapy, magnetic levitation of organic liquid droplets and clinical imaging applications. For instance, magnetic nanoparticles (MNPs) were loaded to cell-encapsulating microscale hydrogels (M-gels) creating composite smart materials responsive to magnetic fields. However, MNPs in M-gels can interact with encapsulated cells affecting their viability and functionality. Although applications of M-gels are interesting, successful release of magnetic nanoparticles from these biomimetic composite materials has to be proven for successful applications such as tissue engineering. Hence, novel approaches are needed to assemble cell-laden microgels without nanoparticles (MNP-free microgels). Here, we present for the first time both experimentally and theoretically assembly of microscale hydrogels exploiting the paramagnetic properties of hydrogels without using magnetic nanoparticles.

34.03
Therapeutic effects of bioactive peptides on ischemic hind limb by recruiting mesenchymal stem cells

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Introduction: Here we developed bioactive self-assembling peptide nanofiber hydrogel (RADA-SP) which could recruit mesenchymal stem cells. The self-assembling peptide, RADAII-16, forms fibers (5–10 nm) and assembles into a 3D scaffold at physiological solution. Substance P (SP) is an injury-inducible factor that acts early in the wound healing process to induce $CD29^+$ stromal-like cell mobilization. By applying these bioactive peptides on ischemic hind limb models, the abilities of recruiting mesenchymal stem cells and therapeutic effects were evaluated.
Materials and methods: The peptides RADA-SP (AcN-RAR-ADADARARADADAGGRPKPQQFFGLM-CONH2) were synthesized and purified (Peptron, Daejeon, Korea). Limb ischemia was produced in ~20 g athymic mice and 1% (wt/vol) peptides were injected into ischemic sites ($n = 6$ in each group: ischemia, SP, RADA, RADA+SP, RADA-SP). The tissues were harvested 1, 3, 7 days after injection for histological analysis.
Results: In RADA-SP group, it was shown that many mesenchymal stem cells were recruited into injected sites compared to other groups. After 7 days, SP of this group was most effectively remained in the injected region. Moreover, $TUNEL^+$ cell density was seven times lower than ischemia group. In Masson's trichrome staining, injection of RADA-SP could prevent fibrosis.
Conclusion: In conclusion, RADA-SP is effective to recruit mesenchymal stem cells and that leads to protect limb ischemia.

34.04
Spontaneous co-assembly of molecularly designed building blocks into hierarchically ordered, dynamic, hybrid macrotubes

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A major limitation in generating materials using bottom-up strategies is the lack of order at the macroscale. In this work, we show that two self-assembling molecules, an elastin-like polymer (ELP) and a peptide amphiphile (PA), can interact to spontaneously to form hierarchically

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ordered, dynamic, bioactive, macrotubes. An RGD-containing ELP and a PA (C16V3A3K3) were synthesised using standard methods. The addition of PA solution into ELP instigated the formation of a hollow tube that expanded from the lower surface up to the air-liquid interface. The tubes were prepared at different pH values, and temperatures. Tubular macrostructures were formed when the zeta potential of the ELP was negative, i.e., above pH 5.5, indicating that an electrostatic interaction is involved in the assembly of the two molecules. When tubes prepared at RT were placed at 4 °C, they became less opaque, suggesting that there is also a temperature-dependent interaction between the two molecules. Continuous assembly for 48 h enabled the formation of strong structures that could easily be manipulated with tweezers and were stable in PBS at 37 °C. SEM images of the tubular structures showed that they were composed of a nanofibrous membrane, about 20 μm thick. When two tubes were assembled next to each other, they spontaneously fused into a larger tube. These self-assembled tubes, which show dynamic and temperature-dependent behavior have great potential for use in regenerative medicine applications.

34.05
Bioactive TGFbeta - CaP- alginate based scaffolds for osteochondral tissue repair: design, realization and multi-level characterization

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The design of an appropriate microenvironment for stem cell differentiation constitutes a multi-task mission and a critical step towards the clinical application of tissue substitutes. We here present a bioactive multiphase osteochondral scaffold, properly designed to offer specific biochemical cues for an efficient and selective cell differentiation towards the bony and chondral lineages respectively. Highly macro-porous alginate scaffolds were fabricated from a mixture of calcium cross-linked alginates by means of a freeze-dry technique. In the chondral layer, transforming growth factor (TGF)-beta1 in citric acid was mixed with the alginate solution. In the bony layer, the alginate scaffolds were coated with a film of calcium-phosphate crystals, to offer an osteoinductive surface to the cells. Optical and scanning electron microscopic analyses were performed to assess the micro–macro architecture of the biphasic scaffold. Uniaxial compressive mechanical tests were conducted to evaluate the elastic modulus of the scaffold. For the biological validation of the developed prototype, mesenchymal stem cells were loaded onto the sample; cellular adhesion, proliferation and differentiation were evaluated. Results have successfully demonstrated the efficacy of the osteochondral graft, which combines interesting functional properties and biomechanical performances, thus becoming a promising candidate for osteochondral tissue-engineering applications.

Acknowledgements: Zwick/Roe

34.06
Method for creating nanofiber-reinforced hydrogel scaffolds by combination of electrospun nanofibers and hydrogels

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Hydrogels as well as electrospun nonwovens are both being widely investigated as tissue engineering scaffolds and they both have their advantages and disadvantages. The present work presents a method developed for efficiently combining the two, thus creating a hydrogel reinforced with nanofibers that has the potential to be injectable and used in tissue engineering applications, such as nucleus pulposus regeneration. The method is based on simultaneous electrospinning of nanofibers and air brush spraying of a gel and is shown to create a homogenous mixture of fibers and gel, avoiding unwanted layered structures. Rheological evaluation of the produced gels shows a clear reinforcing effect by the nanofibers, suggesting potential as scaffolds for nucleus pulposus regeneration. Furthermore, using electrospinning opens up for many possibilities of tailoring fiber morphology and structure also pointing to the potential of the method. Acknowledgements: The funding of the Disc Regeneration project (Grant agreement no. NMP-LA-2008-213904) from the European Community is gratefully acknowledged. Also, RISE Holding is acknowledged for financial support to this work.

34.P01
Combination of protein mimetic signals on self-assembling nanofibers

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There has been a paradigm shift in the fabrication of biomaterials for use as a scaffolding in tissue engineering - from the use of inert synthetic materials to biocompatible native materials and now towards a smart biomimetic new age scaffold. Peptide amphiphiles have been proven to be able to self-assemble into clyindrical nanofibres with its hydrophobic alkyl tail hidden within the core of this supramolecular structure and the bioactive peptide epitopes manifesting themselves on the surface. We show here the ability to present a combination of bioactive epitopes on the surface of the nano-fibers and the effect of them on cellular response. We designed two types of protein mimetic sequences, collagen mimetic sequence and laminin mimetic sequence on negatively charged peptide amphiphiles and initiate their self-assembly into nanofibers by mixing them with positively charged peptide amphiphiles. The elongation of the fibers continues to be driven by beta-sheet formation within the peptide segment to form a stable, high-aspect ratio nanostructure. The entanglement of these nanofibers quickly forms a hydrogel that is able to encapsulate PC12 cells and thus providing an opportunity for its use as an injectable hydrogel. The PC 12 cells showed enhanced neurite extension on self-assembling hydrogels bearing both the protein-mimetic sequences. This is a step towards the fabrication of a self-assembling hydrogel manifesting multiple bioactive signals.

34.P02
Hierarchical scaffolds obtained by non-conventional layer-by-layer electrostatic self-assembly

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The introduction of nano/micro structures in scaffolds is of great interest as a way to achieve a better control over cells environment. Layer-by-Layer (LbL) is a very versatile technique for surface modification by the self-assembly of polyelectrolytes (PE). However, until now, few works have reported the introduction of coatings or reservoirs of bioactive molecules on porous structures using PE. This work introduces a non-conventional approach of LbL to create nano/micro fibrillar-like structures of chitosan and alginate inside and linked onto polycaprolactone scaffolds previously produced by rapid prototyping, resulting in hierarchical scaffolds. Several assembling parameters were studied, verifying that the washing step and the concentrations were variables influencing more cell behaviour. The behaviour of SaOs-2 cell line was assessed on these hierarchical scaffolds by quantifying dsDNA and ALP activity, and observing cell distribution after 1 and 7 days in culture. Cell seeding efficiency was improved with the introduction of hierarchi-

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cal structures and, after 7 days, the cells had colonized the whole scaffold. ALP/dsDNA was higher than on 2D culture plates and, in general, was not influenced by the introduction of the structures. In conclusion, the methodology proposed permits to modify the surface or add a new hierarchical structural level in scaffolds for TE that could be used to control cell adhesion, proliferation and differentiation.

34.P03
Hierarchical design of bone extracellular matrix mimetic nanofibers promote osteogenic differentiation of mesenchymal stem cells

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Native bone is comprised from a collagen-based network which is mineralized with a special type of calcium phosphate in a hierarchical manner. Both instructive signals, including small peptide sequences, growth factors, etc., on the extracellular matrix and bare hydroxyapatite were separately reported in the literature promoting mesenchymal stem cell (MSC) differentiation into osteogenic lineage. However, reconstitution of the hierarchy that imitates the native bone structure in laboratory and clinics, however, still remains as a challenge. In this study, we engineered self assembling peptide molecules bearing MSC-specific peptide signals. Onto these nanofibers, we were able to mineralize bone-like hydroxyapatite (HAP). We characterized the mineralized nanofibers using FT-IR, Raman spectroscopy, XRD, electron diffraction, and SEM. We then evaluated the differentiation of MSCs into osteogenic lineage by employing alkaline-phosphatase activity assay, qRT-PCR, immunocytochemistry, and western blot. We reveal that HAP-peptide nanofiber composites demonstrate robust performance for MSC differentiation.

34.P04
Designing functional self-assembling peptides as biomaterial-scaffolds for bone repair

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Aim of this project is to study designed functionalized oligopeptides immobilized on biomaterial scaffolds that promote cell adhesion, proliferation and differentiation. The oligopeptides consist of a core derived from the adenovirus fiber shaft protein responsible for their amyloid character. The peptides were engineered to contain the characteristic RGDS motif of fibronectin to promote integrin-mediated cell adhesion. We performed TEM for the characterization of structural features of the peptides and the fibrils and SEM for the visualization of the surfaces. We seeded MC3T3-E1 cells on glass slides and added the mature peptide solution. Either the RGDS-containing peptide, or a control peptide or fibronectin, or BMP-2 was added to the cells. On day 8 cells were harvested and counted, and gene expression of bone sialoprotein, osteocalcin and Col24a1 was performed. The oligopeptides self-assemble into a few micrometer-long fibrils at a concentration of 2 mg/ml, pH = 7.4. Preliminary experiments show a significant increase in proliferation of cells cultured in presence of the peptide with the RGDS sequence. Cells cultured in presence of fibronectin and in mixture conditions of the RGDS peptide and BMP-2 behaved similarly. Bone sialoprotein and Col24a1 were both expressed in presence of the RGDS-containing peptide and in mixture of the peptide with BMP-2. Furthermore, we investigated the osteogenic response of immobilized peptides on structured hybrid biomaterial-scaffolds.

34.P05
Characterisation and in vitro and in vivo response to self assembled peptide hydrogels

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The importance of recapitulating a 3-D environment representative of conditions in vivo, has driven the recent development of new materials designed for both in vitro cell and tissue culture and in vivo applications in regenerative medicine. Bioinspired supramolecular assembly is an attractive method for the fabrication of such materials and one class of these materials which are attracting increasing attention in biological applications are hydrogels based on peptides, as a result of their inherent structural and biochemical diversity, low cost and ease of preparation. Fabrication of such self-assembling peptide materials include the use of, for example, a pH switch or the presence of enzymes to trigger the process amongst others. Here, we demonstrate the use of both a pH switch and an enzyme induced self assembly of both capped (Fmoc) and uncapped-tripeptide systems to give ordered nanostructures which further interact to form peptide hydrogels. Our goal is the development of a suite of materials whose rheological properties and other characteristics can be tailored by the manipulation of self assembly conditions during materials fabrication. We demonstrate the use of these hydrogels for culturing specific cell types in vitro and the application of these systems in vivo.

34.P06
Sustained release of ranibizumab from self-asssembled peptide amphiphile microgels

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Ranibizumab is a recombinant VEGF antibody used in clinics for the treatment of wet form of age related macular degeneration. It is intravitreally administered to ocular compartments and it needs frequent injections. However, intravitreal administration could cause side complications as well as patient discomfort. These necessitate alternative treatment strategies based on relatively noninvasive ranibizumab delivery that is more effective and sustainable in the eye vitreous than the current clinical regimen. Herein, we developed self-assembled peptide microgels to sustainably release ranibizumab from these microgels at high local dose. Release profile of ranibizumab at different peptide concentrations was used to evaluate the release performance from the microgels for improved and modulated treatment of wet form of age related macular degeneration.

34.P07
Controlled aggregation of microstructures and hMSCs as a bottom-up approach towards cell and tissue organization

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Current methods to fabricate scaffolds for tissue engineering applications have limitations in terms of 3D structure complexity, remodeling and cellular distribution. In this study, we propose a bottom-up approach towards a cell-driven assembly of microparticles at the macroscale. We speculate this might allow the formation of more complex 3D structures prone to remodeling. We show that physical properties like size, shape and wettability of the structures modulate the circularity, branching and compaction of the cell-driven assembly. As a proof of

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concept microstructures were fabricated of SU-8 negative photoresist (MicroChem, Germany). Interaction of cells with structures of different sizes and shapes was investigated by culturing human mesenchymal stem cells (hMSCs) at different concentrations with varying number of structures. The number of structures that were assembled into aggregates and the compaction of the aggregates were monitored over time. Aggregates showed more circularity and higher aggregation speed when cells were cultured on smaller structures or on more hydrophilic structures. By controlling cell attachment sites, this bottom-up approach could be applicable to the fabrication of 3D cell-laden constructs as carriers for systemic cell delivery or as self-assembled scaffolds for tissue constructs of clinical relevant sizes.

34.P08
Mechanical properties of self-assembling peptide hydrogels and their effects on cell behaviors

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Development of self-assembling biomimetic molecules and integration of those in materials for tissue engineering is one of the most interesting research areas in materials science. Our research is aimed to study self-assembling peptides and understand possible non-covalent interactions taking place in self-assembly mechanisms such as hydrogen bonds, electrostatic and hydrophobic interactions in aqueous conditions. In this work, the non-covalent interactions between oppositely charged peptides are investigated experimentally and simulation of possible aggregate formations is demonstrated. Development of a new peptide hydrogels with interesting mechanical properties and with low contamination risk is one of the main targets of this research. We aim to highlight concentration and time dependence of peptide hydrogels and understand the relationship between mechanical properties and bioactive parameters. We also focus on biocompatibility and biodegradability of the designed hydrogels, which have great potentials for applications in regenerative medicine.

34.P09
In situ chondrogenic differentiation of bone marrow stromal cells in bioactive self-assembled peptide gels

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The gradual increase in the aging population has resulted in many osteoarthritis patients. However, articular cartilage is a specific tissue which lacks nerves and blood vessels and has limited self-repair abilities. Recently, there are many studies on peptide hydrogel scaffolds which are biocompatible and low immunogenic for cartilage tissue engineering. In this study, we used self-assembled peptide gels having unique peptide sequences and introduced the bioactive motifs to the end of the sequences of peptide gels, which are collagen mimetic peptides (CMPs). CMPs that have a unique collagen-like triple helical conformation have been shown to associate with collagen molecules and fibers via a strand invasion process. In order to examine the in situ chondrogenic differentiation of rabbit bone marrow stromal cells (rBMSCs) by functional motifs, biochemical analysis were conducted. The results showed that the secretion of cartilage specific extra cellular matrix and the gene expression concerned with chondrogenic differentiation were increased in group of self-assembled peptide gels introduced CMPs motifs. From the results, it was confirmed that CMPs modified self-assembled peptide gels could enhance effectively the chondrogenic differentiation in situ, consequently they could be a good scaffold for cartilage tissue engineering.

34.P10
Catechol-driven layer-by-layer assembly for surface hydrogel coating

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Surface engineering of materials can introduce the preferable functionality by physical, chemical, or biological decoration into the elaborated architecture having inert exterior, which can boost the end role of the consequent product in a wide range of areas. Due to processing limitation of functional materials in production, bulk material manufacturing with subsequent surface modification of interesting species is often desirable. In particular, surface modification with hydrogels has unique advantages of a high degree of hydrophilicity and biocompatibility in the context of biomedical applications. In this study, we devised the novel layer-by-layer assembly using mussel adhesive-inspired driving force, which led to spontaneous formation of surface-bound hydrogel on virtually all types of substrates including plastics, metals, and ceramics. First, we synthesized many types of catechol-polymer conjugates through simple EDC chemistry. The catechol-polymer conjugates was sequentially adsorbed on the substrate by layer-by-layer fashion with each subsequent oxidation of catechol groups. The resulting hydrogel on the substrate exhibited anisotropic swelling and tenacious adherence to various substrates. This surface-bound hydrogel has promising potential for biomedical applications such as local drug reservoir, biocompatible coating, and protective lubricious surface layer.

34.P11
Modular adipose tissue engineering for soft tissue reconstruction

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Adipogenic tissue reconstruction or augmentation has been implemented when complex trauma, oncologic resections, and congenital or acquired deformities often associated with massive loss of adipose tissue are accompanied. Currently, the transplantation of autologous fat tissue is the main clinical modality for rehabilitating the defect sites. However, the lack of sufficient vascularization after transplantation causes significant graft volume shrinkage, resulting in poor long-term clinical success. Here, we demonstrate a multiscale (from nanometer-scale to macro-scale) and multifunctional assembly route to hierarchically mimic 3D adipose tissue. Biologically optimized module tissues were used as building units for fabricating 3D biohybrid construct of higher order. The microstructured scaffold was prepared via a DPMD process and allowed to deliver angiogenic growth factor in a sustained manner. These bioactive scaffolds with periodic porous internal structures were used to assist the 3D hierarchical assembly of the composite module tissues. The produced multiscaled and multifunctional modular tissue construct enable the successful formation of vascularized adipose tissue in vivo.

34.P12
Void filling strategies of fibroblasts in the onset of regeneration

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In regeneration, strategies are identified to fast and effectively fill voids. Fibroblasts are the cells paving the ground for later reconstitu-

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tion of more complex tissues. In this study, we aimed at analyzing if fibroblasts from different species and ages have similar strategies to initiate micro-void filling. Rat and human primary dermal fibroblasts (RF and HF) were seeded into Macroporous collagen-I scaffolds, using cells from young and old donors. After 1 week in a bioreactor, scaffold volumes were assessed. Cell number and ECM production were evaluated by staining for cell nuclei, fibronectin (Fn), second harmonic generation imaging for collagen (Col) and immunoassay for Fn. Cell spreading behavior was analyzed and correlated with ECM formation. HF seeded scaffolds showed a contraction up to 39%, more pronounced for young cells (vs old cells). Little contraction was seen for RF. New Col was mostly found in young HF which filled the scaffold whereas RF stayed located on the fibers. Still, single cell Fn production was the highest for old RF ($p = 0.005$ vs young RF, $p = 0.014$ vs HF).Scaffold contraction was linked to cell spreading across the pores, as different cellular forces are required to deform a scaffold wall or contract a pore. Pore filling was faster if cells could go from spreading on the pore surface to spreading across the pore void. The cell and pore size ratio seems to represent a measure to (i) prevent scaffold volume loss and (ii) to void tissue filling.

34.P13
Room temperature fabrication of glass based scaffolds using the combination of sol-gel reaction and rapid prototyping system

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The scaffold should have bioactivity, osteoconductivity, biodegradability, suitable surface chemistry, highly porous structure with a well interconnected pore network, irregular shape fabrication ability, and appropriate mechanical properties. Bioactive glasses (BGs) meet the first four criteria. Sol-gel derived BGs much satisfy these conditions compared to melt-derived glasses due to their greater surface area and nanostructure. Meanwhile, conventional fabrication techniques for BG scaffolds cannot fully satisfy the other three criteria for bone scaffold. Rapid prototyping (RP) can generate a physical model directly from computer-aided design data and can overcome these structural limitations. Fabrication of sol-gel derived BG scaffolds using RP technique has suggested to this end. Almost all process for fabricating ceramic scaffold needs a heat treatment at high temperature not only to remove organic binder or template but also to enhance their mechanical strength. However, this sintering process imposes restrictions for using scaffold in tissue regeneration, such as limited functionality, unstable mechanical properties and low reproducibility. The purpose of this study is to develop a novel fabrication process of sol-gel derived BG scaffolds using RP technique without sintering to clear all these limitations.

34.P14
Assessing and manipulating self-assembling peptide viability as a scaffold for cell proliferation

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Rationally designed self-assembling peptides, which are biologically inspired, aim to mimic natural extra cellular matrices for use as three-dimentional tissue engineering scaffolds. Two fundamental issues inherently dictate their usefulness within this field. Firstly it is of paramount importance that the molecular self-assembly is understood so that the range of non covalent interactions can be manipulated in order to produce stable aggregates within biologically relevant conditions. Secondly the peptide material must be biocompatible and provide an environment in which nutritional transfer is not impeded and cells may exude metabolites. This work presents an iterative approach to the study of self-assembling ß-sheet peptides of the P11-X family. Utilising various spectroscopic techniques such as U.V. spectroscopy, FTIR spectroscopy and Circular Dichroism we are able to study quantitatively how permutations of peptide primary structure effect the self-assembly of the peptides. The subsequent nano-structures formed are then assessed using a variety of optical tools and the peptide hydrogels are further studied by assessing their rheological properties. The microenvironments in which the P11-X peptides provide are also investigated by assessing its cytocompatibility and is characterised by various cell types, including fibroblasts. Such a systematic study gives valuable information into the rational design of further peptidic molecules to form optimised materials for cell growth.

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35. Surface Topography

35.01
Keynote: Biomimetic surfaces exhibiting extreme wettability properties for tissue engineering applications

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Surfaces exhibiting hierarchical topographic organization, from the nano to the micro scale, may present extreme wettability properties, ranging from (lotus-like) superhydrophobic to superhydrophilic behaviour. Such surfaces may be used as innovative substrates to infer cell behaviour and protein adsorption onto surfaces beyond the conventional hydrophobic-to-hydrophilic character. We proposed a rapid procedure to produce superhydrophobic substrates in polymeric films that could change the wettability up to contact angles near zero through further modification using plasma or UV/ozone radiation. By spatially control the regions of surface modification it is possible to produce surfaces with gradients in wettability or pattern wettable areas surrounded by superhydrophobic spaces. We have been suggesting the use of such devices for *ex-vivo* biomedical applications, such as platforms for combinatory analysis of 2D or 3D biomaterials and cells, including miniaturised hydrogels and scaffolds with multiple combinations of compositions and properties, and also to produce open microfluidic devices. The developed superhydrophobic substrates may be also used to process polymeric particles in mild conditions and with unique structural characteristics, specially designed for tissue engineering applications

35.02
MicroRNAs potentially regulate TGFβ/BMP & Wnt/Ca^{2+} signaling pathways on modified titanium implant surfaces leading to improved osteogenicity

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Background: Sand-blasted, large grit, acid etched (SLA) and modSLA (hydrophilic SLA) titanium implant surfaces are recognized to have improved osseointegration and osteogenic properties. These properties are guided by activation of several molecular signaling pathways. This study explored the signaling pathways activated following early exposure (24 h) of osteoprogenitor cells to modSLA and SLA surfaces and their possible regulation by microRNAs.
Materials and methods: Human osteoprogenitor cells were cultured on modSLA, SLA and smooth (SMO) surfaces. The relative expression of key genes involved in signaling pathways, and miRNAs related to cell development and differentiation was evaluated. Target predictions for the differentially regulated miRNAs were performed using TargetScan and sorted for genes of TGFβ/BMP and Wnt/Ca^{2+} pathways (for downregulated miRNAs) and inhibitors of osteogenesis (for upregulated miRNAs).
Results: The study confirmed that TGFβ/BMP, Wnt/Ca^{2+} and Notch pathways are activated within 24 h of exposure to modified surfaces. Several miRNAs showed differential expression on modSLA and SLA surfaces. Target predictions for the down-regulated miRNAs revealed several potential genes of the TGFβ/BMP and Wnt/Ca^{2+} pathways as potential targets.
Conclusion: This study demonstrated that modified titanium surfaces induce differential regulation of miRNAs that potentially regulate the TGFβ/BMP and Wnt/Ca^{2+} pathways leading to improved osteogenicity.

[Correction after online publication 3 September 2012: The sentence should be "The study confirmed that TGFβ/BMP, Wnt/Ca^{2+} and Notch pathways are activated within 24 h of exposure to modified surfaces.", not "The study confirmed that TGFβ/BMP, Wnt/Ca^{2+} and Notch pathways are activated within 2 h of exposure to modified surfaces."]

35.03
Human tenocyte response to nano-topographic interfaces and implications on medical device design

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Introduction: Cell-substrate interactions at the nano-bio-interface are becoming increasingly important in the design of the next generation of biomaterials. However, it is important to fully comprehend the influence of nano-topography on cellular behaviour. Herein, the influence of nano-topography on tenocyte behaviour was evaluated.
Materials and methods: Anisotropically ordered ridges and grooves, ranging from 35 to 1900 nm, were imprinted on PLGA films using nano-imprinting lithography. Subsequently, the influence of surface topography on primary human tenocytes morphology, alignment, migration and phenotype was evaluated on days 1, 5, and 10.
Results: No significant difference in cell metabolic activity and viability between the different topographies at each time point was observed ($p > 0.05$). Cellular orientation, parallel to the substrate topography, was observed for groove depths of 300 nm and above, whilst for groove depth lower than 300 nm, the cells adopted a random conformation.
Conclusion: This study has established a range of nano-textured substrates that provide an instructive environment for cellular alignment, while maintaining cell viability. Gene expression assays are on-going to fully comprehend the influence of different nano-textured substrates on cellular behaviour.
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35.04
Nanofiber compliance affects human mesenchymal stem cell motor neuron differentiation

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Little is known about the combined effects of substrate topography and compliance signals in directing stem cell differentiation. In this study,

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human fetal mesenchymal stem cells (fMSCs) were cultured on pullulan-dextran nanofibers and hydrogels with varying stiffness and induced to differentiate along the motor neural lineage. By varying crosslinker, sodium trimetaphosphate (STMP), content from 10, 12 to 16% (denoted as STMP10, 12 and 16), scaffold stiffness was significantly altered (0.24 ± 0.06 kPa–0.92 ± 0.18 kPa, $p < 0.05$). As compared to hydrogels, nanofibers significantly enhanced fMSC viability (~79% vs 12% at day 3, Live-Dead assay, $p < 0.05$). At day 8, the expression of neuronal marker, Tuj1, was enhanced on nanofibers as compared to tissue culture polystyrene (2D controls). Specifically, Tuj1 expression increased with increasing nanofiber stiffness (STMP16 > 12 > 10). However, the expression of glial markers, GFAP and O4, decreased by day 16. A significant increase in mature motor neuron marker, ChAT, was detected in cells on nanofibers than on 2D controls. Specifically, STMP16 nanofibers enhanced ChAT expression (STMP16 > 12 > 10). Inhibition of the ROCK pathway by treating cells with Y27632 abrogated nanofiber compliance effect on ChAT expression. Taken together, our results suggested the advantages of nanofiber topography in supporting fMSC culture. Furthermore, nanofiber compliance affects fMSC motor neural commitment through the involvement of ROCK pathway.

35.05
Patterned superhydrophobic surfaces for the combinatorial assessment of 3D biomaterials-cells interactions

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Cells-biomaterials interactions are dependent on a wide range of factors, namely substrate mechanical properties. Usually in the study of mechanotransduction phenomena 2D models are used and surface modification of the biomaterials is performed to suppress the effect of materials chemistry. However, 3D structures provide a more accurate mimicry of the physiological media. In high-throughput analysis cell encapsulation is commonly used, although in different stiffness hydrogels the maintenance of chemical features is difficult to achieve. Wettable spots were patterned in superhydrophobic surfaces in order to deposit polymeric precursors and generate combinatorial porous scaffolds. On-chip dynamic mechanical analysis was performed, as well as simultaneous quantification of porosity of several scaffolds. After selecting groups of scaffolds with a wide range of mechanical and morphological properties, a new array was created, consisting of these scaffolds with fibronectin adsorbed through the whole solid structure. Protein adsorption was studied by image analysis. The adhesion of two different cell types was studied. A tendency for an increased metabolic activity/cell number rate could be observed for pre-osteoblast cells in high stiffness scaffolds, while an opposite tendency was verified for fibroblasts. In both cell types, mainly for pre-osteblasts, a tendency for increased metabolic activity and cell number in the scaffolds in the presence of fibronectin was observed.

35.P01
'Smart' shape-memory surfaces for mechano-structural control of cell functions

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'Smart' polymers are environmentally sensitive polymers that respond to small changes in environmental stimuli with large, sometimes discontinuous changes in their physical state or properties. Shape-memory polymers (SMPs) are a class of smart polymers that have the capability to change from a temporary shape to a memorized permanent shape upon application of an external stimulus. Especially, the use of SMPs as self-repairing or re-writable materials has found growing interest in environmentally-friendly technologies. Here we propose a novel technique that explores dynamic cell behavior in response to surface changes in nanotopology using biocompatible poly(caprolactone) (PCL) films that actuate on demand under biological conditions. Permanent surface patterns were generated by crosslinking the PCLs in a mold. Temporary surface patterns were later embossed into the crosslinked PCLs. The application of body heat quickly and completely transitioned temporary surface patterns to permanent patterns. To observe the effects of a shape-memory-activated change in surface topography on cell behavior, surfaces with a temporary grooved pattern were used as a cell culture substrate and subsequently triggered to revert to a permanent flat surface in the presence of growing cells.

35.P02
Zwitterionic oligopeptides for cell micropatterning and electrochemical rapid cell detachment

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Aim: To manipulate cells *in vitro* for engineering tissues, we developed oligopeptide-modified cell culture surfaces from which adherent cells can be rapidly detached by application of an electrochemical potential.
Methods: We designed two oligopeptides, CGGGKEKEKEK for cell repulsive surface and CGGGKEKEKEKGRGDSP for cell adhesive surface. These oligopeptides bind to a gold surface via a gold-thiolate bond and form self-assembled monolayers via the electrostatic force between the alternating charged lysine (K) and glutamic acid (E). We examined nonspecific adsorption of proteins to the cell repulsive surface, micropatterning of cells and its electrochemical detachment.
Results: Quartz crystal microbalance measurements revealed that, owing to the ionic solvation in the alternating charged sequence, the nonspecific adsorption of proteins on the surface modified with cell repulsive peptide was significantly reduced as compared to that to a surface without the modification. By spatially patterning these two oligopeptides, cell micropatterns were formed on the surface. The cell micropatterns were then detached by applying a negative potential within 2 min and transferred to a collagen gel.
Conclusion: This cell micropatterning and detachment approaches could be a useful tool for tissue engineering applications.

35.P03
Tailoring the trajectory of cell rolling with cytotactic surfaces

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Cell separation technology is a key tool for biological studies and medical diagnostics that relies primarily on chemical labeling to identify particular phenotypes. An emergent method of sorting cells based on differential rolling on chemically patterned substrates holds potential benefits over existing technologies, but the underlying mechanisms being exploited are not well characterized. In order to better understand cell rolling on complex surfaces, a microfluidic device with chemically patterned stripes of the cell adhesion molecule P-selectin has been designed. A high-resolution, automated visual tracking system was developed to analyze the behavior of cells rolling on these patterned surfaces under flow. This behavior was then correlated to a number of established predictive models, suggesting that the technique

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can be adapted to a wide array of adhesive molecules. This combination of computational modeling and widely available fabrication techniques represents a crucial step toward the successful development of continuous, label-free methods of cell separation based on rolling adhesion.

35.P04
Regulation of PC12 differentiation by controlled 3D micro/nano laser structuring of culture substrates

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The control of the outgrowth of neuronal cultured cells is of critical importance in a wide spectrum of neuroscience applications including tissue engineering scaffolds and neural electrodes. However, the study of neuron cell outgrowth on more complex topographies remains limited. Evidence of phenotype alteration of stem cells and differentiated neuronal cells when cultured on traditional flat culture substrates that lack structural cues, emphasize the necessity to shift from 2D to 3D or multi-scale cell culture models. The aim of the present study was to investigate the cellular response and differentiation (neuritogenesis), of the pheochromocytoma, PC12, cell line on femtosecond laser fabricated 3D micro/nano structured Si surfaces. Variation of the laser fluence, leads to substrates exhibiting different roughness ratios and wetabilities. PC12 cells were cultured on these substrates for 4 and 7 days and treated with nerve growth factor (NGF). The results showed that, although in all cases cells had been treated with NGF, cell differentiation was only observed on the hydrophilic patterned surfaces of low and intermediate roughness, whereas it was strongly inhibited on the highly rough superhydrophobic surfaces. Our results indicate a method to tune cell differentiation into a neuronal phenotype by proper selection of the surface free energy of the culture substrate and may be promising for the design of cell culture platforms with controlled differentiation environment.

35.P05
Proliferation and osteogenic differentiation of human mesenchymal stem cells on gradient micropatterned surface

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Surface properties of biomaterials and scaffolds have been reported to affect mesenchymal stem cells (MSCs) functions. Besides the surface properties such as surface functional groups, wettability, nano- or micro-structured morphologies and elasticity, cell density has also been shown to affect the proliferation and differentiation of MSCs. However, most previous studies have examined the effect using separate culture wells and dishes at different cell densities. In the present study, a cell-culture polystyrene plate with micropatterned non-fouling poly (vinyl alcohol) (PVA) areas was prepared to allow the formation of a cell density gradient of MSCs. And the effect of cell density on the proliferation and osteogenic differentiation of MSCs was investigated. To prepare the micropatterned surface, photoreactive PVA was grafted onto cell-culture polystyrene plates by UV photolithography. Human MSCs were cultured on the patterned surface in osteogenic induction medium for up to 14 days. After 1 day of culture, a cell density gradient ranging from 2.6×10^3 to 112.5×10^3 cells/cm^2 was formed on the micropatterned surface. MSCs at lower density proliferated faster than those at higher density. A high cell density initiated the osteogenic differentiation of MSCs more rapidly than did a lower cell density. The micropatterned surface will be useful to directly compare the effect of cell density on MSC functions.

35.P06
Selective cell control by laser-generated surface topographies

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To achieve a perfect integration of the implant or tissue-engineered substitute into the body, tissue formation in contact with the biomaterial has to be controlled. In this manner, the inhibition of fibroblasts is demanded, since these cells contribute to fibrous encapsulation which can lead to implant loosening and failure. Simultaneously, competitive cell types, which support implant integration and functions, have to be stimulated. Since conventional biomaterials do not fulfil all specifications with respect to cell control, functionalization methods are under development. One approach focuses on the fabrication of defined surface topographies. Thereby, laser-processing or microreplication techniques are very beneficial, due to a large variety of geometries, low mechanical damages, and reproducibility. This study concentrates on topographical effects on cell behaviour with respect to adhesion, localization, morphology, proliferation, and differentiation. It is demonstrated that fibroblasts can selectively be inhibited by nano- and microstructures. Simultaneously, neuronal cells or osteoblasts are not negatively affected. Therefore, these findings suggest favourable surfaces for orthopaedic applications or electronic devices.

35.P07
Co-culture *in vitro* study on surface modified PEOT/PBT rods to mimic foreign body response *in vivo*

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Researchers in the field of tissue engineering aim to reduce foreign body response to avoid formation of a fibrocellular capsule triggered by an implantation. We, instead, aim to take advantage of such a response to direct cell migration and tissue formation/plasticity, to create a vascular graft in '*in vivo* bioreactor' strategies. Initial cell and material interaction can be modulated by material's chemical composition and surface characteristics. Here, we analyzed alteration of polymeric rod surface by different surface modifications for topography alteration and hydrolysis. PEOT/PBT was used to fabricate rods for its easy tunable mechanical and physical properties. Scanning electron microscopy (SEM) and atomic force microscopy showed different surface topography and roughness between the treatments, with an increase of roughness up to 40-folds versus the unmodified rods. Total amount of protein absorbed by the rods increased after surface modification, due to changes in surface area and surface chemistry, which was confirmed by x-ray photon spectroscopy. *In vitro* co-culture studies with fibroblast and macrophages was done to mimic the initial inflammatory events leading to a fibrocellular capsule formation *in vivo*. DNA and presto blue assay showed an increase in cell attachment and metabolic activity and SEM images showed better cell distribution on surface treated rods.

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35.P08
Proliferation of human bone marrow mesenchymal stem cells on PHBV-based binary and ternary composites

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Combination of bioresorbable polymers and bioactive fillers allows to adjust mechanical properties and degradation rates of those materials to match bone tissue. Additionally presence of bioactive particles like calcium silicate (CS) and its mineral form wollastonite in polymer matrix improves bioacceptance of the composites. The aim of this study was to evaluate the biological performance of poly(3-hydroxybutyrate-co-3-hydroxyvalerate)(PHBV) – CS composites for bone applications. For this films of binary (PHBV and CS) and ternary composites (PHBV, CS and poly(glycolide-co-lactide, PLGA) and neat PHBV were prepared by solvent casting technique. The resulting surface morphology of PHBV-CS and PHBV-CS-PLGA composite films differed from that of the neat PHBV. Addition of CS reduced surface roughness by one order of magnitude from micro- (PHBV) to nanometer range (composites). Human bone marrow mesenchymal stem cells (HMBC) were seeded on the surface of the films and cultured in expansion medium for a period up to 7 days. The number of cells was assessed by measuring total culture DNA content. No difference between the tested materials in total culture DNA was found 6 h after seeding. However, after 7 days of culture the total DNA content was significantly enhanced on neat PHBV if compared to both composites. These results suggest that the surface morphology can play significantly bigger role in colonization by HBMCs than presence of low concentrations of bioactive filler.

35.P09
Influence of silicon carbide topography on inflammatory reactions and osteogenic differenciation of human mesenchymal stem cells

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Surface properties are key factors in biomaterial tissue integration as they modulate cell adhesion, gene expression and cytoskeletal organization. Biomorphic silicon carbide ceramics (bioSiCs) obtained from different natural resources are characterized by a biomimetic structure, good biocompatibility and high degree of pore interconnectivity. The surface properties of different bioSiCs were evaluated by scanning electron microscopy, interferometric profilometry and static contact angle measurements. The adsorption of albumin and fibrinogen onto the materials were studied as well as the complement system activation. Human mesenchymal stem cells (MSC) were cultured on bioSiCs. Apoptosis (caspase-3) and differentiation (osteocalcin, osteopontin) were evaluated at different times by ELISA. Cell viability was analyzed by calcein-propidium iodide staining. Results show a higher adsorption of albumin than those of fibrinogen. The complement activation was moderated and no cell apoptosis were observed indicating good tissue interactions. Levels of osteocalcin were significant higher than those of positive controls after 15 of cell culture. However no significant levels of osteopontin were detected except for juncus bioSiC samples. As shown in confocal microscopy images cells cultivated on juncus samples grow by contact guidance. BioSiC surface roughness were able to differentiate MSC and juncus precursor seems to be the most efficient in osteoblast differentiation.

35.P10
Effect of nano-patterned polymer surface on the phenotype control of pre-osteoblasts

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Tissue engineering generally requires the use of an ECM-mimicking scaffold and the surface nanotopography of the scaffold is one of the most critical factors affecting cell phenotype. We hypothesized that controlling the surface nano-structure of polymer matrices could be critical in the regulation of pre-osteoblast phenotype. Nano-structured polymer surfaces were produced via self-assembly of polystyrene-b-poly(ethylene oxide)/dodecylbenzenesulfonic acid complex systems and their structures were dependent on the composition and/or molecular weight of block copolymers. Changes in the size of polystyrene domains under 100 nm, which was considered the cell adhesion domain, strongly influenced the adhesion, proliferation, and differentiation of MC3T3-E1 cells cultured on the nano-patterned surfaces. The growth rate of the cells decreased as the PS domain size increased despite the same center-to-center distance between the domains. In addition, osteoblastic differentiation was enhanced when cells were cultured on the nano-structured surface containing larger adhesion domains. This approach to controlling pre-osteoblast phenotypes by varying the surface nano-structures could be beneficial for the development of novel scaffolds in tissue engineering applications.

35.P11
Microstructure and chemistry affects the *in vitro* bioactivity of calcium phosphate bone graft substitutes

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Despite some controversy as to the methods reliability, its relevance to the understanding of mechanisms behind osteointegration, and the actual nature of the calcium-rich precipitate formed on materials Kokubo's Simulated Body Fluid has been used to evaluate the Bioactivity of numerous types of materials ranging from calcium phosphate (CaP) ceramics and Bioglasses through to Chitosan and natural Pearl. The objective of our study was to compare the Bioactivity of CaP of varying chemistry and strut-porosity through determining the rate of formation of hydroxycarbonate apatite (HCA) crystals on the material surface and the extent to which these crystals formed after the material was soaked in simulated body fluid for between 3 and 30 days. The propensity for the formation of either HCA or Octacalcium Phosphate (OCP) as a continuous, un-broken layer over the implant surface immediately after implantation into bone is in theory likely to be significant to the processes of osteointegration; the greater the surface area covered by this layer the better the opportunity for interdigitation with host bone. In practice this assumption is still under debate as the *in vivo* studies in support of the outcomes of the bioactivity test are often contradictory. In our study formation of HCA varied among different materials. Under the conditions of our test the rate of HCA growth was related to the strut-porosity and the absolute amount formed was dependent upon the chemistry of the substrate.

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35.P12
Multi-structured titania features for stem cell differentiation and osseointegration

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Functionalisation of orthopaedic implants with topographies to stimulate apposite integration of the device with surrounding bone (osseointegration) would have considerable therapeutic benefits. Until recently, only non-specific roughening of titanium was possible, but more defined titania nanofeatures can now be produced using through-mask anodisation [1, 2] and self-assembled block copolymer templating. Culture of mesenchymal stem cells on pillar-like features of 8 nm and 15 nm height produced using the latter two techniques promoted osteogenic differentiation. This was assessed using early- and late-stage bone markers (including osteocalcin and the osteogenic transcription factor phospho-Runx2), computational analysis, focal adhesion quantification and metabolomic analysis [2]. Metabolomics allows examination of changes in global metabolite profile, and was used to identify functional changes in stem cell physiology. Most interestingly, SEM revealed that cells could interact directly with 8 nm features using nanoscale membrane projections. In addition, we have generated surfaces with a combination of micron and nano-scale titania features (micropits and nanopillars) that promoted osteogenic differentiation. This study illustrates the sensitivity of stem cells to nanotopographical cues, and highlights the potential for use of these surfaces in orthopaedics.[1] Sjöström et al. Acta Biomaterialia. 2009;5:1433–41. [2] McNamara et al. Biomaterials. 2011;32:7403–10.

35.P13
Structural shear stress evaluation of hyperbolic scaffolds for tissue engineering applications

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Tissue engineering represents a new, emerging interdisciplinary field involving combined efforts of several scientific domains towards the development of biological substitutes to restore, maintain, or improve tissue functions. Scaffolds provide a temporary mechanical and vascular support for tissue regeneration while shaping the in-growth tissues. These scaffolds must be biocompatible, biodegradable, with appropriate porosity, pore structure and pore distribution and optimal structural and vascular performance, having both surface and structural compatibility. Surface compatibility means a chemical, biological and physical suitability to the host tissue. Structural compatibility corresponds to an optimal adaptation to the mechanical behaviour of the host tissue. The design of optimised scaffolds based on the fundamental knowledge of its macro microstructure is a relevant topic of research. This research proposes the use of geometric structures based on Triple Periodic Minimal Surfaces for Shear Stress applications. Numerical Shear Stress simulations are performed on these geometries varying the thickness and radius of the scaffold's geometric definition. Geometries based on these surfaces enables the design of vary high surface-to-volume ratio structures with high porosity and mechanical/vascular properties.
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35.P14
Modulation of cell attachment and detachment on patterned thermo-responsive polymeric surface for cell separation

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Thermo-responsive polymeric surfaces can allow adhered cells and cell sheet to be spontaneously recovered by lowering temperature without proteolytic enzymes. In this study, hydrophilic thready micro-patterns were immobilized on the thermo-responsive surface for controlling a cell adhesion and proliferation. In this study, cell attachment and detachment control on the patterned surface for cell separation was accomplished. 3T3 cells, human umbilical vein endothelial cells (HUVEC), and HeLa cells were used for controlling the cell attachment and detachment. Adhered 3T3 cells spontaneously detached themselves on the patterned surface after reducing temperature as well as a conventional thermo-responsive surface. On the other hand, HUVECs adhered on the patterned surface until 12 h incubation at 37 °C, but the adhered HUVECs spontaneously detached themselves through the next 12 h incubation at 37 °C. In addition, the attachment of HeLa cells was hardly observed on the patterned surface. Based on these results, we developed cell separation system using the patterned surface without any antibody labels or proteolytic enzymes. In conclusion, the patterned surface in this study has a potential for breakthrough in the next generation of biomaterials.

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36. Metals and Metal Surfaces

36.P01
In vivo test of nanoparticulate bioactive glass coating in porous Ti-implants

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Titanium and its alloys are the most widely used materials for bone substitutes in orthopaedics and dentistry. They are biocompatible but in unmodified state they are not bioactive thus their osseointegration is limited. To improve their bioactivity different surface modification are used, from mechanical to chemical, and among them is application of bioactive material such as calcium phosphate or bioactive glass. To improve the stability of an implant a porous coating can be applied that allow the bone to grow into the pores. In our study, to promote osseointegration, we infiltrated nanoparticulate bioactive glass into the porous Ti-layer on Ti6Al4V implants. Implants, with or without BAG, were then implanted into rabbit tibia of New Zealand white rabbits. After 10 weeks the rabbits were sacrificed and the implants were cut to prepares samples for histological and SEM examination. The EDS analyses also performed to quantitatively evaluate the bone-to-implant contact and bone-to-pore ratio. The analyses confirmed that bioactive glass induce bone ingrowth. Samples infiltrated with bioactive glass had 38% of the pores occupied while the one without bioactive glass coating only 22%.

36.P02
Improving conditions for MSC growth on porous TiO_2 scaffolds

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Mesenchymal stem cells (MSCs) represent, in combination with scaffolds, an outstanding tool for bone tissue engineering. However, the ballance between culture conditions and loadbearing properties remains a challenge. Ultra porous TiO_2 scaffolds have high strength, excellent biocompatibility and allow for attachment and growth of MSCs. The aim of the present study is to improve MSC growth in scaffolds focusing on viability, differentiation and extra-cellular matrix (ECM) deposition. Human adipose-derived MSCs were either seeded on uncoated scaffolds or on Ca-alginate coated scaffolds in 1% Na-alginate (NovaMatrix). Lactate dehydrogenase (LDH) activity in medium was measured every 2nd day to asses cell death. Day 14 relative expression of skeletal precursor markers RUNX2 and SOX9, as well as the osteogenic markers COL1, and ALPL was determined by qPCR. Type 1 collagen (COL1) protein expression was evaluated by confocal microscopy. Viability and proliferation rate was evaluated based on ethidium bromide/acridine orange staining (day 7 and 14). Alginate coating improved MSC growth and viability as indicated by low cell death, reduced LDH activity, and increased proliferation. Moreover differentiation towards skeletal progenitors was induced as shown by significantly increased expression of RUNX2 and SOX9. COL1 staining showed increased ECM deposition. In conclusion, seeding MSCs in an alginate improves growth conditions and primes MSCs towards osteogenic differentiation.

36.P03
The potential of magnesium alloys for hard tissue regeneration

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The magnesium alloys has been studied for their ability to be degradable in biological environments. In our study, we select and test some magnesium alloys Mg-Ca0,8, Mg-Ca1,8 and ZK30 in order to investigate the microstructure, mechanical properties, corrosion and how alloying elements influenced the characteristics of this new potential biomaterials for hard tissue regeneration. To obtain Mg-Ca alloys with different nominal calcium contents, calcium particles were added to the melt commercial pure Mg (99.7%) at 680 °C under a gas atmosphere of SO_2. After holding and stirring for 10 min the melt was cast into a metallic mould preheated to 250 °C. Commercially available MgZn6Zr0.5 was used for preparation of the ZK30 alloy. Disk samples ($10 \times 10 \times 3$ mm^3) were prepared for the microstructure characterization with the surface being polished up to 2500grit and finally etched with 50ml solution of picric and acetic acid. An optical microscope (Olympus BX51) was used for microstructure analysis. Surface morphology characterization before and after corrosion test in simulated medium was done using scanning electron microscopy. The corrosion results are connected with different medium used for testing corrosion resistance and demonstrate the ability of these alloys to be potentially used as orthopaedic biomaterials, if the problem of hydrogen bubbles will be controlled.

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36.P04
A preliminary study of the dental implant therapy -initial osteogenesis of human mesenchymal stem (HMS0014) cells on titanium discs and implants with different surface modifications-scs

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HMS0014 cells were GBR-engineered to proliferate and differentiate into osteoblast(Ob)-like cells, which initiated hard tissue matrix deposition in both monolayer and 3-D Cellmatrix/PuraMarix cultures. The osteogenesis initiated with attachment/adhesion of HMS0014 cells on either Titanium(Ti) or Ti alloy discs/implants modified with osteoconductive/osteoinductive surface substrates was histologically assessed. The results obtained were as follows: (i) The HMS0014 cells actively proliferated/differentiated into mature Obs to initiate mineralisation since day 1 in both monolayer and 3-D cultures; it was prominently progressed between day 7 and day 14 of cultures. (ii) The SEM of 60-min specimens demonstrated a loose distribution of proliferating spherical-to-polygonal (d = 10–40 μm) cells sending out many minute filopodia and some lamellipodia to attach with the substrate. (iii) In the 180-min specimens, the HMS0014 cells actively proliferated and spread into flat polygonal cells with prominent lamellipodia and den-

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dritic filopodia (30×90–100×200 μm^2) to employ cell-to-substrate and intercellular attachments. (iv) On the other hand, the present immunohistochemistry of the attached HMS0014 cells demonstrated the co-expression of F-actin and CD51 (αV integrin) in both the 60-min and 180-min specimens. We concluded that the present GBR method enhanced HMS0014 cells to initiate a direct bone-to-substratum contact on Ti discs/implants which were subject to different surface modification.

36.P05
Gentamicin and bone morphogenic protein-2 (BMP-2)-delivering heparinized-titanium implant with enhanced antibacterial activity and osteointegration

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The aim of this study is to develop novel Ti implants that enhance osteoblast functions, while simultaneously decreasing bacterial infections. First, the surface of pristine Ti was functionalized with heparin-dopamine by mimicking a mussel adhesion mechanism. Gentamicin sulfate (GS) and/or bone morphogenic protein-2 (BMP-2) was then sequentially immobilized to the heparinized-Ti (Hep-Ti) surface. The compositions of pristine Ti and Hep-Ti with or without gentamicin and/or BMP-2 were characterized by X-ray photoelectron spectroscopy (XPS) and the growth of Staphylococcus aureus on the substrates was assayed. Osteoblast functions of all Ti substrates were investigated by cell proliferation assays, alkaline phosphatase (ALP) activity, and calcium deposition. The results showed that the growth of bacteria on GS/Hep-Ti and GS/BMP-2/Hep-Ti was significantly lower compared to that on the pristine Ti and BMP-2/Hep-Ti. In addition, BMP-2/Hep-Ti and GS/BMP-2/Hep-Ti significantly enhanced ALP activity and calcium mineral deposition of osteoblast cells. Taken together, GS/BMP-2/Hep-Ti could achieve the dual functions of excellent antibacterial activity and osteoblast function promotion. Therefore, dual drug (antibiotics and osteoinductive protein)-eluting Ti substrates such as GS/BMP-2/Hep-Ti are a promising material for the enhanced osteointegration and implant longevity in orthopedics and dentistry.

36.P06
In vitro and *in vivo* combination effects of immobilization of PDGF-BB and BMP-2 onto titanium implant on osteoblast function, osseointegration and bone regeneration

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The aim of this study was to examine the combination effects of immobilization of PDGF-BB and BMP-2 onto heparinized-Ti implant on *in vitro* osteoblast function and *in vivo* osseointegration and vertical augmentation of the alveolar ridge. Successful immobilization of PDGF-BB and/or BMP-2 onto heparinized-Ti (Hep-Ti) was confirmed by SEM and XPS, and the two growth factors were released in a sustained manner. The *in vitro* studies revealed that osteoblasts grown on PDGF-BB and BMP-2 immobilized Hep-Ti had a significant increment in ALP activity, calcium deposition, osteocalcin and osteopontin levels as compared to those grown on PDGF-BB alone- or BMP-2 alone-immobilized Hep-Ti. The *in vivo* study showed that osseointegration and bone formation in PDGF-BB and BMP-2-immobilized Hep-Ti was greatly enhanced as compared to that in PDGF-BB-immobilized Hep-Ti. PDGF-BB and BMP-2 immobilized Hep-Ti also displayed improved implant stability, bone volume around the defect area, and intra-thread bone density (ITBD) as compared to BMP-2-immobilized Hep-Ti, but no significant differences were seen between the two groups. From these results, we concluded that the combination effects of immobilization of PDGF-BB and BMP-2 onto Ti on bone formation and regeneration were better.

36.P07
Electrophoretic deposition of bioactive nanocomposite coatings on titanium as a hard tissue implants

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As a coating technique, electrophoretic deposition (EPD) has significant role for biomedical applications, particularly for production of bioactive ceramic coatings on metallic substrates for hard tissue implants. The main advantages of EPD are possibility of controlling the coating thickness and morphology, as well as deposition on metals of complex shapes. Since the primary inorganic component of bone is similar to hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$,HAP), it has been used as coat material for metal implants, like titanium and its alloys. However, the brittleness of HAP limited its wider applications as hard tissue implant. Therefore, it has been observed that biopolymers, such as lignin (Lig), can improve the adhesion and biocompatibility of composite HAP/Lig coatings. However, an antibacterial activity is important property to ensure successful bonding with natural bone. The possibility to prevent the implant infections by using the antimicrobial properties of silver has generated an interest in the development of composite HAP coatings containing silver (Ag/HAP/Lig). The structure, morphology, corrosion stability in simulated body fluid, cytotoxicity and antimicrobial activity of electrodeposited HAP/Lig and Ag/HAP/Lig coatings on titanium were investigated by XRD, SEM and EIS techniques, as well as MTT test and antimicrobial test in suspension, respectively. The obtained results indicated the potential for biomedical applications as a hard tissue implants.

36.P08
In vivo evaluation of titanium implants coated with calcium phosphate or collagen type 1 in osteoporotic rats

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Osteoporosis is defined as a condition in which bone mineral density is severely affected. Consequently, healing process of bone implants placed in osteoporosis is likely negatively influenced by the compromised condition. It can be anticipated that modification of the implant surface, e.g. by inorganic/organic coatings, in osteoporosis can more significantly improve the implant-bone response compared to healthy conditions.
Materials and methods: Sixty female Wistar rats were divided into an ovariectomized (OVX) and sham-operated groups. Bone alterations were assessed to be significant by *in vivo* micro-CT after 4 weeks in OVX rats. Implants coated with CaP (radiofrequent magnetron sputter deposition) or collagen type-1 (electrostatic spray deposition) were placed into femoral condyles and retrieved after 12 weeks for micro-CT and histological analysis. Non-coated implants served as controls. Results: Histomorphometrical bone area (%) was lower around implants in OVX compared to sham rats. Bone-to-implant contact

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(%BIC) was higher for CaP and collagen-coated implants compared to non-coated in OVX and in sham rats. There was no difference in regard to BIC between coated implants in OVX and sham groups.
Conclusion: An osteoporotic condition has a significant effect on the amount of bone as present in close vicinity around implants surfaces. The use of implant surface coatings has a favorable effect on bone-implant interface in osteoporotic as well as in healthy rats.

36.P09
Fabrication of the functionally graded metal-ceramic materials with controlled surface topography, chemistry, and wettability for bone substitution

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Osseointegration is a key property of biomaterials intended for bone tissue substitution. The present work is focused on the surface modification of metallic implants using a combination of various methods such as cold spray (CS), selective laser sintering (SLS), pulsed electroerosion treatment (PEET), and magnetron sputtering to control surface topography (roughness and open porosity), surface chemistry, and wettability, i.e. those characteristics which affect osseointegration. The obtained results show that Ti coatings deposited by CS can be divided into three groups with a characteristic value of average roughness Ra: (i) 4 μm (single particles and agglomerates on the surface), (ii) 22 μm (thin coatings), and (iii) 80 μm (thick coatings). PEET with pulse discharge energies of 0.025 and 0.38 J resulted in the average values of surface roughness of 3 and 8 μm, respectively. During SLS, Ti powder paths were sintered by a laser beam in mutually perpendicular directions to form surface network structures. By varying the distance between the tracks, samples with blind porosity 1.0–5.1′10^{-3} mm^3 were obtained. In order to modify the surface chemistry, multifunctional bioactive nanostructured TiCaPCON films, 1-2 μm thick, were deposited atop the CS, PEET, and SLS samples by sputtering a composite TiC0.5 + Ca3(PO4)2 target. The influence of the surface chemistry and surface topography on adhesion, proliferation, and early stages of osteoblasts differentiation was studied.

36.P10
Osteoblasts defeat fibroblasts on titanium surface during osteointegration steps

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Introduction: Titanium is an excellent implantable material for osseointegration. However, it is still vague why titanium is such adequate material for osseointegration. Soft tissue, associated with fibroblasts, is found at the interface of failed osseointegration, but so far only osteoblasts have been noticed in this field. We hereby focused on the competition between osteoblast and fibroblast.
Method: Two types of cell lines of NIH3T3, a fibroblast, and MC3T3-E1, an osteoblast-like cell, were introduced GFP and DsRed expression vectors, respectively, and their permanent expressing clones, FibGreen and OstRed, were established. FibGreen and OstRed were seeded together on each glass disk vapor-deposited with Ti, Au, Al2O3 or ZrO2, respectively, and were cultured under static or shear stress conditions.
Results: There was no difference in adhered cell number between FibGreen and OstRed on each disk under static condition. On the other hand, under shear stress condition, adhered cell number of OstRed was more than the number of FibGreen on the Ti disk, but not on other disks. The same result could be observed when FibGreen and OstRed were seeded on collagen type 1 under shear stress condition.
Conclusions: In this study, we revealed Ti has advantage of osteoblast adhesion under shear stress condition. Our studies have contributed to progress of osseointegration researches by suggesting two new factors which affect osseointegration, shear stress and cell competition.

36.P11
Atomic layer deposition (ALD) of calcium phosphate (CaP) thin films: a novel method for titanium implant coating

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CaP coating done by plasma spraying (SP) method is the most common in clinical use in order to improve biocompatibility and bone bonding properties of Ti implants. However it presents some serious disadvantages, such as the heterogeneity of the coating, including variable crystallinity, dissolution and microcracks, which sometimes lead to delamination and fragmentation of the CaP layer. This can initiate harmful biological reaction and loosening of an implant causing high cost and morbidity. PS CaP coatings are usually approx. 50–300 μm thick, which circumvents the use of nano- and micropatterning of the implant in order to guide the behavior of cells which come in contact with the implant.In our study we wanted to address these issues and by modifying a method first described by Putkonen et al. (2009) we have deposited 40–60 nm thick CaP coating by ALD on Ti surfaces. FIB-SEM studies showed that the coating conformity was excellent and that it followed even the smallest grooves of the polished Ti substrate. Through pull-out tests we have found that the measured adhesion strength was 37.0 ± 8.1 Mpa resulting in failure between the coating and adhesive. This result may indicate the adhesion strength of the coating and is significantly higher than adhesion strength of SP CaP according to the literature. AFM studies show that the coating does not affect significantly the micro-structure of polished Ti surface; however, an increase of roughness was observed in nano-scale.

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37. Mechanotransduction in TERM

37.01
Keynote: In situ mechanotransduction via vinculin regulates stem cell myogenesis

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Human mesenchymal stem cell (hMSC) proliferation, migration, and differentiation have all been linked to extracellular matrix stiffness, yet the signaling pathway(s) necessary for mechanotransduction remain unproven. We used kinase binding accessibility and cysteine shotgun assays to screen 47 focal adhesion proteins that may function as mechanosensors. Both assays strongly indicated that the talin-vinculin-MAPK1 signaling pathway, which has been implicated in mechanosensing via in vitro experiments, might be a mechanism of regulating hMSC fate in situ. RNA interference-mediated vinculin knockdown significantly decreased stiffness-induced MyoD, a muscle transcription factor, but not Runx2, an osteoblast transcription factor. Reintroduction of vinculin into knocked-down cells indicated that MAPK1 binding site-containing vinculin domains are necessary but not sufficient for hMSC myogenesis. Vinculin knockdown was not observed to interfere with focal adhesion assembly, significantly alter adhesive properties, or diminish cell traction force generation in hMSCs, indicating that its knockdown only adversely affected MAPK1 signaling. However, vinculin sensing without perturbing mechanotransduction may be specific to multiponent versus senescent cells. Together these data identify adhesion proteins that are potential mechanosensors and provide some of the first in situ evidence that one candidate sensor, vinculin, can regulate cell fate and function in a force-sensitive manner.

37.02
Keynote: Differentiation and dynamic loading alter nuclear mechanics and mechanotransduction in mesenchymal stem cells

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Passive and active mechanical inputs from the cellular microenvironment (in addition to soluble differentiation cues) inform lineage transitions in progenitor cells. In this presentation, we will describe recent findings related to how fibrochondrogenic differentiation of mesenchymal stem cells (MSCs), mediated through the addition of soluble differentiation factors, alters nuclear mechanics and structure, cell contractility, and response to applied stretch. Both direct and indirect measures of nuclear mechanics show that the MSC nucleus stiffens appreciably as the cell undergoes fibrochondrogenesis. Moreover, baseline cell contractility (measured via elastomeric micropost arrays) and nuclear connectivity (via altered expression of members of the LINC complex) increase with differentiation. These findings suggest that differentiation itself evokes inherent mechanical changes in progenitor cells that may 'prime' them to differentially transduce exogenous mechanical stimuli. Further work shows that dynamic tensile loading, applied in the absence of soluble differentiation signals, elicits some of these same changes in cell mechanics and nuclear structure and connectivity. Collectively, this work indicates that both the temporal changes in cell and nuclear mechanics that occur with differentiation and mechanical perturbation may alter MSC mechanosensitivity and guide lineage specification in progenitor cells. This work was supported by NIH & Human Frontiers in Science Foundat.

37.03
Elasticity, recruited cell interactions and 3D tissue repair

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Tropoelastin is the soluble precursor to elastin, the main elastic protein found in mammals. Elastin facilitates the reversible deformation of elastic tissues and can withstand decades of repetitive forces. Tropoelastin has a defined 3D shape that exquisitely balances multiple roles, including tissue elasticity, organized assembly and cell interactions. We found that tropoelastin's extensional elasticity can be utilized to expand undifferentiated cells, including progenitors and mouse hemopoietic stem cells. Mechanotransduction is facilitated through the actin-myosin cytoskeleton. An elastic circuitry extends continuously from the extracellular tropoelastin to deep inside the cell, involving elastic communication from the tropoelastin extracellular environment though intracellular myosin II to the nucleus. We found distinct regions of the molecule that are responsible for elasticity and cell binding. Also, we identified a specific non-canonical, integrin-binding motif at the tip of the human protein that dominates interactions with a variety of human cells. Additionally, in a series of recent PNAS papers we decoded the shape of the cell-binding and elastic regions and manipulated their connecting bridge through novel molecules that manifest sophisticated effects on cellular and macro scales. We are using, adapting and modifying these specific interactions to generate functional elastic scaffolds that promote cell attachment, spreading and proliferation in 3D.

37.04
Stress-fiber remodeling in 3D: 'contact guidance vs stretch avoidance'

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When engineering heart valves in vitro, matrix anisotropy is considered vital for long-term in vivo functionality. However, it is not fully understood how to guide matrix anisotropy. Experiments suggest actin-mediated cell traction contributes. Although F-actin orientation in 2D can be guided via uniaxial cyclic stretch, 3D data are lacking. We questioned how cyclic stretch influences actin and collagen orientation in 3D tissues. Using a novel micro-tissue model system, cell-populated fibrous tissues were dynamically/biochemically loaded. Flexible membranes with a rectangular array of silicone posts constrained a mixture of human vena saphena cells, collagen I and matrigel. Constrained tissues were uniaxially stretched (±agents). F-actin orientation was quantified throughout the whole tissue thickness using fiber-tracking software, and was fitted using a bi-model distribution function. Uniaxial cyclic stretching after a static constraint period resulted in F-actin to be orientated perpendicular to stretching, only at tissue surfaces. Strikingly, F-actin orientation was biaxial in the core. Immediate cyclic stretching (before polymerization of the collagen) resulted in strong stretch avoidance, of both F-actin and collagen, throughout the tissue.

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We systematically investigated the effect of matrix perturbation by adding MMP1, ROCK-inhibitor, or decreased collagen density. Results suggest F-actin avoids cyclic stretch in 3D, unless collagen contact guidance dictates otherwise.

37.05
Investigating mechanotransduction in 3D culture using different encapsulating hydrogel systems

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The regulation of cellular morphogenesis via the mechanical properties of the extracellular matrix (ECM) is poorly understood and our group has been working towards elucidating the dominant physical factors of the ECM that influence cell spreading in 3-D culture. In this study, we applied three different types of encapsulating hydrogel biomaterials to study the role of mechanics in 3-D cellular morphogenesis, including biological fibrin gels, semi-synthetic PEG-fibrinogen gels, and completely synthetic transglutaminase-PEG-peptide gels. The mechanical and biodegradation properties of the materials were characterized by in situ rheometry and fluorescence spectrometry. Hydrogel constructs were cast with human dermal fibroblasts and cultivated for up to 7 days. Cell spreading in the 3D cultures were documented by f-actin labeling using scanning confocal microscopy. Each matrix type was able to support the time-dependent cell spreading within the material, but each type of hydrogel system exhibited distinctly different patterns of multicellular organization within the gels. Moreover, the mechanics of each hydrogel had a strong influence on cell morphogenesis; however, biodegradation appeared to have a similar impact on the morphogenesis across the different biomaterial platforms. We conclude that the bulk material properties, independent of the type of material, may be the dominant and influential factors affecting morphogenesis patterns of mesenchymal cells in 3D culture.

37.06
Mechano-transduction pathway interference with BMP-2 signaling cascade

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The bone morphogenetic proteins (BMP) growth factors are essential signaling molecules in embryogenesis, tissue regeneration or cancer metastasis. Until now, the cellular effects of these proteins have mostly been investigated by adding them in solution in the cell culture medium even though growth factors are naturally not free to diffuse and are rather bound to ECM components including proteins and polysaccharides. In this work, we design a new biomimetic nanoassembly based on polyelectrolyte multilayer film made of a polypeptide and of hyaluronan which combines both matrix-bound presentation of growth factor bone morphogenetic protein 2 (BMP2) and modulation of the material's mechanical properties. We investigated the interplay between the stiffness of a biomaterial and the matrix-bound presentation of the growth factor BMP2 and its impact on cellular processes. Whereas C2C12 myoblast cells are round and poorly spread on soft films, cells displayed an increase of spreading and migration. By using a combined set of techniques including SiRNA strategy and pharmacological approach, we study how the mechano-transduction pathway can potentially interfere with the BMP-2 signalling cascade. We demonstrated a crosstalk between specific integrin and BMP2 receptors. Our results pointed out that signaling can be enhanced by the spatial association of integrin and BMP2 receptors.

37.P01
Development of biomaterials for cellular differentiation using a metabolomics approach

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Understanding how to mimic the physical characteristics of the extracellular matrix in vitro is an invaluable tool with regards to being able to target stem cell differentiation along selective cell lineages. One such physical characteristic is the innate elasticity of the cell substrate. Studies have shown that the rigidity of a substrate has considerable influence over cellular behaviourisms such as migration and differentiation. Here we make use of liquid chromatography coupled to high mass accuracy mass spectrometry (LC-MS) as an established method for monitoring small changes in cell stasis. As such, this can be exploited to envisage the cellular metabolome (the entire array of metabolites that exist within a cell at any point in time). The interpretation of external cues by stem cells causes the activation or deactivation of several proteins, consequently causing shifts in the metabolome. This effect renders the metabolic profile of a cell to be highly indicative of its phenotype at that point in time. This is potentially of significance in stem cell research as the cells are metabolically quiescent in their self-renewing state in their natural niches and the metabolome is thought to become up regulated during differentiation. Investigating MSC metabolites during directed differentiation has enabled scrutiny of the cell behaviour as phenotype is altered and has the potential to influence the manner in which biomaterials are designed for cell culture in vitro.

37.P02
Mesenchymal stem cell differentiation is determined by the biophysical nature and biochemical composition of the pericellular environment

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Both hydrostatic pressure (HP) and substrate stiffness (SS) have been shown to independently regulate MSC differentiation. The objective of this study was to explore the interplay of SS, HP, and the role of integrin binding on both mechanotransduction and the differentiation of MSCs. Bone marrow-derived MSCs were seeded into 1, 2, or 4% agarose hydrogels. The hydrogels were subjected to 10 MPa of HP for 4 h/d at a frequency of 1 Hz for 3 weeks. Scaffolds were cultured in a chemically defined media supplemented with either human TGF-β3 (with or without the integrin blocking RGDS peptide) or 16% FBS. MSCs cultured in the presence of FBS underwent osteogenesis in stiffer hydrogels. Increasing the SS of the hydrogels maintained in the presence of TGF-β3 led to a decrease in sGAG and collagen synthesis. HP was found to significantly increase sGAG production in the stiffest 4% agarose hydrogels. The addition of RGDS negated the effects of both SS and HP on chondrogenesis of MSCs. In conclusion, this study demonstrates that stiffer gels support an osteogenic phenotype but suppress chondrogenesis of MSCs. Both the application of HP and the addition of RGDS overcame the inhibition of chondrogenesis in stiffer hydrogels, raising the possibility that HP exerts its pro-chondrogenic effects by interfering with integrin binding or its downstream effects. Further support for this hypothesis is found in the observation that HP does not enhance chondrogenesis in the presence of RGDS.

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37.P03
Si-HPMC hydrogels for tissue engineering

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Tissue engineering is a multidisciplinary field combining chemical, engineering and life science expertises in order to develop functional structures for damage tissues. Cells are generally seeded into the extracellular matrix (ECM). These biomaterials are prepared as a temporary scaffold to improve mass transfer, support cell growth and enhance the cells to produce molecules they are supposed to. Until recently, it was believed that biomaterial selection was performed based on its macroporous, biocompatibility and degradability properties and its ability to withstand biomechanical stress. Engineering and material issues are now under focus to develop suitable scaffolds for tissue engineering. Indeed, Discher et al.1 have shown that material stiffness induces critical effects on cell behavior and differentiation. Therefore, LIOAD has been working on the development of hydrogels as ECM for tissue engineering. They prepared a cellulose based polymer capable of self cross-linkage (Si-HPMC) to avoid any toxicity issues resulting from using cross-linking chemicals or photo cross-linking. The objectives of the present work are to modulate and study the stiffness of Si-HPMC hydrogels by adding particles as reinforcement. These reinforced networks, will then be used to grow stem cells in 3D and their differentiation toward a chondrogenic lineage will be studied.

37.P04
Substrate stiffness modulates phenotype and gene expression in neonatal cardiomyocytes in vitro

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Biomaterials to be used as instructive environment to regulate cell fate in vitro can potentially play an important role to set up alternative therapies in the field of cardiac regenerative medicine. Synthetic materials usually display mechanical properties which are far from those of the native myocardium and thus could affect host cell survival and activity. Here, the mechanical properties of inert poly-ϵ-caprolactone (PCL) planar layers were tuned to change surface stiffness without changing matrix chemistry. These substrates were challenged with neonatal cardiomyocytes to study the possible effects of substrate stiffness on such cell behavior. The results show that on softer substrates (0.91 ± 0.08 MPa and 1.53 ± 0.16 MPa) cardiomyocytes exhibited well organized sarcomeric structures and a higher overall cell survival rate. On the other hand, a reduced number of beating areas was detected on stiffer materials (49.67 ± 2.56 MPa and 133.23 ± 8.67 MPa), very likely as a result of the impaired sarcomeric structure and the reduced electromechanical coupling. Also, the differential expression of mid-stage cardiac genes as well as a number of genes involved in cell-matrix interaction could also be observed. Altogether, these data indicate that a shift in cardiomyocyte cell signaling induced solely by the substrate stiffness can occur as a result of changes in matrix mechanical properties, thus opening new arguments for the use of biocompatible polymers in cardiac tissue engineering.

37.P05
Smart thermo-responsive polymers with tuneable stiffness to control stem cell adhesion, proliferation and fate

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Bone marrow-derived mesenchymal stem cells (MSCs) have been proposed as a promising candidate to be utilized in tissue engineering and cell therapy applications due to their multipotency and immunomodulatory activities. In the present investigation, inert poly-?-caprolactone (PCL) planar layers were manufactured to obtain thermo-responsive films displaying tuneable surface features (with Young moduli ranging from KPa to MPa values) without changing matrix chemistry. Substrate mechanical properties were finely tuned by dynamically changing culture temperature around the body tissue range and challenged with MSCs in proliferation and differentiation media. Stem cell response to matrix elasticity was assessed in terms of cell adhesion, proliferation and differentiation ability. Moreover, to decipher the molecular mechanisms that regulate adult stem cell plasticity, the expression of a number of genes involved in stem cell adhesion, maintenance and differentiation function was assessed by targeted real- time PCR arrays. Significant differences in human MSC signalling could be found as a response to substrate properties, although stem cell responsiveness as a bulk to differentiating signals was not impaired. Altogether these data suggest that matrix mechano-physical cues can indeed interfere with stem cell behavior and reveal the complexity of cell response to such stimuli.

37.P06
Effects of substrate stiffness on myoblasts phenotype and differentiation

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The sensitivity of different stem and progenitor cells to substrate stiffness has been compellingly demonstrated. When contractile cells (i.e. muscle cells) are grown on surfaces with different stiffness, an effect of such parameter on cell phenotype and function is hypothesized In this respect, in the present study inert poly-e-caprolactone (PCL) planar layers of various elastic modulus (with Young modulus ranging from kPa to MPa) were tested with rat skeletal myoblasts (Myb01) in order to elucidate a possible role for matrix or tissue stiffness in striated muscle differentiation. The cells demonstrated to adhere without significant preferences to all the substrates and the spontaneous occurrence of myotube formation could be noticed on all the surfaces tested, independently of substrate stiffness. Furthermore, numerous studies have also implicated a role for surface nanotopography affecting the cell response, with both increased and decreased adhesion. Surface topography may be key in determining cellular response by modulating focal complex formation and maturation. Therefore, Myb01 were challenged on PCL films with nanometric lines and their adhesion, distribution and cell morphology were analyzed. Preliminary results showed that substrate nanotopography can influence cell morphology and distribution but the mechanisms governing this effect remain so far undisclosed.

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37.P07
Superhydrophobic platforms for the combinatorial analysis of biomaterials-cells interactions using arrays of 3D scaffolds with distinct mechanical and morphological properties

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High-throughput studies of cells mechanotransduction are usually performed using 2D biomaterials. However, cells-extracellular matrix interactions in the body occur in 3D environment. By using cells entrapped in hydrogels, it is not easy to isolate the mechanical effect from the chemical cues of biomaterials. We used a cytocompatible and non-expensive platform based in the patterning of wettable spots in superhydrophobic surfaces to deposit porous biomaterials in these regions. Freeze-dried alginate/chitosan scaffolds were used to create an array of mechanical properties and porosities. Adaptation of dynamic mechanic analysis equipment allowed performing on-chip single scaffold analysis. Micro-computed tomography allowed acquiring data for whole chips simultaneously. Results were validated using individual scaffolds and single-formulation chips. A sub-array with combined modulus/porosity properties was selected. Fibronectin (Fn) in different concentration was adsorbed in the scaffolds, and fibroblasts and osteoblast-like cells were seeded. The independent study of variables influence in cell response was performed by image-based methods. In the absence of Fn fibroblasts did not respond to mechanical properties in the chip range. Osteoblast-like cells showed higher cell adhesion in stiffer substrates. The adsorption of Fn was studied qualitatively by image methods. Results related with Fn amount and scaffolds' mechanical properties were analyzed for each cell type.

37.P08
Extrinsic biophysical stimulus can override the influence of local substrate in determining stem cell fate

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The aim of this study was to explore how cell-matrix interactions and extrinsic mechanical signals interact to determine stem cell fate in response to transforming growth factor-β3 (TGF-β3). Bone marrow derived mesenchymal stem cells (MSCs) were seeded in agarose and fibrin hydrogels and subjected to dynamic compression in the presence of TGF-β3. Markers of chondrogenic, myogenic and endochondral differentiation were assessed. Free-swelling agarose constructs stained positively for chondrogenic markers, and appeared to be progressing towards terminal differentiation as indicated by collagen type X and mineral staining. Dynamic compression suppressed differentiation along this endochondral pathway. In contrast, fibrin constructs supported differentiation along an alternative myogenic pathway in long-term free-swelling culture. Given that fibrin clots support a chondrogenic phenotype in vivo within mechanically loaded joint defect environments, the influence of long term dynamic compression on MSC differentiation was investigated. Mechanical signals generated by this extrinsic loading ultimately governed MSC fate, directing MSCs along a chondrogenic pathway as opposed to the default myogenic phenotype supported within unloaded fibrin clots. In conclusion, this study demonstrates that external cues such as the mechanical environment can override the influence of specific substrates, scaffolds or hydrogels on determining mesenchymal stem cell fate.

37.P09
Evaluation of cell response on permanent and pulsed atmospheric pressure stressed cells

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Introduction: Many studies display that mechanical stimuli already fulfill the needs for cell differentiation, without any need for additional growth factors. Thus, we concentrated on mechanical stimuli of atmospheric pressure on cells using a tailor-made pressure chamber. Aim of the study was to determine pressure limits of cells and possible side effects to find supporting conditions for cell proliferation or differentiation.
Materials and methods: Cells of the cell lines U937 and MG63 were permanently kept at 37°C and buffered with HEPES during the experiments. The atmospheric pressure was applied on 1- respectively 2-well-chamberslides in the pressure chamber. For static pressure experiments, cells were permanently stimulated at 200, 300 or 400 kPa for 1 respectively 12 h and cultured for 2 weeks afterwards. For dynamic pressure experiments, pressures between 300 and 400 kPa were applied at frequencies of 0.25–4 Hz. Cell growth was observed for 5 days and evaluated by trypan blue staining and MTT assay.
Results: We could not observe any negative or positive effects on cell proliferation after the static pressure mode experiments. In the dynamical pressure experiment, 24 h after last pressure exposure, cells restored the original conditions and gave the same read-out compared to the untreated control.
Discussion: Thus, our tailor-made pressure chamber seems to be appropriate to find more specific parameters that induce cell differentiation of adult stem cells.

37.P10
Epigenetic modification of beta-catenin in 3D Titania mesenchymal cell cultured scaffolds under variable flux conditions in bioreactor chambers

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Mesenchymal stem cell fate determination is heavily regulated by the WNT pathway through β-Catenin.Biomaterial topography triggers cytoskeleton biochemical and physical responses, which will ultimately modify gene expression leading to an osteoblastic phenotype. It is already known that several cytoskeleton related genes are mechanoregulated. In this study as a result of exploring epigenetic regulations by methylation levels of Beta-Catenin, a more accurate insight of osteogenic control could be achieved. Engineering a stem cell niche is a task that considers not just a 3D scaffold which will mimic a cellular microenvironment, but also the dynamic forces exerted on cultured stem cells.A functional tissue engineering (FTE) method could be completed by identifying methylation patterns within dynamic culture models.Titania (TiO2) scaffolds were synthesized with pore sizes ranging from 300–400 μm by Direct Ink Writing and cultured with human mesenchymal stem cells. In order to evaluate the existence of potential differential methylation rates, scaffolds were placed in a bioreactor chamber under pulsed flux conditions (2, 6, and 12 h). A non flux control group was used. Methylation specific polymerase chain reaction (MSP) and direct sequencing showed how CpG islands related to Beta Catenin are being directly modified under variable flux conditions

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when compared to a static culture. Canonical WNT pathway could be therefore being mechanoregulated through Beta-Catenin.

37.P11
Ingrowth of bone cells within cryogels under compression and perfusion

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Bone tissue engineering involves mimicking and creating a complex biomechanical environment for cell-cell and cell-matrix interactions. Such an environment should promote maturation of the cell-scaffold construct in vitro. Various bioreactors have been designed aiming at not only providing better nutrient and gas exchange but also applying mechanical strain on the constructs. The combination of strain types, such as tension, compression, or shear, and the strain dynamics, for instance, constant, cyclic, intermittent, in a bioreactor give a great opportunity to optimize the culture environment for different type cells. The combined application of mechanical force in bioreactor direct cellular activity and maintain cell phenotype in some engineered tissues. Previous studies showed that for an optimal supply of the cells with nutrients and gas within the scaffold, the cultivation is ideally performed in a bioreactor system, since static cultures are insufficient to mimic the in vivo conditions. Our aim in this study is to evaluate the interaction of biodegradable cryogels with cells at different regimes in bioreactors for possible tissue engineering applications. 3D-cell ingrowth within biodegradable cryogel-scaffolds with interconnected macropores was studied in bioreactors at different regimes (static,dynamic) with an osteoblast-like cell-line. Dynamic conditions (perfusion and/or compression) have a significant impact on cell ingrowth and extracellular matrix synthesis.

37.P12
Primary cilia removal inhibits osteoblastic matrix-forming response to mechanical stimuli

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Introduction: Mechanical forces have been shown to regulate bone cell differentiation and are used in many laboratories to accelerate bone tissue engineering. One suggested sensory mechanism responsible for mechanotransduction is the primary cilia, a microtubule-based structure extending from the apical surface of most mammalian cells.
Aims: To confirm whether or not primary cilia removal inhibits the matrix forming response of bone cells to mechanically induced fluid shear stress.
Materials and methods: MLO-A5 late stage osteoblastic cells were seeded into gelatine-coated 6-well plates containing complete alpha MEM with ascorbic acid and beta-glycerophosphate. At days 3 and 7, half the cells were treated with chloral hydrate to remove the primary cilia. At days 5–7 and 9–11, cells were subjected to 2 h/day of oscillatory fluid shear stress (FSS). At day 12, calcium and collagen deposition were assayed using Alizarin red staining, and second harmonic generation imaging respectively.
Results: Cells subjected to FSS had higher collagen production and calcium deposition compared with static controls. Removal of the primary cilia resulted in the inhibition of the mechanically induced increases in extracellular matrix production.
Conclusion: The primary cilia play a role in facilitating mechanically induced matrix formation by bone cells; this has implications for bone diseases such as osteoporosis as well as in the design of bioreactor conditions for bone tissue engineering.

37.P13
Arterial shear stress accelerates the differentiation of endothelial progenitor cells adhered on VEGF-bound surface

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Introduction: Endothelialization of intravascular artificial devices may promise to express nonthrombogenic potential. To this end, we are developing the surface technology of in situ capturing of endothelial progenitor cells (EPCs) on VEGF-bound device surface under arterial flow, followed by rapid proliferation and differentiation into endothelial cells (ECs). In fact, our extensive stent implantation study in porcine model showed capture of EPCs and subsequent endothelialization. In this study, we investigated how differentiation potential of EPCs on VEGF-bound surface is influenced by a simulated arterial shear stress.
Materials and methods: EPCs, obtained from human peripheral blood, were cultured on VEGF-bound surface under a simulated arterial flow (shear stress 15 dynes/cm^2; corresponds to arteries) for 24 h using the parallel plate-type device.
Results: EPCs adhered well and proliferated on VEGF-bound surface. The loading of shear stress suppressed the expression of mRNAs (CD34 and CD133) specific to EPCs, promoted the expression both mRNAs {CD31 and von Willebrand factor (vWF)} and protein (vWF) specific to ECs. The loading of shear stress promoted the expression of ephrinB2 mRNA specific to arterial ECs.
Conclusion: The arterial shear stress accelerated differentiation of EPCs in vitro, strongly suggesting that the hydrodynamic shear stress is one key factor for rapid endothelialization in vivo. This supports evidence of rapid endothelializatoin in porcine model.

37.P14
Evaluation of the osteogenical effects of a low-frequency electromagnetic field on adipose-derived stem cells

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An electromagnetic field is one type of biophysical stimuli (signals) which affect cellular activities. Because of these effects, electromagnetic field therapy has been approved for bone disorders in animals and humans. In previous study, we found specific conditions of the electromagnetic field to be both positive for osteogenic differentiation of adipose-derived stem cells (ADSCs). To further understand mechanisms of ADSC osteogenic differentiation using the electromagnetic field, we evaluated the effects at this positive condition in vitro / in vivo. Gene expression profiles of osteogenically induced ADSCs were examined using microarray analysis. About 242 genes were affected during electromagnetic field stimulation. Additionally, pathway analysis indicated the potential roles of Wnt/BMP signalling in ADSCs during osteogenic differentiation. Acceleration of osteogenic differentiation of ADSCs was confirmed by the mouse calvarial defect model.

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Pre-treatment of ADSCs using the electromagnetic field increased bone formation compared to the non-treated group based on histology assay. In this study, we investigated the mechanisms of the electromagnetic field on osteogenic differentiation of ADSCs and confirmed the effects in vivo, providing significant information toward improved understanding of the use of an electromagnetic field for better bone tissue engineering applications.

37.P15
Remote activation of Wnt signalling pathways in human mesenchymal stem cells using magnetic nanoparticle technology

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The Wnt signalling pathway plays important roles in embryonic development, tissue patterning and the differentiation of stem cells in many adult tissues. This makes wnt signalling an attractive target for regenerative medicine and tissue engineering. Wnt signals are transduced through a number of cell surface receptors, including the Frizzled class of receptors which has been shown to play a role in mechanotransduction. Our previous work has demonstrated the potential of magnetic nanoparticle technology to remotely target membrane bound mechanosensitive proteins and receptors (Hughes, McBain et al. 2008). The application of an alternating magnetic field causes a translational torque in the membrane bound particles. The mechanical stimulus is then transduced through mechanosensitive proteins to initiate biochemical signals and consequent signalling pathways. Here we demonstrate the use of functionalised magnetic nanoparticles to target and activate the frizzled receptor in human Mesenchymal Stem Cells (hMSC). Cultures of hMSC's were labelled with 250 nm magnetic nanoparticles functionalised with defined peptide targets and antibodies. Cultures were stimulated for different durations in an oscillating magnetic force bioreactor (MICA Biosystems) to create a targeted compressive force on the Frizzled receptors. Gene expression analysis and immunofluorescence demonstrated a time course of activation of downstream signalling pathways following nanoparticle treatment and loading.

37.P16
Pathways implicated in the mechanosensing of chondrocytes

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Aim: In a recent study we characterized the mechanosensing of chondrocytes. Here we describe some events in the pathways implicated.
Methods: Rat chondrocytes were cultured in collagen hydrogels of different stiffnesses (2–20 Pa) in normoxia and hypoxia, in monolayer and embedded inside hydrogels. First, culture on hydrogels was performed using antibodies to block a series of integrins. In a second experiment, custom RTPCR array plates were used to confirm the up-regulation of integrins selected and to detect changes in expression of other proteins implicated in different signaling pathways.
Results: The results allowed us to identify myosin II and integrins alpha1, alpha5, beta1 and beta3 as participants in the mechanosensing, since their blockade inhibits the sensing of the stiffness, and they are up-regulated in the process. RTPCR arrays detected the role of Paxilin, RhoA, Fos, Jun and Sox9. We detected no expression of src in the monolayer cultures, but we found a role for this protein in 3D. The expression of HIF1-α was not modified under normoxia but was found to participate under hypoxia. Finally, FAK, showed a direct relationship with the expression of aggrecan in hypoxia and an inverse one in normoxia.
Conclusions: We suggest some of the molecular signaling events that are triggered during the process of chondrocyte mechanosensing.

37.P17
Mechanosensing of chondrocytes in collagen hydrogels

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Expansion of chondrocytes is an unsolved issue in the production of in vitro cartilage cells since it yields fibroblast-like chondrocytes. The physical properties of the substraste has become an interesting field for the research of the preservation of the cartilage phenotype. We have developed a model for the culture of rat cartilage cells in collagen hydrogels, which resulted to have 2–20 Pa as their stiffness. Experiments were performed using normoxia and hypoxia conditions and 2 and 3 dimensional cultures. We could observe a clear effect of the matrix stiffness in the chondrocyte phenotype, showing an improvement of the expression of cartilage related genes in the more compliant gels, which have been already described for higher stiffness ranges. Surprisingly, the exposure to hypoxia completely inverted the mechanosensing showing an improved phenotype in the more stiff hydrogels. This mechanosensing was maintained during the plastic expansion of chondrocytes, which showed the same capacity up to the 8th passage. Using blockade experiments we detected non muscle myosin II, and integrins alpha1, alpha5, beta1 and beta3 as participants in the mechanosensing. We also described a direct role of Paxilin, RhoA, Fos, Jun and Sox9 in the mechanosensing. Finally, src resulted to have a role in the switch to 3 dimensional culture, and HIF-1 and FAK in the effect of hypoxia. In summary the chondrocyte phenotype can be modulated by switching the mechanical properties of the substrate.

37.P18
Correlation of fluid dynamic with single cell mechanics and cell response to mechanical stress

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There is increasing evidence that mechanical factors can significantly influence the functional development of tissues and play a major role in controlling stem cell fate and lineage determination. However, when used as therapeutic products, how cells response to its mechanical environment during cold shipping of cell suspension is not well studied. Here we would like to report our findings on how different vibration parameters, including frequency, acceleration rate and displacement, would affect the flow dynamic of cell suspension and cell viability. Further experiments combining of fluorescence microscopy and atomic force microscopy revealed the variation of cell membrane mechanics with the progress of cell death, allowing correlation of change of mechanical properties of single cell membrane with the dynamic of the flow and the observed biological consequences. Our study revealed that investigated human mesenchymal stem cells demonstrated different membrane stiffness, as evidenced by the variation of young's modulus measured by AFM, and it was dependent on its membrane integrity and on whether phosphatidylserine was presented at the outside wall of cell membrane. Cells in suspension are sensitive to vibration at certain frequency and acceleration rate and cells proceeding to the oncosis routes are more susceptible to mechanical damage.

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37.P19
Train the chondrocytes: Mechanostimulation reverses the catabolic phenotype of human matrix-embedded chondrocytes - a preliminary report

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The goal of this study was to determine the effect of cyclic mechanical stimulation on matrix-embedded human osteoarthritic chondrocytes. Articular cartilage was obtained from osteoarthritis patients subjected to total knee arthroplasty. Osteoarthritic chondrocytes were embedded in a collagen I-matrix. After 14 days of cultivation, cell-seeded matrices were either mechanically stimulated (for 4 days) or further cultivated without stimulus. For measurement of gene expression, chondrocytes were isolated and mRNA levels of genes known to be affected in diseased cartilage (MMP-3, MMP-13, Col II, aggrecan) and its alteration upon mechanical stress was investigated via RT-PCR. Moreover, morphology and distribution of chondrocytes in the differentially treated matrices were histologically determined. Results were expressed as ratios of aggrecan and Col II to MMP-3 (MI3) and MMP-13 (MI13), respectively. Both, MI3 as well as MI13, significantly increased by mechanostimulation compared to control indicating an 'anabolic shift'. Moreover, chondrocyte morphology proved to be drastically altered by appearing round rather than spindle-shaped in histological slices of stimulated compared to control matrices. Our findings suggest the importance of mechanical stress for metabolism and function of chondrocytes and indicate that the supposed catabolic phenotype of matrix-embedded osteoarthritic chondrocytes might be reversible by mechanostimulation.

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38. Developmental Biology and Tissue Engineering

38.01
Keynote: Engineering of renal-like tissue using in vitro developmental models

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Engineering of kidney-like tissue could have a broad impact on the treatment of declining or poor renal function. For many years, kidney development has been analyzed using in vitro and ex vivo models for morphogenesis and differentiation. These are generally 3D cell culture or modified organ culture systems. Our lab has been employing combinations of such models, including several developed by us, to "engineer" renal-like tissue. Nephronal structures capable of recruiting a glomerular vasculature are evident. Markers of differentiation appear as does tubular function as measured by organic anion transport capacity. Inherent in the strategy is the potential for propagation of engineered renal-like tissue.

38.02
Formation of tissue structure by engineering epithelial progenitor cells of glandular organs by biomaterial cultivation

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Epithelial glandular organs mediate enzymes, metabolites, and fluid transport and feature ramified structures. By forming complex branches, different cells are well-organized and the network is established to fulfill physiological functions. To regenerate a functional glandular organ, recapitulating the formation of the tissue-specific structure is required. The current study is to explore the feasibility by engineering epithelial progenitor cells in a biocompatible biomaterial system without exogenous serum or growth factors. The fetal mammary gland (MG) was selected as the model organ. Among many biomaterials, chitosan demonstrated a superior effect in promoting branching in MG epithelial progenitor cells. The morphogenetic effect to promote MG branching was chitosan-specific because it was not observed in other analogues. The molecular weight and specific linkages in the chitosan polymer were important parameters in mediating the morphogenetic effect. The engineered epithelial progenitor cells of MG from different anatomical locations all effectively generated structure formation. Blocking endogenous fibroblast growth factor 10 (FGF10) inhibited the morphogenetic effect of chitosan, indicating that the chitosan effect was FGF10 dependent. This work demonstrates the feasibility of establishing a clinical translatable biocompatible system that efficiently recapitulates tissue morphogenesis by engineering epithelial progenitor cells with biomaterials.

38.03
Engineering MSC condensations: an example of skeletal developmental engineering

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The primordial skeleton appears first as a formation of high density regions of mesenchymal stem cells (MSCs) whose size, shape and arrangement foreshadow the future skeleton. Skeletal condensations result from a transient upregulation of cell adhesion molecules and extracellular matrix proteins. Here methods for artificially inducing high-density MSCs or MSC/chondrocyte condensations by manipulating the cell-cell interaction strength are explored. Our primary approach is the formation of nanofilms around cells using the layer-by-layer technique. Cells are incubated in alternating solutions of a polyelectrolyte pair, using titration to maximize cell viability during nanofilm buildup. The nanofilms were composed of carbohydrates and proteins, allowing electrostatic and covalent interactions which promoted rapid assembly of tissues from cell solutions. Nanofilm buildup of oxidized chondroitin sulfate, poly-L-lysine and others was followed by quartz crystal microbalance with dissipation (QCM-D). The formation of films on chondrocytes and stem cells increased the interaction between adjacent cells and resulted in both an increased size of the cell agglomerations and cell packing density as well as reduced space between each agglomeration. By tailoring the surface properties of cells, we can engineer the interaction of one or more populations of cells and form cellular structures based on the relative adhesion strength between each population of cells.

38.04
Mechanical stimulation programs mesenchymal stem cells during valvulogenesis

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Embryonic valvular malformations are among the more serious of congenital heart defects, predisposing the valve to diseases or disorders that compromise its hemodynamic function. How these primitive cushions mature into thin fibrous leaflets may provide a blueprint for effective valvular repair and regeneration strategies. We aim to understand and direct the differentiation of atrioventricular cushion (AVC) mesenchyme and chick bone marrow mesenchymal stem cells (cBMSC) through mechanically stimulated in vitro tissue models. We have developed ultrasound techniques to characterize in-vivo strain profiles for approximating developing cushion tissue deformation and found that the HH27 septal LAV leaflet were estimated at 12%. We then isolated and exposed HH25 AVC mesenchyme to 12% equibiaxial strain at 2 Hz for 24 h, with static cultures as controls. We quantified mRNA expression of TGFB3, BMP2, and VEGFA via qPCR, normalizing to 18S. We found a significant downregulation in TGFB3 with strain, but a significant upregulation of both BMP2 and VEGFA ($P < 0.05$). Interestingly, mechanical strain had an opposite effect on cBMSC's, significantly upregulating TGFB3, but such that their absolute level approached that of the AVC under strain. Taken together, these results suggest that mechanical forces regulate the differentiation of valvular precursors via the BMP2/TGFB3 pathway, and this mechanism may be used to direct stem cells toward valvular lineages.

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38.05
Tissue reconstruction potency of Oct4 expressing somatic cells

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While various adult stem cells have been reported to exist in different tissues, discussions continue as to whether Oct4 expressing stem cells naturally exist in the adult body. Therefore, the potential of oct4 expressing somatic cells is still elusive. To obtain Oct4 expressing somatic cells, cells were isolated from adult tissues representative of the three different germ layers, derived from B6-Oct4/GFP transgenic mice. The isolate was triturated to disrupt mature cells and then propagated as non-adherent clusters or spheres in a serum-free culture medium. Regardless of the tissue from which they had been isolated, Oct4 expressing spheres were observed after 5–7 days. GFP (Oct4) expressing cells in spheres from each tissue contained cells positive for SSEA-1, E-cadherin and expressed alkaline phosphatase activity. The gene profiles expressed are recognized as embryonic stem cells markers and suggestive of pluripotency. Furthermore, Oct4 expressing cells showed widespread demethylation of Oct4 and Nanog gene promoter regions similar to that seen in ES cells. To examine the differentiation potential of cells, Oct4 expressing cells were seeded on biodegradable scaffolds and implanted into immunodeficient mice. Oct4 expressing somatic cells generated 3D-structured tissues derived from all three germ layers in vivo.

38.P01
Chondrogenesis of mesenchymal stem cells mediated by the combination of SOX trio gene

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Target gene transfection for desired stem cell differentiation has recently become a major issue in cell therapy. For the safe and stable delivery of genes into human mesenchymal stem cells (hMSCs), we employed a non-viral gene carrier system such as polycataionic polymer, poly(ethyleneimine) (PEI),polyplexed with a combination of SOX5, 6, and 9 fused to green fluorescence protein (GFP), yellow fluorescence protein (YFP), or red fluorescence protein (RFP) coated onto PLGA nanoparticles. The transfection efficiency of PEI-modified PLGA nanoparticle gene carriers was then evaluated to examine the potential for chondrogenic differentiation by carrying the exogenous SOX trio (SOX5, 6, and 9) in hMSCs. Additionally, use of PEI-modified PLGA nanoparticle gene carriers was evaluated to investigate the potential for transfection efficiency to increase the potential ability of chondrogenesis when the trio genes (SOX5, 6, and 9) polyplexed with PEI were delivered into hMSCs. SOX trio complexed with PEImodifiedPLGA nanoparticles led to a dramatic increase in the chondrogenesis of hMSCs in in vitro culture systems. For the PEI/GFP and PEI/SOX5, 6, and 9 genes complexed with PLGA nanoparticles, the expressions of GFP as reporter genes and SOX9 genes with PLGA nanoparticles showed 80% and 83% of gene transfection ratios into hMSCs two days after transfection, respectively.

38.P02
Depolarization of pre-differentiated human mesenchymal stem cells modulates differentiated state while maintaining lineage plasticity

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Bioelectric signaling regulates biological functions from the cell level (proliferation, differentiation, migration) to the organ level (tail and limb regeneration). Recent studies have uncovered a functional role for bioelectric events in differentiation of mammalian stem and progenitor cells, which may be capitalized upon to control stem cell behavior for tissue regeneration. Because the differentiated cells present in a wound also participate in healing, we investigated the effects of membrane potential modulation on pre-differentiated human mesenchymal stem cells (hMSCs) to determine how mature cells respond to electrical modulation. When depolarized by high potassium levels, pre-differentiated osteoblasts and adipocytes exhibited lower expression of tissue-specific markers than untreated cells, an effect comparable to cell de-differentiation by removal of differentiation stimulants. Gene expression profiling of depolarized osteoblasts revealed several pathways that may mediate this depolarization response. We also investigated whether depolarized cells retained the ability to undergo subsequent transdifferentiation. When transdifferentiated toward the adipogenic lineage, depolarized osteoblasts acquired an adipogenic phenotype and decreased their osteoblastic phenotype. Membrane potential modulation can thus be used as a control mechanism to induce stem-derived cells to de-differentiate and maintain a degree of plasticity despite their previous differentiated state.

38.P03
Wnt signal pathway network genes expression in cell reprogramming and maintenance of pluripotency

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Wnt signaling activity contributes the regulation of many cellular functions, including proliferation, migration, cell fate specification, maintenance of pluripotency and induction of tumorigenicity. Cell reprogramming can convert somatic cells to ESC-like cells that was named 'induced pluripotent stem cells (iPSCs), by using direct transduction of a cocktail composed of only four pluripotent transcription factors: Oct4, Sox2, Klf4 and c-Myc. Recent findings show that modulation of the Wnt/beta-catenin signalling pathways strikingly enhances somatic-cell reprogramming. Although Wnt signal transduction can promotes cell reprogramming process in vitro, their roles in differentiation processes and maintenance of pluripotency remain poorly explored. In our study, we performed gene expression including Fzd1, Ccnd1, BtrC, Axin1, APC and Adar that these genes were defined to play functions in Wnt signal pathway network in mouse induced pluripotent stem cells (miPSCs), mouse embryonic stem cells (mESCs) and mouse bone marrow-derived mesenchymal stromal cells (mBMMSCs) with comparing mouse embryonic fibroblasts (MEF) using quantitative reveres transcription polymerase chain reaction (qRT-PCR). We found that the expression of these genes was significantly reduced in miPSCs but not mESCs which is condition of maintenance for pluripotency. Our results indicated that although Wnt signal transduction can enhance cell reprogramming in vitro, probably these processes are conversely inhibited.

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38.P04
Integrative studies on the role of Ccbe1 in cardiogenesis: from the embryo to ES cell derived cardiac tissue.

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A central challenge for the future perspective of cardiac regenerative medicine is the generation of large numbers of patient-specific cardiac myocytes. Ccbe1 encodes a secreted molecule that was firstly identified using an Affymetrix GeneChip differential screen for chick heart precursor cells expressed genes (Bento et al., 2011). In mouse and chick, Ccbe1 is expressed in major cardiac progenitor lineages that contribute to distinct heart structures during heart organogenesis (Facucho-Oliveira et al., 2011). Moreover, analysis of gain and loss of function performed in both mouse and chick embryos showed abnormal cardiac morphogenesis and aberrant chamber formation further elucidating the role of Ccbe1 for cardiac development. Similarly, in mouse and human ES cells, increased levels of Ccbe1 expression were detected after cardiac lineage commitment demonstrating well-coordinated expression of various early and late cardiac specific markers and Ccbe1. Modulation of mCcbe1 activity in differentiating mES cells using media supplementated with mCcbe1 recombinant protein has demonstrated a remarkable inductive potential of mCcbe1 to enhance cardiogenesis.Taken together, this data strongly suggest that Ccbe1 has the ability to direct the expression of cardiac inducers and to control cardiac progenitor expansion in vitro and in vivo, allowing the generation of non-genetically manipulated cardiac cells from a renewable cell source for regenerative cardiovascular medicine.

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39. Autologous vs. Allogeneic Approaches

39.P01
Evaluating immunogenicity of human endothelial colony forming cells for application in cell-based therapies

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Endothelial Colony Forming Cells (ECFC) are able to promote vascular repair processes. To predict the potential use of autologous and allogeneic ECFC sources for cell-based therapies, we focused on the immunological characterization of ECFC under inflammatory conditions. ECFC were stimulated 24 h with 10 ng/ml of the pro-inflammatory cytokines TNFα, IL-1ß and IFNγ. Mesenchymal Stromal Cells (MSC) from the same donor and unrelated Umbilical Vein Endothelial Cells (HUVEC) were used as reference cells. Expression of surface molecules was measured using FACS analysis. Without stimulation, all cell types showed HLA-ABC and ICAM-1 expression, but were negative for VCAM-1 and HLA-DR. TNFα and IL-1β induced the up-regulation of HLA-ABC, ICAM-1 and VCAM-1. ECFC triggered with TNFα showed significantly lower VCAM-1 levels compared to HUVEC. In both endothelial cell types, exposure to IFNγ enhanced HLA-ABC, ICAM-1 and HLA-DR expression, but did not affect VCAM-1 expression. In contrast, MSC up-regulated VCAM-1 after IFNγ stimulation. HLA-DR was up-regulated exclusively after IFNγ-treatment in ECFC and HUVEC to a similar extent, while MSC remained HLA-DR deficient after cytokine stimulation. Thus, ECFC and HUVEC demonstrated a higher degree of HLA-DR expression in an inflammatory microenvironment compared to MSC. Whether the differences in HLA-DR or VCAM-1 expression detected after cytokine triggering could influence allo-recognition has to be studied in future functional assays.

39.P02
Immunogenicity of allogeneic mesenchymal stem cells and dermal fibroblasts

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Mesenchymal stem cells (MSC) are multipotential cells with utility in tissue engineering and regenerative medicine. Increasing evidence suggests that dermal fibroblasts (DF) may have similar functions to MSC with potential implications for cell based therapies. However, the immunological properties of both allogeneic MSC and DF are poorly defined and recent studies investigating their immunogenicity remain inconclusive and this has hampered their clinical application. This study investigated the immunology of MSC and DF using an allogeneic mouse model. Immuno-modulatory properties were assessed using adaptations of the one-way mixed lymphocyte reaction (MLR) while immunogenicity was assessed by lymphocyte transformation assays (LTA). Bone marrow MSC and DF were isolated from C3H and Balb/c mice, expanded and characterised by flow cytometry. Their phenotypic profile was similar to that used for defining MSC with the exception of CD 105 [negative in DF]. Tri-lineage differentiation into adipocytes, chondrocytes and osteocytes plus gene expression of lineage-specific markers confirmed their multipotency. In one-way MLRs, both allogeneic MSC and DF significantly suppressed Balb/c lymphocyte proliferation. However, allogeneic MSC, but not allogeneic DF stimulated Balb/c lymphocyte proliferation in LTA. Hence the clinical utility of allogeneic MSC could be limited by their immunogenicity and further studies of the potential of DF in tissue engineering applications are warranted.

39.P03
Topical administration of allogeneic mesenchymal stem cells (MSCs) seeded in a collagen scaffold augments wound healing and increases angiogenesis in the diabetic ulcer

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Non-healing diabetic foot ulceration is the most frequent reason for hospitalisation in people with diabetes. We hypothesized that topically applied allogeneic MSCs increase wound healing and support angiogenesis. Allogeneic non-diabetic bone-marrow derived MSCs were seeded in a type 1 collagen scaffold. The cells were applied topically to a full thickness cutaneous wound in the alloxan-induced diabetic rabbit ear ulcer model using a dose escalation strategy. The experiments were performed under license with ethics committee approval. The groups included: untreated wounds, collagen scaffold alone, collagen seeded with 50 000, 100 000 or 1 000 000 MSCs. Percentage wound closure after 1 week was assessed using wound tracings. Diabetic wound neovasculature was analyzed using stereology.1 000 000 MSCs demonstrated significantly increased percentage wound closure when compared to untreated wounds. Collagen and MSC seeded collagen scaffolds demonstrated increased blood vessel density and decreased radial diffusion distance when compared to controls. Blood vessels were longer and more convoluted in wounds treated with collagen seeded with 1 000 000 MSCs. Allogeneic non-diabetic MSCs seeded in a collagen scaffold demonstrate augmented cutaneous wound healing in a pre-clinical model. Collagen and collagen seeded with MSC treatments result in increased angiogenesis when compared to untreated wounds but augmented wound healing was observed only at higher cell doses.

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39.P04
Autologous circulating angiogenic cells treated with osteopontin and delivered via a collagen scaffold enhances wound healing in the alloxan-induced diabetic rabbit ear ulcer model

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Diabetic foot ulceration is the leading cause of amputation in people with diabetes mellitus. Peripheral vascular disease is present in the majority of patients with diabetic foot ulcers. Circulating angiogenic cells (CACs), previously known as early endothelial progenitor cells are derived from peripheral blood and support angiogenesis and vasculogenesis, providing a potential topical treatment for non-healing diabetic foot ulcers. A scaffold fabricated from Type 1 collagen facilitates topical cell delivery to a diabetic wound. Osteopontin is a matricellular protein involved in wound healing and increases the angiogenic potential of CACs. A collagen scaffold seeded with CACs was developed. Subsequently the effect of autologous CACs that were seeded in a collagen scaffold and topically delivered to a hyperglycaemic cutaneous wound was assessed. Appropriate license and ethical approval was obtained. The alloxan-induced diabetic rabbit ear ulcer model was used to determine healing in response to the following treatments: collagen seeded with autologous CACs exposed to osteopontin, collagen seeded with autologous CACs, collagen alone and untreated wound. Stereology was used to assess angiogenesis in wounds. The cells exposed to osteopontin and seeded on collagen increased percentage wound closure as compared to other groups. Increased angiogenesis was observed with the treatment of collagen and collagen seeded with CACs.

39.P05
Immunomodulatory potential of tissue engineered constructs based on human adipose tissue derived stem cells and starch-based scaffolds

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Natural-origin materials such as corn starch have been suggested as valid alternatives to benefit, in comparison to synthetic materials, the engineered device's biocompatibility and functionality. Promising results in terms of new tissue formation have been obtained using starch and poly-caprolactone (SPCL) scaffolds in combination with mesenchymal stem cells (MSCs) from different origins. Considering the importance of the host immune response to implanted tissue engineered constructs (TECs) and the immunomodulatory role assigned to MSCs and to adipose derived stem cells (ASCs) in particular, this work aimed at understanding the impact of SPCL-hASCs based TECs in bone marrow murine-derived macrophages (MØ) and dendritic cells (DCs) activation. The profile of inflammatory and anti-inflammatory cytokines expressed by the MØ and the DCs, in contact with the scaffolds and the SPCL-hASCs based TECs, was evaluated by quantitative polymerase chain reaction (qRT-PCR). After 12 h and 24 h, of direct contact, it was found that the level of expression of IL-10, IL-4, IL-6 and TNF both in MØ and DCs was below the detection limit. These findings suggest that the combination of SPCL scaffolds with hASCs offer a promise strategy for allogeneic approaches in the TERM field, also corroborating the reduced immunogenic properties of hASCs.

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40. Embryonic Stem Cells in TERM

40.01
Keynote: Generation of skeletal hard tissues from pluripotent stem cells

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Cartilage has limited abilty to regenerate after damage but cartilage injury and osteoarthritis often initiated by injury, are major untreatable causes of disability. Pluripotent stem cells e.g. human embryonic stem cells (hESc) and induced pluripotent stem cells (iPSc) can differentiate to any cell type and provide an expandable source of cells for cell therapies. We developed a chemically-defined protocol which efficiently generates chondroprogenitors from hESCs in vitro (Oldershaw et al 2010 Nat. Biotech 28,1187) and have now shown that iPSc can also respond to the same signals. Pluripotent cells were expanded in feeder-free conditions and transferred to defined feeder- and serum-free culture. Chondrogenesis was initiated by a sequential series of growth factors, mimicking developmental and differentiation signals, which drive the pluripotent cells to a Safranin O, COLL II + cell type at high eficiency. ES/iPS derived chondrogenic cells show high expression of e.g. SOX9, p300, Znf219. COLL II and aggrecan but lack Col X, (hypertrophy marker). The cells responded to pellet and other 3D culture systems and differentiated cells repaired surgical osteochondral defects in nude rats. Pluripotent cells can also produce tendon-like cells by the application of tension in the presence of defined growth factors. Thus pluripotent cells provide a source of skeletal tissues for cell therapy or research on the effect of diverse agents on normal traumatised or diseased tissue.

40.02
Human tendon perivascular cells are multipotent and express embryonic stem cell associated markers

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Tendon-derived stem cells are commonly considered to be of mesenchymal origin, having the capacity to differentiate into adipocytes, chondrocytes, osteoblasts and tendon cells. Earlier on we have shown that human tendon perivascular cells express markers associated with neural stem cells such as Nestin and Musashi1. Here we describe a so far unrecognized type of human tendon perivascular stem cells (hTPSC) expressing markers commonly associated with embryonic stem cells (ESC). By immunohistochemistry and single cell PCR on human tendon tissue we demonstrate that hTPSCs express Oct4, Nanog, Klf4, Cmyc and Sox2 in vivo. In cell culture, these cells give rise to clonal spheroid cell aggregates harboring cells expressing the stem cell markers mentioned and markers associated with all three embryonic germ layers, such as Insulin and Glucagon, Collagens type 1 and 3 and GFAP and Galactosyl ceramidase. In differentiation experiments, hTPSC can give rise to adipocytes, osteoblasts, oligodendrocytes, astrocytes, endothelial cells and insulin producing cells. Despite their ESC-like marker expression, these cells do not form tumors upon injection into immunodeficient mice. These findings suggest that TPSC represent a more undifferentiated cell type than mesenchymal stem cells. hTPSC may be a valuable source for future applications in tissue engineering and cell therapy.

40.03
Spatial and temporal regulation of signaling pathways in hESCs using microfluidic technologies

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In vivo, tissues emerge from coordinated sequences of stem cell renewal, specialization and assembly regulated by multiple signals. While this complex milieu is poorly replicable using standard *in vitro* techniques, microscale technologies offer potential for conducting sophisticated experiments at biologically relevant scales with insights into cellular responses. We developed a microbioreactor providing complex sequences of time- and space-resolved concentration gradients in 3D culture settings coupling application of fast dynamic changes of environmental signals with versatile, high-throughput operations and imaging compatibility. Our device comprises a matrix of conical microwells housing 3D cell constructs exposed to stable concentration gradients generated by an integrated microfluidic platform. Mathematical modeling of flow and mass transport predicts the gradients shape and the fast dynamic concentration changes. hESC driving the Wnt/β-catenin–dependent expression of Venus were exposed to gradients of multiple factors. Wnt3a, Activin, BMP4 and their inhibitors were chosen for being the main player in early stage lineage commitment in the developing embryo. Results will be presented correlating the behavior of differentiating EBs to their exposure to time- and space-resolved concentration gradients of mesodermal-inducing morphogens. We hypothesize that the application of complex regulatory patterns would provide predictable in vitro models of development and disease.

40.04
Defined, growth factor free production of cardiac cells and tissues from human pluripotent stem cells

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Human pluripotent stem cells (hPSC) offer the potential to generate large numbers of functional cardiomyocytes from clonal and patient-specific cell sources. We showed that temporal modulation of Wnt signaling is both essential and sufficient for efficient cardiac induction in hPSCs under defined, growth factor-free conditions. Short hairpin RNA (shRNA) knockdown of β-catenin during the initial stage of hPSC differentiation fully blocked cardiomyocyte specification while Gsk3 inhibition at this point enhanced cardiomyocyte generation. Furthermore, sequential treatment of hPSCs with Gsk3 inhibitors followed by inducible expression of β-catenin shRNA or chemical inhibitors of Wnt signaling produced a high yield of virtually pure (up to 98%) human cardiomyocytes from multiple hPSC lines. Cardiomyocytes produced by temporal modulation of canonical Wnt signaling exhibited electrophys-

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iological properties of ventricular cardiomyocytes and contraction stress was similar to that of primary rat cardiomyocytes. Tissues generated from these cells spontaneously contracted in unison for over 6 months. The robust ability to generate functional cardiomyocytes and cardiac tissues under defined, growth factor-free conditions solely by genetic or chemically-mediated manipulation of a single developmental pathway should facilitate scalable production of cardiac cells and tissues suitable for research and regenerative applications.

40.05
Human embryonic stem cell-derivatives in a hydrogel-based skin model

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Cases of massive skin loss upon injury or disease still represent a serious healthcare challenge worldwide, and despite the many alternatives available, results are not yet satisfactory. Skin Tissue Engineering, especially now combined with Stem Cell Research, holds great expectation towards an efficient and high quality skin regeneration. Human embryonic stem cells (hESCs) are quite attractive for this purpose, as they possess both immunoprevileged features and unique self-renewal and differentiation potency, being able to provide unlimited biological material. In this specific work, an innovative hydrogel-based model, together with hESCs-derived epidermal cells is proposed for skin regeneration. hESCs were moved from Mouse embryonic fibroblasts (MEFs), cultured in a feeder-free system comprising Matrigel and mTeSR media, and characterized by immunocytochemistry for pluripotency markers. After expansion, hESCs were differentiated towards the epidermal lineage,by culturing them in Collagen IV coated dishes, and upon supplementation of both Retinoic acid and bone morphogenetic protein 4. Keratin 18/14 positive cells were obtained, confirming the success of the used protocol. Differentiated epidermal cells were purified and cultured onto the hydrogel. Following several culture methodologies optimization, hESCs-derived epidermal cells were able to adhere, proliferate and form a stratified epidermis-like structure demonstrating the potential and expectation for Skin Regeneration.

40.P01
Cross-talk of ES-derived neural stem cells with endothelial cells in a fibrin-based 3D co-culture system

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In the neural stem cell (NSC) niches, interactions between NSCs and endothelial cells (ECs) are orchestrated by paracrine and cell-cell cues. ECs are believed to modulate NSC fate decision, offering great potential to NSC-based therapies in the treatment of spinal cord injuries. To study the effects of ECs on NSC proliferation and differentiation, we propose a fibrin (Fb)-based 3D coculture system in an effort to mimic the 3D in vivo microenvironment and get insight into the outcome of NCS-EC co-transplantation.Cocultures were performed using a human EC line and neurospheres (NSs) derived from a mouse ES cell line, embedded in Fb drops. Indirect cocultures were performed in transwells. Direct cocultures were assembled suspending ECs and NSCs in Fb drops. Cocultures were followed over 14DIV in serum-free or serum-reduced media. In indirect cocultures ECs were removed at day 4 of cell culture.In serum-free medium, no differences on cell proliferation were found between co- and monoculture. In serum-reduced medium, cocultures showed a decrease on the % of $BrdU^+$ cells. At 4DIV, mainly $Nestin^+$ and β-III $tubulin^+$ cells migrating from NSs were found in all conditions tested. At 14DIV, most of the cells were β-III $tubulin^+$. NSCs were still present, as well as, $GFAP^+$ and O_4^+ cells though in lower number. Results suggest that ECs, in serum-reduced medium, might be modulating NSC differentiation into the neuronal lineage at expenses of their proliferation. FCT (PTDC/SAU-BEB/6532).

40.P02
Differentiation of human ES cells into megakaryocytes and platelets in protocols derived by multiplexed CombiCult technology.

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Human embryonic stem (hES) cell derived hematopoietic progenitors represent a renewable source of material for use in therapies and in screening for novel regenerative drugs. Here we describe the discovery of novel serum-free feeder-free protocols that direct differentiation of hES cells to functionally active megakaryocytes and platelets. Additionally these protocols feature the replacement of commonly used cytokines with small molecule bioactives. Novel differentiation protocols were discovered using a high throughput screen called CombiCult™ (Combinatorial Cell Culture) that is capable of multiplexing very large numbers of cell differentiation protocols to identify critical combinations that result in high efficiency differentiation to a given phenotype. Human ES cells were grown on microcarriers and shuffled randomly through 40 different culture conditions, with concomitant labeling using nanomaterial tags. Ten thousand distinct protocols were sampled in one experiment. Following screening to identify microcarriers bearing cells positive for the megakaryocyte specific marker cd41a and analysis of the tags to deduce cell culture history, 90 novel protocols were identified. A number of protocols efficiently directed the differentiation of hES cells into megakaryocytes and platelets through the use of small molecule bioactives instead of traditionally used cytokines making these protocols more amenable to therapeutic and large scale-applications.

40.P03
Efficient derivation of mesenchymal stem cells from human embryonic stem cells for tissue engineering

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Introduction: Human embryonic derived mesenchymal stem cells (ESC-MSC) are promising alternatives for bone marrow derived mesenchymal stem cells (BM-MSC) and adipose tissue-MSC (AT-MSC). In this study, MSC are derived from ESC via different methods and compared on their: (1) cell surface markers and (2) multilineage differentiation capacity.
Materials and methods: ESC-MSC were derived by: (1) an embryoid body (EB) step, (2) ST2-coculture, (3) monolayer differentiation, (4) mesoderm induction via short-term growth factor (GF) treatment and (5) selective addition of GF. Cell populations were enriched with MACS/FACS. MSC were phenotypically characterized by flow cytometry ($CD73^+$, $CD90^+$, $CD105^+$, $CD34^-$, $CD45^-$, $CD20^-$, $CD14^-$). Multilineage differentiation was studied in 2D/3D cultures and evaluated by fluorescence microscopy, histology and qRT-PCR.
Results: 75.9% of the BM- and AT-MSC populations possess the MSC phenotype. EB formation, the ST2 coculture method and monolayer derivation is insufficient for large-scale differentiation: only 0–5% ESC-MSC can be derived. Short-term GF treatment followed by $CD34^+$

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MACS isolation resulted in 53,3% MSC. Selective addition of GF resulted in 42,8% MSC. Enriched ESC-MSC by FACS were able to differentiate into the adipo-, chondro- and osteogenic lineage in 2D and 3D cultures.
Conclusion: The most efficient derivation method of ESC-MSC resulted in cells which are excellent alternatives to study cell/biomaterial interactions for TE.

40.P04
Cartilage tissue engineering using pluripotent human embryonic stem cells

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Autologous chondrocytes implantation (ATI) is one of the most successful methods to treat cartilage defects in clinic. However, the limit of cell resource and complexity of the procedure has limited the wide application of this in clinical practise. Embryonic stem cells (ESCs) are one of the most attractive cell types for tissue engineering due to their two distinct properties: unlimited self-renewal and pluripotency. We developed a protocol, using chemical defined medium, to generate chondroprogenitors from human ESCs (hESCs) in vitro and test the ability of these cells for cartilage repair in vivo. hESCs were initially cultured and expanded on feeder cells and then transferred to a feeder-free, serum-free culture system. Chondrogenesis was intiated by applying a sequential series of growth factors, which drive the hESCs through mesendoderm and mesoderm to a chondrogenic cell type. These cells were characterised by qRT-PCR, immunostaining and microarray technology. To test the cartilage formation capacity, these cells were implanted into to an osteochondral defect in the patella groove of nude rats and assessed after different times. Chondroprogenitors derived from hESCs show typical chondrocytes properties and gene expression. These cells could be cultured in a 3-dimentional fibrin gel and still retain their phenotype. Furthermore, these cells formed cartilage, and strongly stained with Safranin O and expressed Collagen II when implanted into cartilage defects in nude rats.

40.P05
Chondrogenic differentiation of human embryonic stem cells in chondrocyte co-culture

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Chondrogenic differentation of human embryonic stem cells (hESCs) can be achieved in embryoid bodies by adding selected growth factors to the medium. Chondrocyte-secreted factors have been considered to promote the chondrogenic differentiation. Thus we studied whether coculture with primary chondrocytes induces hESCs to differentiate into chondrocyte lineage. Coculture of hESCs was established in a transwell insert system in feeder-free culture conditions while hESCs grown alone in the wells were used as control. The hESCs were weekly replenished with chondrocytes for 3 weeks, and chondrogenically-committed cells (hCCCs) were evaluated by morphology, immunocytochemistry, qRT-PCR, and analysis of chondrogenic, osteogenic and adipogenic markers. The expression of chondrocyte- and remnants of hESCs-specific genes were frequently measured during the monolayer expansion of hCCCs from passage 1 to 10. Human CCCs displayed similar morphology to chondrocyte and expressed chondrocyte-specific genes, which were declined following passaging, similarly to chondrocytes. Human CCCs formed a chondrogenic cell pellet and differentiated into chondrocytes, which secreted abundant extracellular matrix. Moreover hCCCs could rapidly proliferate. However hCCCs lost their potential to differentiate towards osteoblasts or adipocytes. Our results show that coculture of hESCs and primary chondrocytes could induce chondrogenic differentiation, while osteogenic or adipogenic capacity decreased.

40.P06
Tissue engineering in hostile environments: the effects of inflammation on embryonic stem cell-derived osteogenic cells

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Proinflammatory cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α) are associated with the innate inflammatory response and are critical for control of bone remodelling. Little work has been performed showing the effect of these cytokines on the activity of stem cells, particularly embryonic stem cells. This work compares the response of osteogenically differentiated mouse embryonic stem cells (osteo-mESCs) and mouse primary calvarial cells to an inflammatory environment created by adding Il-1β, TNF-α and interferon-gamma (IFN-γ) to the culture media. The cells were monitored for viability, nitrite release and prostaglandin E2 (PGE2) release. The presence of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) were determined by immunocytochemistry and RT-PCR.Early results show the two cell types have very different responses to the presence of proinflammatory cytokines. The primary cells showed significant increases in nitrite and PGE2, suggesting activation of iNOS and COX-2. The osteo-mESCs do not show detectable and significant increases in nitrite and PGE2, for the first 14 days of osteogenic culture. By day 21, levels of PGE2 and nitrite are significantly increased, although still lower than seen with the primary cells. The discovery of the osteo-mESCs possibly having a better tolerance to the inflammatory environment would have an important impact in the use of such cells for osteochondral tissue engineering strategies.

40.P07
Understanding cell-to-cell communication in hESCs – creating basis for the move to 3D culture

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Human embryonic stem cells (hESC) are undifferentiated pluripotent cells derived from the inner cell mass of blastocysts, with the capacity to both self-renew and to differentiate into all somatic cell lineages. During their in vitro cultivation, hESC within colonies employ various communication mechanisms to influence their activities. Here we have investigated morphologic aspects of intercellular communication at the electron microscopic level. Cells of CCTL14 line of hESC were routinely cultured in DMEM media supplemented with serum replacement and 4 ng/ml FGF2 on the layer of mouse embryonic fibroblasts serving as feeder cells. Colonies of hESC were processed for transmission and scanning electron microscopy on days 3 and/or 4 after seeding. Neighbouring cells in colonies communicate directly via intercellular junctions. All types of intercellular connections were found: occluding, adhering and communicating (gap junctions). Interactions with distant cells were mediated by various types of vesicles shedding from the cell surfaces, and intensive formation of coated vesicles was observed. A special type of very long intercellular bridges resembling 'tunnelling nanotubes' was observed occasionally between distant cells. Intercellular junctions and numerous morphologic signs of material exchange

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reflect intensive intercellular communication in hESC colonies. The nature of intercellular bridges is also addressed.Study was supported by MSM 0021622430.

40.P08
Attenuation of transport limitations and controlled differentiation of multicellular tissue constructs derived from embryonic stem cells via convective perfusion

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Embryonic stem cell (ESC) spheroids recapitulate much of the signaling and adhesions of native tissue, which may enable new opportunities to derive multicellular constructs; however, the requirements and limitations that arise within ESC spheroids have not been fully characterized. The objective of this study was to develop a platform for the production of three-dimensional tissue structures from ESCs for transplantation in regenerative therapies. Embryoid bodies (EBs) were formed from ESCs with precisely controlled size and formation kinetics via forced aggregation in micro-wells, followed by maintenance of uniform size EBs in rotary orbital suspension culture; the resultant homogeneous populations enabled systematic analysis of EB microenvironments during the course of ESC differentiation. After 7 days of differentiation, EBs exhibited an increased elastic modulus compared to day 2 EBs, which was paralleled by changes in the viscoelastic properties, such as creep and stress relaxation responses. Additionally, during the course of EB differentiation fluidic resistance increased, concomitant with decreased permeability and diffusivity, indicating that changes in EB structure increasingly limit transport. Preliminary evidence suggests that convective transport using microfluidic perfusion bioreactors may attenuate 3D transport limitations and may enhance the differentiation efficiency of stem cells toward specific phenotypes and germ lineages, including mesoderm.

40.P09
Generation of 3D mineralized cellular constructs from mESCs in a perfusion bioreactor using simvastatin

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Current approaches for bone differentiation use Bone Morphogenetic Proteins, which are expensive, need appropriate carriers, have suspicion of carcinogenicity and biocompatibility issues. We have produced 3D implantable, mineralized cellular hydrogel constructs from mESCs by achieving osteoinduction with simvastatin and culture in a novel perfusion bioreactor, which provides a well-mixed environment with low shear stress. Simvastatin, a widely used drug to lower cholesterol, has been shown to have anabolic effects on bone formation in nanomolar concentration and can cross the cell membrane with passive diffusion. mESCs were encapsulated in alginate and cultured in the perfusion bioreactor for 3 days in HepG2-CM. Osteoinduction was performed using aMEM supplemented with 15% FBS, 1% P/S, 10 mM b-glycerophosphate and 0.1 nM simvastatin, for 26 days. Bone formation was assessed by gene expression analysis using quantitative real time PCR, Alizarin Red staining for calcium deposition and alkaline phosphatase activity. The osteoinductive properties of simvastatin on mESCs were demonstrated by the increased ARS staining, the mineralization stage–dependent ALPase activity, and the increased Ocn and Osx gene expression. Treatment with 0.1 nM simvastatin enhanced significantly bone differentiation of mESCs in the perfusion bioreactor without the presence of other osteoinductive supplements. Perfusion bioreactor can support high cell numbers with high viability.

40.P10
Expansion and cardiac differentiation of human iPS cells using a suspension culture system

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Recently, regenerative medicine is expected as a promising therapy for severe heart disease. On the other hand, the collection of huge amounts of beating cardiomyocytes and the fabrication of pulsatile thicker tissue, which directly contributes to the pulsation of damaged heart, are expected as next-generation cardiac regenerative medicine. The aims of this study are to establish easy and effective methods for collecting the huge amounts of cardiomyocytes from human iPS cells (hiPSCs) using suspension culture. hiPSCs (RBRC-HPS0001 or HPS0002) were detached from culture dishes by an enzymatic treatment and were cultured in a spinner flask. An optimized culture condition including the dissociation treatment of enzyme, the culture medium, and the agitation rate enabled to create typical embryoid bodies (EBs), and the number of cells increased upto 3 to 4-folds on day 6. When the cultured EBs were dissociated into single cell suspension and were re-seeded into the spinner flask, hiPSC were also cultivated in the EB formation. Furthermore, an immunocytochemical analysis suggested that the suspension culture in the spinner flask might be useful for expansion of hIPSCs with undifferentiated state. When the expanded EBs were cultured in the medium supplemented with FBS, spontaneous beating cells were observed. These findings suggest that the suspension culture in the spinner flask might contribute to collect enough amounts of cardiomyocytes for the cardiac regenerative medicine.

40.P11
Mouse embryonic stem cell differentiation on top of acellular lung matrix slices

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Introduction: The use of decellularized lung scaffolds has emerged as a new alternative in the field of tissue engineering for the fabrication of bioartificial lungs.
Objective: To establish a cell culture approach based on acellular lung matrix slices to study the pulmonary differentiation of mouse embryonic stem cells (mESC).
Methods: Rat acellular lung scaffolds were prepared according to Cortiella et al., 2010. Decellularized lung lobes were embedded in OCT and snap-frozen in liquid nitrogen. Cryosections of 40 μm were used to culture mESC-derived cells. mESC were pre-differentiated into definitive endoderm derived cells for 6 days. Afterwards, day 6-endoderm derived cells were seeded on top of acellular lung matrix slices and maintained until day 21 in the presence of growth factor stimulation. Cell attachment, proliferation/survival, spatial distribution and cell fate commitment on top of acellular lung slices were evaluated at day 8 and 21 of the differentiation process.
Results: Pre-differentiated mESC, expressing the typical markers of definitive endoderm Foxa2 and Sox17, adhered successfully to lung

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matrix. Cells proliferated and further differentiated into lung progenitor cells expressing Foxa2, TTF1 and p-Cytokeratin.
Conclusions: The use of acellular lung matrix slices is a simple approach to study the differentiation of stem cells interacting with the lung matrix. This system also provides useful information to further improve whole lung recellularization.

40.P12
Cardiac differentiation of chicken spermatogonial stem cells

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Multipotent adult germline stem cells (maGSCs) have been successfully established and show phenotypic characteristics similar to embryonic stem cells (ESCs). In the present study, we successfully isolated chicken spermatogonial stem cells (SSCs) from testicular cells and performed subsequent analysis to identify pluripotent cells by investigation with cytochemical reagents including periodic acid-Schiff (PAS), alkaline phosphatase (AP), and antibodies to stage-specific embryonic antigens (Oct4, SSEA1, SSEA3, SSEA4, STRA 1–60, and STRA 1–81). Our results show that maGSCs highly express these markers related to ESCs and could spontaneously differentiate into three embryonic germ (EG) layers in vitro. The maGSC-derived cardiomyocytes expressed some cardiac-specific markers such as sarcomeric alpha actinin, specific for alpha-cardiac actinin; conexin-43, the major protein of gap junctions which are thought to play a crucial role in the synchronized contraction of the heart and in embryonic development; and cardiac troponin T, the tropomyosin binding subunit of the troponin complex which regulates muscle contraction in response to alterations in intracellular calcium ion concentration. Our results contribute information related to the ability of maGSCs to differentiate into cells such as contraction cardiomyocytes similar to ESCs providing a new source of distinct cardiomyocyte types for basic research and potential therapeutic application.

40.P13
Transition of human pluripotent stem cells from an integrin to e-cadherin mediated attachment system using StemAdhere™ defined matrix for hPSC

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Human pluripotent stem cell (hPSC) cultures using defined media such as mTeSR™1 or TeSR™2 have typically necessitated the use of crude preparations of extracellular matrices (ECMs) to permit cell attachment. The most commonly used matrix with mTeSR™1 and TeSR™2 hPSC maintenance culture systems is Matrigel™(BD Biosciences), which is undefined and derived from mouse tumor. The main components of Matrigel™ are collagen and laminin, both of which interact with cells via surface integrin receptors. StemAdhere™ Defined Matrix for hPSC (developed and manufactured by Primorigen Biosciences) mediates cell attachment onto plastic cultureware through an interaction with extracellular E-cadherin expressed by hPSC. Using optimized protocols, we observed that upon transition of hPSC cultured in mTeSR™1 or TeSR™2 on Matrigel™ to StemAdhere™, an 'adaptation phase' lasting 2–4 passages was observed; this adaptation was characterized by reduced cell expansion and plating efficiency. After the adaptation phase, hPSC cultured in mTeSR™1 or TeSR™2 on StemAdhere™ exhibited plating efficiency (60–100% per passage) and expansion rates (8 to 12-fold expansion per passage) equal to or greater than those seen for hPSC grown on Matrigel™. hPSCs from long term cultures in mTeSR™1 and TeSR™2 on StemAdhere™ maintained high expression (>90%) of the pluripotency markers SSEA3 and OCT4, remained karyotypically normal, and generated all three germ lineages in teratoma formation assays.

40.P14
Suspension culture of human pluripotent stem cells in controlled, stirred bioreactors

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Therapeutic and industrial applications of pluripotent stem cells and their derivatives requires large cell quantities generated in defined conditions. To this end we have translated single cell inoculated suspension cultures of human pluripotent stem cells to stirred tank bioreactors. These systems are widely used in biopharmaceutical industry, allow straight forward scale up and detailed online monitoring of key process parameters. To ensure minimum medium consumption, but in parallel functional integration of all probes mandatory for process monitoring i.e. for pO2 and pH, experiments were performed in 100 ml culture volume in a 'mini reactor platform' consisting of four independently controlled vessels. By establishing defined parameters for tightly controlled cell inoculation and aggregate formation up to 2×108 hPSCs / 100 ml were generated in a single process run in 7 days. Expression of pluripotency markers and cells ability to differentiate into derivates of all three germ layers in vitro was maintained, underlining practical utility of this new process. The presented data provide key steps towards scalable mass expansion of human iPS and ES cells thereby enabling translation of stem cell research to (pre)clinical application in relevant large animal models and valuable in vitro assays for drug development and validation as well.

40.P15
Improving expansion of 'high-stemness' murine embryonic stem cells (mESCs) through multifactorial tuning of bioprocess parameters

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ESCs are routinely cultured on tissue culture plastic in artificially high glucose (25 mM) and O2 (20%) conditions, which deviate from the in vivo levels. Critical to the expansion of undifferentiated mESCs is the provision of leukemic inhibitory factor (LIF) as well as the metabolic state of the cells. We established a semi-automated perfusion bioprocess for the 3D culture mESCs and demonstrated that metabolic stress attenuated 'stemness' and proliferation even in the presence of sufficient LIF. We further optimised the bioprocess by cultivating mESCs in reduced (5%) O_2 levels to mimic in vivo conditions. In static cultures, 5% O_2 attenuated LIF activity, reducing mESC proliferation; however, when signalling activity was modified using both '2i' inhibitor and retinol A conditions, self-renewal was recovered. In contrast, in the perfusion bioreactor cultures self-renewal was restored without signal modification. Finally, we demonstrated that a combination of 5% O2, perfusion and signal modification improves proliferation and pluripotency levels. In conclusion, arbitrarily determined 'standard' ESC culture conditions are sub-optimal, whereas perfusion feeding allows the tuning of multiple bioprocess parameters enhancing LIF-mediated self-renewal and 'stemness'. Critically, different bioprocess cues can be used to modulate ESC fate.

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40.P16
Smart culture of mouse embryonic stem cells

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Current culture methods for mammalian cells use trypsin as means to detach them from the culture surface. This inflicts damage to cell membrane receptors (mainly integrins) which can be detrimental for further adhesion to a new substrate. In addition, using trypsin may contaminate cell cultures destined for clinical applications. Therefore, an alternative, affordable, and scalable method of passaging is a new and active area of research. Stimuli responsive polymers can be used to avoid the deleterious effects of trypsin/EDTA as the cells detach from the culture substrate upon a stimulus (temperature, pH and light). We show that the thermo-responsive polymer poly (MEO2MA-co-OEGMA) was grafted successfully from a glass surface and that a switchable response to temperature achieved. 3T3 fibroblasts adhered, spread and proliferated at 37°C and detached when the temperature was lowered to 10°C. A similar effect was observed with mES cells and no differentiation was observed following two passage cycles.

40.P17
Novel self-assembled polymeric nanofibers for mouse embryonic stem cell culture

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Embryonic stem (ES) cell research has emerged as a vibrant area in biomedical breakthrough. This is evidently because ES cells have the ability to self-renew indefinitely in an undifferentiated state. Conventionally, ES cells have been cultured on gelatin or under a monolayer of mouse embryonic fibroblasts, which differ from the environment of a whole organism and consequently, cells isolated from higher organisms frequently alter their metabolism, morphology and gene expression profile. Also, the existing complex culture systems formed from animal-derived biomaterials pose problems for replacement therapies.In this work, we present new artificial nanofiber structures formed from self-assembly of amphiphilic biodegradable peptide-copolymers that were designed for ES cell culture. This newly developed synthetic system allows elimination of animal-derived products and provides a complex network of nanofibers in a scale similar to the native extracellular matrices.Undifferentiated mouse embryonic stem (mES) cells were cultured using the polymeric nanofibers in order to evaluate their potential applications in ES cell research. It was assessed the morphology, proliferation, viability, self-renewal and pluripotency of mES cells. The new synthetic nanofibers promoted mES cells growth and seem to retain their pluripotent state, indicating that if this system was improved it may lead to an alternative and easy to use substrate for mES cell culture and regenerative therapies.

40.P18
Identification of differentially expressed genes in bovine blastocysts

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To date, no embryonic stem cells (ESCs) from farm animal species have been generated. For tissue engineering purposes, such cell lines would have enormous potential. ESCs have been generated from the inner cell mass (ICM) of rodent and primate preimplantation embryos. As a first step in the generation of bovine ESC lines, we focused on genes responsible for the differentiation of ICM versus trophectoderm. Therefore, we investigated the differential expression between ICM and trophectoderm in bovine blastocysts. Bovine blastocysts (freed of zona pellucida) were incubated with FITC-labelled Concanavalin A that specifically binds to the outer cell layer of blastocyst stage embryos: the trophectoderm. Trypsin treatment and pipette-assisted disaggregation followed by incubation with microbead conjugated anti FITC IgG and subsequently magnetic sorting resulted in an ICM-pool without trophectoderm cells and a trophectoderm-pool without ICM cells. Extracted RNA was synthesized to cDNA and amplified followed by suppression subtractive hybridization. Differentially expressed genes were sequenced and identified. These genes and their proposed functions will be presented.

40.P19
Discrimination between ES cells and feeder cells using dielectrophoresis

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Culturing embryonic stem (ES) cells effectively requires feeder cell layer. However, to apply the ES cells in a clinical setting, it is important to get rid of the feeder cells after the expanding culture of ES cells. In this study, we focused on dielectrophoresis (DEP) to discriminate the ES cells from the feeder cells. Dielectrophoresis is a phenomenon in which a force is generated on a cell when it is subjected to a non-uniform electric field. The direction and amplitude of this force depends on the cell and medium electric properties, on the voltage and frequency of electric field. If the cell is polarized more than the medium in the electric field, the cell is attracted to regions of stronger electric field (positive-DEP). Negative-DEP is contrary. Therefore, we hypothesized that using DEP force enables the discriminating the ES from feeder cells. To test this hypothesis, dielectrophoretic properties of ES and feeder cells were measured by custom-made DEP cell analysis chip. From the results, the DEP properties of ES and feeder cells showed different dependencies on the frequency of applied electric field. Using the different frequency-dependencies of the DEP force and a flow-induced shear force, it was possible to get rid of the ES from feeder cells. In conclusion, our cell sorting approach based on DEP phenomena has a possibility to enable better cell sorting technology without magnetic or fluorescent labeling.

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41. Many Roads to iPS

41.01
Keynote: Potential uses of iPS cell technology for treating and modeling human disease

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The isolation of embryonic stem cells (ESCs) from early stage blastocysts created an unprecedented interest among scientists and the general public. These cells have the ability to proliferate and remain undifferentiated *in vitro* if cultured under the right conditions. They can also be transformed into any cell type of the mammalian body thanks to the application of specific protocols. However, both practical and ethical concerns hampered the research and application of human ESCs, which stimulated researchers worldwide to find new ways to produce ESC-like cells *in vitro* from somatic cells. Takahashi and Yamanaka were the first to generate induced pluripotent stem cells (iPSCs) by nuclear reprogramming of other cell types with defined combinations of transcription factors. Ever since, a large number of laboratories worldwide have validated the technique, and in fact iPSCs can now be produced routinely from multiple species (including humans) and using multiple methods. The implications of reprogramming in general and of human iPSCs in particular are vast. On one hand, the technique can be used to understand epigenetic transformations that run in opposite directions. On the other hand, human iPSCs are providing outstanding models for drug/toxicity screening and disease mechanistic studies. They also hold promise for future cell-based therapies once the technology progresses further and relevant safety concerns are overcome.

41.02
Non-invasive generation of human induced pluripotent stem cells from urine

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The transformation of somatic cells into induced pluripotent stem cells (iPSCs) using exogenous factors, also termed reprogramming, may be used for personalized medicine in the future and can produce valuable *in vitro* models of human diseases. So far, human iPSCs have been generated from different cell types with varied frequencies, indicating that the cells of origin are an important determining factor. In addition, there is intense debate regarding whether human embryonic stem cells (ESCs) and iPSCs are equivalent and donor cell heterogeneity may further complicate this due to difficulties in setting standards for performing such comparisons. The ideal cell source for reprogramming should be easily accessible, easily reprogrammed, and universal (any age, sex, ethnic group, and body condition). Here, we report the generation of human iPSCs from cells obtained non-invasively from human urine. Urine-derived cells from 12 donors yielded iPSCs with excellent differentiation ability. Therefore, we propose urine to become the preferred source for generating iPSCs in many instances. The ease of this method may facilitate the standardization of iPSC technology, will boost the generation of cell based disease model systems and is also an advance in the direction of clinical use of iPSCs.

41.03
Cardiomyocyte-derived iPS cells supported by hydrogel possess an enhanced capacity to repair the damaged myocardium

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In cardiac regeneration, cardiomyocyte (CM) transplantation may not be able to restore the damaged myocardium without a proper biomaterial carrier for augmenting the efficacy of cell delivery. The purpose of this study was to define the cardiac regenerative capacity of CM-derived induced pluripotent stem (iPS) cells after acute myocardial infarction (AMI), when transplanted using an engineered hydrogel cell carrier. Injectable hydrogels were made from a PEG-fibrinogen (PF) liquid precursor that can be photopolymerized *in situ*. The contractile properties of the combined PF-iPS cells implants were first optimized *in vitro* using cardiac biomarkers. The *in vivo* efficacy of the implants was tested in an AMI model in immune-deficient mice, injecting PF, iPS cells, and PF-iPS cells combinations. The fractional shortening (FS) was drastically reduced in the saline control group 30 days after AMI (17.1 ± 2%), whereas the groups containing cells or PF showed a relatively slower time-dependent reduction of the FS (PF 10.7 ± 3% and PF-iPS cells 5.7 ± 1%). All treatment with cells (iPS cells 6.1 ± 1% and PF-iPS cells) showed a partial recovery of cardiac function. Histological analysis showed transplanted iPS cells were functionally integrated with the host tissue. This study confers the *in vivo* efficacy of combined PF-iPS cells transplantation. Given the synergetic effects observed herein, we believe that these two strategies can offer a wider range of therapeutic options in the future.

41.04
Proliferation and initial differentiation of mouse iPS cells in various-types of alginate hydrogel microcapsules

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Encapsulating iPSCs in alginate hydrogel-based capsules is scalable method and able to prevent excess aggregation and protect cells from shear stress caused by agitation in mass culture systems. However, few reports have referred to capsule type optimization in terms of its biological and physiological influence. We therefore cultured mouse iPSCs in three types of calcium alginate gel capsules (Φ = 500–600 μm), that is, standard capsules without any surface layers (NAKED capsule), those having alginate-PLL layers on the surface (COATED capsule), and those with the same surface layers and liquid core by EDTA treatment (Hollow capsule). During 10 days of culture in the presence of LIF and serum, all types of capsules kept cells in pluripotent state better than suspension culture did as evidenced by qPCR analyses. Outer alginate-PLL layers were very effective in keeping cell inside and enabled 6–10 times growth, but 30% number of cells leaked from NAKED capsules with up to three times growth. In the absence of serum and LIF, only COATED capsule kept cells undifferentiated, but all other types made cells differentiated to various germ layers. This was likely that formation of primitive endoderm was inhibited in COATED capsules but occurred in other types of cultures. These biological effects caused by the differences in capsule types in an important knowledge in opti-

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mizing processes for mass propagation and differentiation of iPS cells using hydrogel microcapsules.

41.05
Engineering the 3D morphogenic environment of pluripotent stem cells for directed differentiation

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Pluripotent embryonic stem cells (ESCs) can be differentiated as self-assembled cell aggregates in suspension culture conditions referred to as 'embryoid bodies' (EBs). We have previously demonstrated the ability to entrap microparticles (MPs) of synthetic degradable polymers as a means to engineer the 3D microenvironment of EBs. More recently we have examined ECM-based MPs as delivery vehicles for exogenous growth factors as well as sequestering agents for endogenous morphogens produced within EB microenvironments. Delivery of BMP4 or Noggin from gelatin MPs exhibited opposing effects to promote mesoderm or ectoderm morphologies and gene expression, respectively, that were comparable or exceeded that of soluble treatment controls. The percentage of Brachyury-T^+ cells (early mesoderm marker) was increased with BMP4 treatment, and moreso with delivery from MPs than solubly. Introduction of heparin-conjugated and gelatin MPs alone significantly affected the secreted morphogen profile (BMP4, IGF2, VEGF) of EBs. Divergent gene expression patterns were observed for heparin-gelatin and gelatin MPs compared to each other, and VE-cadherin$^+$ cells were enhanced locally adjacent to heparin-gelatin MPs. Furthermore, magnetic MPs could be physically entrapped during EB formation to allow for subsequent external manipulation via magnetic forces to create patterns of multicellular aggregates. Altogether these results demonstrate novel ways to engineer pluripotent ESC 3D microenvironments.

41.06
Neural differentiation by human pluripotent stem cells is regulated by scaffold presentation

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Cell differentiation and tissue formation involve cell interactions which control cell fate. We have previously shown that neural differentiation of human pluripotent stem cells is significantly enhanced in three dimensional (3D) cell aggregates compared to conventional two dimensional (2D) monolayer cultures. However, aggregates are not uniform and central regions can suffer from necrosis. We have developed a scaffold that significantly improves the cell growth environment and enables routine 3D cell culture in a robust and reproducible manner. The material is a porous polystyrene scaffold into which cells invade and grow readily in 3D. We have found that scaffold presentation plays an important role in stem cell maintenance and differentiation. Scaffolds were presented as membranes in specially designed well inserts where the wall of the insert chamber was either 'open' (containing a port to allow mixing of media around the membrane) or 'closed' (media above and below the membrane are in separate compartments). We observed the growth and differentiation of human pluripotent stem cells in response to retinoic acid and the formation of neural derivatives. The extent of differentiation and location of cells in the scaffold was regulated differently when using the alternative well insert designs and may relate to waste product and lactic acid accumulation. These data demonstrate how subtle changes to scaffold presentation can directly impact on cell behaviour during 3D culture.

41.P01
Mesenchymal progenitors derived from human induced pluripotent stem cells

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Mesenchymal stem cells (MSCs) have a high potential for therapeutic efficacy in treating diverse injuries and diseases. However, most of these clinical applications require substantial quantities of cells, but the number of MSCs obtainable from a single donor is limited. Reports on the derivation of mesenchymal progenitors from human embryonic stem cells (ESCs), and more recently also from human induced pluripotent stem cells (iPSCs), are thus most intriguing, since the infinite proliferative capacity of ESCs opens the possibility to generate large amounts of highly uniform batches of MSCs. In the present study we derived mesenchymal progenitor cells (iMPs) using three different protocols: (i) embryoid body formation, (ii) indirect co-culture with MSCs, or (iii) forced differentiation of colonies. Starting from an existing human iPS cell line and a newly established cell line reprogrammed from hMSCs, respectively, we derived iMPs exhibiting typical MSC/fibroblastic morphology, that could be expanded for >35 PDs before reaching senescence and were able to differentiate *in vitro* along the osteogenic, chondrogenic, and adipogenic lineages. Our system of using iPS cells reprogrammed from hMSCs allows us to compare key characteristics of iMPs with the original MSCs from the same donor and draw more specific conclusions on their similarities and differences than would be possible with iMPs and MSCs from different sources and donors.

41.P02
Generation of induced pluripotent stem cells from human umbilical cord blood-derived mesenchymal stem cells

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The generation of induced pluripotent stem cells (iPS) cells from mouse and human somatic cells by expression of defined transcription factors (Oct3/4, Sox2, Klf4, c-Myc) is a powerful tool to understand the mechanism of regaining pluripotency and investigate the potential of these cells for replacement therapies. Considering that tumorigenesis by ectopic expression of c-Myc and that insertional mutagenesis by genome integration, the generation of iPS cells with a minimal number of factors may hasten the clinical application of this approach. Herein, we show that iPS cells from human umbilical cord blood-derived mesenchymal stem cells only with Oct3/4 and Sox2 without c-Myc and Klf4(oncogenes) transduction. These colonies has high mRNA levels of ESC pluripotent markers (Nanog, Oct3/4, Sox2, hTERT, TDGF1, GDF3, Rex1), comparing to that of parent cells, umbilical cord blood-derived mesenchymal stem cells. Moreover, these iPS cells have the activity of alkaline phosphatase and normal karyotype. Additionally, these cells could differentiate into cell types from all three germ layers *in vitro* and *in vivo*. In summary, we report the successful generation of induced pluripotent stem cells from human umbilical cord blood-derived mesenchymal stem cells without oncogenes, c-Myc and klf4, transduction.*
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41.P03
Efficient derivation of osteoprogenitor cells from induced pluripotent stem cell for bone regeneration

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IPSCs represent a promising cell source for bone regeneration. To generate osteoprogenitor cells, most protocols use the generation of embryoid bodies (EBs). However, these protocols give rise to heterogeneous population of different cell lineage. In this study, we determined if a direct plating method without EB formation step could be an efficient protocol for generating a homogeneous population of osteoprogenitor cells from iPSCs.Murine iPSC colonies were dissociated with trypsin-EDTA, and obtained single cells were cultured on gelatin-coated plates in MSC medium and FGF-2. Adherent cells obtained by this direct-plating technique were termed as direct-plated cells (DPCs). DPCs were evaluated for cell-surface protein expression using flow cytometry. Expression levels of Oct-3/4 mRNA in iPSCs and DPCs were analyzed by real-time PCR. DPCs were cultured for 14 days in osteogenic medium. Osteogenic differentiation was evaluated by Alizarin Red S staining and ALP activity. Expression of osteogenic genes, ALP, osterix, Runx2, and osteocalcin was analyzed by RT-PCR.Expression level of Oct-3/4 in DPCs was robustly down-regulated compared to that in iPSCs. Flow cytometry analysis revealed DPCs had similar characteristics to MSC, suggesting DPCs lost pluripotency. Moreover, the DPCs exhibited high osteogenic potential.Our novel direct plating method in the absence of EB formation step could be amenable to large-scale production of osteoprogenitor cells for bone regeneration.

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42. Adipose-derived Cells for Regenerative Medicine: Pre-Clinical Overview (in coop. IFATS)

42.01
Keynote: Regenerative properties of adipose derived stroma cells and aging

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Adipose-derived stem cells (ASC) share numerous features with MSC from bone marrow and represent a good alternative and attractive source of stromal cells for clinical purposes. ASC are relatively easy to obtain from adipose tissue and in higher number compare to MSC from bone marrow. They display a greater angiogenic potential and can be grown in a defined medium as spheres including at clonal level. In recent investigations, we investigate the effects of aging on regenerative properties of ASC. We demonstrate that aging doesn't change number, phenotype, growth and differentiation of human ASC and causes little senescence. Paracrine activity of these cells is highly disturbed. Manipulating oxidative stress drive the fate of these cells and can reverse aging phenotype. We set-up a clinical trial to test the tolerance and feasibility of a treatment of critical ischmia leg with autologous ASC. The phase I/II is now finished.

42.02
Keynote: Effect of burn injury on mesenchymal stem cell niches: the mechanism behind hetertopic ossification

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Introduction: Heterotopic ossification (HO) is a devastating sequela of burn injury. Mechanisms for HO propose that stem cell progenitors become aberrantly activated to form bone. We hypothesize that the inflammatory response to burn injury enhances the osteogenic capacity of mesenchymal stem cells (MSCs) and that local ATP inhibition at the burn site mitigates this osteogenic potential.
Methods: MSCs were harvested form mice after: (i) 30% burn (ii) burn with topical ATP inhibitor (Apyrase) or (iii) sham control. Human MSCs were harvested from burn and non-burn patients. Osteogeneic capacity was assessed by gene and protein expression and *in vitro* differentiation assays. *In vivo* heterotopic ossification was assessed by an Achilles tenotomy and ossicle models.
Results: Burn injury increased osteogenic differentiation among MSCs which was mitigated by Apyrase treatment. MSCs harvested after burn injury resulted in increased bone formation *in vitro* in mouse and human cell lines. This enhanced osteogenic potential correlated with increased BMP-2 signaling. Similarly, burn injury enhanced bone formation using *in vivo* HO models.
Conclusion: We demonstrate that MSCs exhibit enhanced osteogenic capacity after burn injury. The mechanism appears to be due increased BMP-2 signaling which can be mitigated through ATP inhibition. We establish a potential role for MSCs in modulating HO and demonstrate that direct manipulation of MSCs may have therapeutic utility in its treatement.

42.03
Primary cilia: does cilium architecture predict lineage specification in adipose-derived stem cells?

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Introduction: Non-motile primary cilia have recently been implicated as critical mechanosensory structures in a variety of mammalian cell types. We have identified the presence of primary cilia on human adipose-derived stem cells (hASC) in both 2-dimensional (2D) and 3-dimensional (3D) culture. Based on our previous work with hASC and tensile strain, we hypothesize that primary cilia dynamically mediate hASC differentiation mechanisms, and their conformation is related to their chemo- and mechanosensing properties.
Approaches: Immunofluorescence images were used to categorically evaluate hASC primary cilia conformation in relationship to lineage specification. siRNA knockdown was used to abrogate cilia-associated proteins polycystin-1 (PC1), polycystin-2 (PC1) and Polaris (IFT88).
Results and discussion: Primary cilia were observed on hASC in both 2D and 3D culture with different cilia conformation based on substrate environment as well as chemical and mechanical stimulation. Additionally, hASC exhibited a diminished calcium accretion with PC-1 and PC-2 knockdown and diminished Runx2 gene expression with IFT-88 knockdown, all hallmarks of osteogenesis. Our data has indicated that primary cilia-associated proteins play in important dynamic role in hASC differentiation, particularly in hASC osteogenesis. The results of this study elucidate the intricacies of primary cilia function in hASC differentiation and which can be applied to developing hASC-derived tissue replacements.

42.04
Human platelet lysate favours stemness of adipose derived stem cells

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Human platelet lysate (hPL) can be produced from outdated thrombocyte concentrates or buffy coats which usually represent waste products in blood banks. Therefore hPL is highly available and in comparison to fetal calf serum not associated with safety- or ethical concerns.In the present study we evaluated the potential of hPL in comparison to fetal calf serum (FCS) to promote proliferation and to preserve stemness of ASC. This is of particular importance since stem cells intended for regenerative applications often require extensive expansion. While previous studies focused on adipogenic and osteogenic differentiation, we have evaluated the expression of chondrogenic markers during expansion and subsequent chondrogenic differentiation. Interestingly, presence of hPL in the expansion medium leads to low expression of collagen type II, collagen type IX, aggrecan and COMP. However, this seems to be essential to maintain stemness and to allow intense cartilage matrix synthesis during subsequent differentiation. Furthermore,

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the presence of angiogenic factors in hPL was used as a basis for investigations of hPL in a co-culture model of ASC and outgrowth endothelial cells (OEC). In contrast to OEC mono-culture, co-culture of OEC and ASC resulted in intense vascularization of fibrin matrix. The use of hPL instead of FCS is investigated to transfer this model to an 'all human system'. In conclusion, hPL serves as a valuable alternative for the propagation of ASC.

42.05
A nerve repair conduit containing differentiated adipose-derived stem cells within engineered neural tissue can support and guide neuronal growth *in vitro* and *in vivo*

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Tissue-engineered cellular bridging devices for surgical implantation into peripheral nerve injury sites could provide an attractive alternative to autografts. A patient's own adipose tissue can be used as a source of cells that provide the trophic support and pro-regenerative behaviour elicited by Schwann cells in an autograft. Adipose-derived stem cells can be differentiated towards a Schwann cell-like phenotype *in vitro* (dADSC). Here we report the development of a living replacement tissue using therapeutically relevant cells in an engineered neural tissue (ENT). ENT is made from a cellular collagen gel that is tethered at each end to permit the cells to self-align; this is subjected to a compression process to produce a stable biomaterial. Experiments using cell death assays, immunostaining and confocal microscopy show that dADSCs can be successfully incorporated within ENT - dADSCs survive and maintain their alignment following the stabilisation process to form sheets of an aligned cellular biomaterial (ENT). Primary rat neurons growing on the surface of ENT extended neurites that were guided by the orientation of the aligned dADSCs. These sheets of ENT were rolled into columns and then packed together within a clinically approved tube, NeuraWrap™. Testing this 'engineered endoneurium' in the rat sciatic nerve model showed that neuronal growth was supported and guided by ENT and demonstrates the potential of the device to offer an alternative to nerve autografts.

42.P01
Efficient transfer of human adipose-derived stem cells by chitosan/gelatin blend films

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Adipose-derived Stem Cells (ASCs) are a potential source of abundant mesenchymal stem cells. Previous investigations have demonstrated enhanced therapeutic effects of ASCs in a three-dimensional spheroid culture formulation. In this study, we hypothesize that a composite membrane made of chitosan/gelatin (C/G) is beneficial to facilitate transfer of human ASCs in spheroids. Increasing chitosan content within the blends enhanced the mechanical properties of the sample, including tensile strength and elongation-at-break ratio. Although ASC spheroids developed shortly after seeding on pure chitosan films, increasing gelatin proportion in the C/G blends promoted cell adhesion onto the membranes. We also found that C/G blends of different ratios supported ASC proliferation in the first 4 days of culture. However, ASCs on all C/G blends started to detach from the films to form spheroids after day 4, while ASCs on pure gelatin films remained attached and continued to grow. Gradual gelatin release from the C/G blend films, leading to enriched chitosan content in the blends, probably encouraged ASC detachment, and spheroid formation. We placed porous collagen matrix on ASC-seeded C/G blends and found that a C/G film composed of 75% chitosan could facilitate significantly more cell transfer into the overlying collagen sponge. Therefore, a blend film containing 75% chitosan and 25% gelatin showed promising results to serve as a biomaterial for human ASC-based cell therapy.

42.P02
Non-adherent progenitors from adipose-derived stem cells (napADSCs): a new prospective for tissue engineering?

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Introduction: Mesenchymal stromal cells (MSCS) are often mislabeled as adipose-derived stem cells (ADSC) despite presenting a more differentiated phenotype. The isolation of non-adherent progenitors from ascs (N.A.P.ASCS) is currently under investigation in our laboratory. In this study we investigate: (i) the proof of stem cell origin of such progenitors, and (ii) the feasibility of NAPASCS adhesion over integra® for cell colonization, future differentiation and engineering of semi-synthetic tissues.
Material and methods: Liposuction samples were obtained and plated in stem cell-specific enriched media and in no adherence conditions. Clonal expansion of single cells was assessed by limiting dilution and asymmetric division was detected by pkh26 staining. Expanded cells were seeded within integra®.
Results: Napadscs may represent an upstream line of mesenchymal progenitors compared to more differentiated, adherent, fibroblast-like MSCS. Their stem cell origin was confirmed *in vitro* by clonal expansion and asymmetric division. NAPASCS adhesion to integra® was achieved modifying culturing conditions.
Conclusions: The identification of napadscs may dissipate the doubts on the stem-cell origin of the more differentiated and commonly used adherent MSCS. The ability of NAPADSCS to adhere to a clinically available dermal regenerative template, and grow within its three-dimensional structure, may prove useful in regenerative surgery.

42.P03
Scaffold-free cartilaginous tissue engineered using osteoarthritic human infrapatellar fat pad derived stem cells

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Scaffold-free approaches have been used to engineer cartilaginous tissues with mesenchymal stem cells from healthy donors, however it is unclear if the self assembly technique can be used to generate such grafts using stem cells isolated from osteoarthritic patients. The aim of this study is to use diseased human infrapatellar fat pad derived stem cells (FPSCs) to engineer scaffold-free cartilaginous tissue using a self-assembly approach. Human FPSCs were isolated and expanded in monolayer culture before being seeded into porous transwell inserts and cultured for 6 weeks under altered oxygen and growth factor supplementation conditions. It was found that continuous supplementation with TGF-β3 was required for optimal development of the engineered tissue. Comparable sGAG levels were observed in constructs maintained at 20% O_2 and 5% O_2 (1.2% wet weight), although collagen synthesis was higher in constructs maintained at 20% O_2 (1.1% wet weight at 20% O_2 compared to 0.7% at 5% O_2). Additional supplementation with BMP-6, in combination with TGF-β3, further

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enhanced the functional development of the engineered tissue. All engineered tissues stained positively for alcian blue and type II collagen and weakly for type I and type X collagen. The results of this study confirm that the self-assembly approach can be used to engineer cartilaginous grafts using diseased human FPSCs.

42.P04
Effects of FBS and PL supplemented medium on osteogenic differentiation of stem cells

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Due to the major clinical demands of stem cells, it is vital to seek for an efficient approach for cell culture, including proliferation and differentiation into specific lineages. Lately, human platelet lysate (PL) is used as a substitute for fetal bovine serum (FBS) since it can avoid zoonose contaminations. Human bone marrow stromal cells (HBMSCs) and human adipose-derived stem cells (HADSCs) are popular in bone tissue engineering. Cocultures of either HBMSCs or HADSCs with angiogenic cells can enhance angiogenesis and osteogenesis compared to monocultures. However, the comparison of FBS- and PL-supplemented medium on osteogenesis of stem cells is inconsistent or even lacking. Moreover, most researchers cocultured HBMSCs with endothelial cells (ECs) in FBS-supplemented medium. Available data are limited on comparison of osteogenesis in cocultures versus monocultures for HADSCs and for cultures in PL-supplemented medium. Consequently, this study aimed to assess the effects of FBS- or PL-supplemented medium on osteogenesis of stem cells. Cell proliferative capacity was higher in PL- compared to FBS-supplemented medium in monocultures and cocultures for both HBMSCs and HADSCs. Conversely, osteogenic differentiation decreased for HBMSCs and increased for HADSCs in PL-supplemented medium despite monocultures or cocultures. Osteogenesis was increased in HBMSCs/ECs compared to HBMSCs in both FBS- and PL-supplemented media, whereas this trend was not observed for HADSCs/ECs.

42.P05
Culture of adipose-derived stem cells using a heparin-based hydrogel

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Adipose-derived mesenchymal stem cells (ADSCs) are one of attractive cell sources in cell-based therapy due to their easy collection and capacity of self-renewal and differentiation potential. However, it is important to keep their primitive properties during *in vitro* culture, so the culture substrates need to control the fate of ADSCs via cell-substrate interaction. Recently, various biomaterials have been studied to maintain the pluripotency of embryonic stem cells, but the application of biomaterials on adult stem cells has not been reported much. In this study, we applied the heparin-based hydrogel as a stem cell culture substrate for cell expansion while maintaining the pluripotency. Heparin is capable of interaction with numerous ECMs and cells by heparin-binding domains. Since heparin is a main component of the hydrogel, it can provide a specific cell adhesion ligand to stem cells without modification of cell adhesive molecules (collagen type I or Fibronectin). Here, we characterized the cell adhesion, proliferation, and pluripotency of ADSC on the heparin-based hydrogel. Also, the differentiation potential of ADSCs on the heparin hydrogel upon adipogenic induction was also characterized.

42.P06
Long-term culture without passaging of freshly-isolated human adipose-derived stromal cells (ASC) creates a niche environment which maintains their phenotypic and functional properties

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In vivo, adipose stromal/stem cells (ASC) are identified as $CD34^+/CD105^-$ cells, but their phenotype changes during culture on plastic and expanded ASC are described as $CD34^-/CD105^+$ cells. In order to preserve ASC progenitor properties, we aimed at mimicking the native ASC environment by favoring cell-extracellular matrix and cell-cell interactions.Long-term (LT) cultures of adipose-derived cells, without passaging after reaching cell confluence, were established and LT-ASC were compared to passagged (pass-) ASC ($n = 7$ donors). CD34 was maintained in 22.3 ± 18% of LT-ASC, whereas it was lost by pass-ASC after the first passage. After 28 days of culture, LT-ASC were able to proliferate (8.27 ± 0.82 population doublings) and expressed mesenchymal markers, such as CD90 and CD73, but remained negative for the expression of CD105. LT-ASC displayed a significantly higher clonogenicity as compared to pass-ASC (11.1 ± 0.6% and 6.7 ± 2%, $p < 0.05$) and they produced 30% more hydroxyapatite deposits under osteogenic conditions ($p < 0.05$). The collagen receptors beta1, alpha1 and alpha2 integrins were expressed in pass-ASC, but weakly in LT-ASC, suggesting different adhesion and migratory properties. These data provide evidences of the importance of establishing a specific *in vitro* niche to allow ASC expansion while preserving their native phenotype and progenitor features. It should also provide a model to study the role of specific proteins (integrins, CD105, CD34) in the biology of ASC.

42.P07
Incorporation and stability of supermagnetic iron-oxide maghemite nanoparticles in mesenchymal stromal cells

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Introduction: Therapy based on aplication of mesenchymal stromal cells (MSC) is a promising approach in regenerative medicine of cartilage, bone, skin and other tissues. MSC, which are loaded with supermagnetic iron-oxide nanoparticles (SPION), are detectable by nuclear magnetic resonance imaging system (NMRi).
Methods: MSCs were isolated from adipose tissue or bone marrow and seeded into the plastic. After 2–4 passages, MSCs were incorpotrated by simple incubation with different concentration of SPION for 48 h ('FeNV' maghemite nanoparticles, diameter 20 nm, concentration 50 μg iron-oxide / 1 ml of medium). Properties of MSC were evaluated by semiautomatic fluorescence microscopy, quantification of SPIO in the cytoplasm was performed by Ferrozine spectrophotometric method, the distribution of SPION in MSC and SPION redistribution during cell division were analyzed by time lapse microscope BioStation (Nikon) and by electron microscopy.
Results and conclusion: After staining with SPION, the cell growth was not significantly influenced. SPION in the MSC did not affect metabolism, cell shape or profile of surface markers. During the cell division,

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nanoparticles from primary cell are equally split into the daughter cells. This type of SPION seems to be safe and stable tool for MSC labeling and monitoring with NMRi in a time scale up to 300 h after staining with high potential for preclinical and clinical studies.

42.P08
Comparison of three different harvesting methods to obtain preadipocytes: impact on viability and differentiation to adipocytes

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Autologous fat transfer is a wide spread technique for soft tissue augmentation. A number of different tools for harvesting fat tissue have been established. Such devices should be easy to handle, time saving, low priced, safe and provide a high amount of viable cells in the aspirate. Aim of this study was to compare three different methods for harvesting fat tissue for lipotransfer: Water assisted liposuction (WAL), Power assisted liposuction (PAL) and manual aspiration. Fat tissue was obtained from nine donors undergoing abdominoplasty. Samples were divided into three sections. Out of each section fat was harvested using either WAL, PAL or manual aspiration. Preadipocytes were isolated using a standard protocol. The amount of viable preadipocytes out of 1 ml of fat tissue was evaluated using cell count. The ability of isolated preadipocytes to differentiate was determined by expression of the adipocyte markers adiponectin, GLUT4 and PPARG. Our results show that there are significant differences using different harvesting methods: number of viable preadipocytes were significantly higher using PAL than WAL or manual aspiration. Furthermore their quality as determined by their ability to differentiate into mature adipocytes differs significantly as adiponectin, GLUT4 and PPARG were significantly higher using PAL. Preadipocytes play an important role in autologous fat transfer. Therefore these results should be considered when choosing the harvesting method.

42.P09
Comparison of human and porcine mesenchymal stem cells from adipose tissue (ASCs) and their growth in the presence of different sera

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Bone tissue engineering aims to restore or repair musculoskeletal disorders through the development of bio-substitutes that require the use of cells and scaffolds which need to be tested in a preclinical setting. We analyzed mesenchymal stem cells isolated from subcutaneous adipose tissue of different animal species (ASCs). In particular, comparing porcine and human, the yield of both minipig (mp) and pig ASCs (p) is lower than the one of human ASCs (h). However, the animal cells proliferate faster than the human ones (doubling time of 76, 90 and 109 h for mpASCs, pASCs and hASCs, respectively) and they produce a high number of colonies (porcine 19%, human 3%). With the idea of a future clinical application we cultured these cells in a self and not self conditions. hASCs grow regularly in 5% autologous and heterologous sera, and when cultured in 10% autologous serum their proliferation increased compared to 10% fetal bovine serum (FBS). In contrast, pASCs grow better in 10% FBS without any benefits from the swine supplements. We conclude that growing hASCs in the presence of human sera could become a feasible procedure to ameliorate a future cellular therapy. Regarding ASCs differentiation potential, both human and porcine ASCs, osteo-differentiated for 14 days, similarly increased the expression of collagen and extracellular calcified matrix. Experiments to evaluate if ASCs are able to osteodifferentiate in presence of autologous serum as in 10% FBS are in progress.

42.P10
Neuropathic pain treatment in a mouse model with human adipose-derived stem cells

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Neuropathic pain is a complex disease associated with neuronal-tissue damage. Adipose-derived Stem Cells (ASCs), have shown the capacity of limiting neuronal damage through their anti-apoptotic effect, together with their capacity of releasing neurotrophic molecules. Our study aimed to detect the effect of human ASCs in a mouse sciatic nerve chronic constriction injury (CCI) model. hASCs were isolated from subcutaneous adipose tissue of 5 healthy women (mean age 37 ± 12) and characterized in order to verify their stemness. 10^6 hASCs were injected into the rodents caudal vein 7 days after CCI, and, at 1, 3, 7, 14, 21 and 28 days post injection, we assessed their effect on mechanical allodynia and thermal hyperalgesia, and it correlated with the alteration of the profile of pro- and anti-inflammatory cytokines. hASCs were able to completely reverse hyperalgesia and reduce allodynia starting 24 h after injection. The effect began to fade 21 days after administration, but it could be restored by a new cell injection (10^6). We also observed a recovery of cytokines balance both for pro- (IL-1β) and anti-inflammatory (IL-10) ones. Here, we demonstrated that hASCs treatment is able to reduce neuropathic pain symptoms and to re-establish cytokine balance in a CCI mouse model; this phenomenon might be due to the recruitment of cells in the lesion area and to their interaction with the resident ones inducing a modulation of pain and inflammation.

42.P11
Modulating behaviors of human adipose-derived stem cells using a graphene oxide-based artificial extracellular matrix

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In this report, we propose that a graphene oxide (GO) film is an efficient extracellular matrix (ECM) for human adipose-derived stem cells (hASCs). Using a self-assembly method, we successfully fabricated GO films. The hASCs grown on GO showed increased adhesion, indicated

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by a large number of focal adhesion, and higher correlation between the orientations of actin filaments and vinculin bands compared to hASCs grown on control substrate. Further, the GO film proved to be a suitable environment for the time-dependent proliferation of hASCs. The enhanced differentiation of hASCs included osteogenesis, adipogenesis, and epithelial genesis, while chondrogenic differentiation of hASCs was decreased. The data obtained here collectively demonstrates that the GO film is a suitable ECM for the adhesion, proliferation, and differentiation of hASCs.

42.P12
The effect of electric stimulation and metallic ions on differentiation of adipose-derived stem cells

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Applied electric fields have been shown to have an important role in many cellular functions, including cell migration, orientation, elongation and differentiation. Neural stem cells have been differentiated towards neuronal lineages by using alternating electric fields (EF). In this study, we have investigated the influence of the applied electric currents and electrolysis of copper to differentiate human adipose-derived stem cells (hADSC) towards neuronal cells. The hADSCs were induced by both mechanical and electrical stimulation along with a small amounts of copper released from the electrode. In the controls, either EF was applied or copper chloride was added to the culture. The morphological changes and neuronal lineage specific marker beta-III-tubulin expression was studied and stimulated cells further characterized by IHC, WB, and PCR. Through the application of electricity and copper electrolysis we were able to differentiate hADSCs to cells with neural phenotype with elongated, neuron-like shape and high level of beta-III-tubulin expression. Cells exposed to electric stimulation without copper release changed their morphology to elongated shape with no beta-III-tubulin expression. Cells with addition of copper alone did not significantly change the morphology and had a limited beta-III-tubulin expression only. We have thus demonstrated that electric stimulation together with electrolytic release of copper induces the neuronal differentiation of hADSC *in vitro*.

42.P13
Molecular mechanisms regulating matrix dependent adipose derived stem cell physiology

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In regenerative medicine soft tissue defects are often repaired by autologous tissue/stem cell transplantation. Actually, synthetic or biologically derived materials are used to improve the surgical outcome, however, often with limited success. This might be due to the use of non-tissue compatible matrices/fillers and – properly – to our limited knowledge on how matrix structure influences adipose derived stem cells (ASC) behavior. Thus we aim to address the question how primary ASC interact with their extracellular matrix (ECM) form a biological point of view. For this we systematically investigated the expression of ECM-interacting alpha integrin proteins in primary human ASC and identified by comparative expression profiling with *in-vivo* and *in-vitro* differentiated adipocytes integrins, specifically expressed in ASC. To this end, functional gene analysis revealed the importance of ITGAV (recognizes type I collagen) for ASC proliferation and ITGA7 (recognizes laminin) for efficient adipocyte differentiation. The investigation of the activated downstream pathways and competitive substrate experiments suggest that the presence of single ECM components specifically support individual ASC characteristics and hence shall be considered by the rational design of tissue specific bioscaffolds.

42.P14
Comparison of polylactide-based 3D composites in the differentiation of adipose stem cells

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The objective of this study was to examine the growth and differentiation of adipose stem cells (ASCs) in knitted and rolled polylactide (PLA)-based scaffolds. The basic PLA96/4 (P) and composite scaffolds consisting of PLA96/4+bioactive glass (PG), PLA96/4+chitosan (PC), and PLA96/4+bioactive glass+chitosan (PGC) were seeded with rabbit ASCs. The constructs were cultured in control and chondrogenic media for 3 weeks. Cell viability was visualized by fluorescent staining. Quantitative real-time RT-PCR was used to measure the relative expression of the genes related to osteochondral differentiation. The formation of cartilaginous matrix components was examined with sulfated glycosaminoglycan (GAG) assay and histological staining.All the composites provided a suitable basis for the proliferation and differentiation of ASCs. Cell aggregate formation was enhanced in chondrogenic medium and in the presence of chitosan in the PC and PGC scaffolds. The accumulation of sulfated GAGs was strongest in the PGC. In the PC and PGC scaffolds, the expression of the genes related to chondrogenic differentiation was higher than in the P and PG scaffolds, where the gene expression was more affected by culture media. The biomaterial composition of the PLA96/4-scaffold has even more effect on the growth and differentiation of ASCs than the culture medium. The advantages of different composites can further be utilized in the development of tissue-engineered osteochondral implants.

42.P15
Immunosuppressive effect of human adipose derived stem cells(ADSC)

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ADSCs have similar potentials as stem cells with bone marrow stem cells, but has more advantages such as easy to get large amount of cells and minimal invasion to the patients. For clinical use of ADSCs in urgent patients, rapid availability from cell banking may offer many options. Recent reports revealed that ADSCs have immune suppressive effect which might be a key for ADSCs allograft from cell banking. This study was conducted to analyze immunosuppressive effect of ADSC for allogenic transplantation for therapeutic purpose. We obtained adipose tissue from donors and isolated and cultured ADSC. We examined phenotypic and gene expression profile by flow cytometry and induced ADSCs to differentiate into various cell types. Immunosuppressive properties were analyzed by mixed lymphocyte culture and lymphocyte proliferative response to non-specific mitogens. Cultured ADSCs shows characteristics of stem cells and can be differentiated into the osteoblast, adipocyte and neuronal cells. ADSC did not provoke in-vitro alloreactivity of incompatible lymphocyte and suppressed mixed lymphocyte reaction. ADSC also suppressed proliferative response of lymphocyte to the mitogen such as PHA, ConA and IL-II. These findings support that ADSC has properties of stem cells and immunosuppressive effect. ADSCs might have potentials for cell based regenerative thera-

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pies for various neurological diseases and also for allogenic transplantation because of their immunosuppressive properties.

42.P16
Immunogenicity of adipose stem cells

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Adipose stem cells (ASC) are capable of differentiating into multiple cell types and they have been shown to have therapeutic potential for diverse clinical applications. ASCs seem to be immune privileged; they do not to elicit immune reactions against themselves. Furthermore, ASCs have shown to be immunosuppressive, diminishing immune reactions. Because of these properties, ASCs are an attractive cell type for cell therapies. In this study, we investigated the immunogenic properties of the ASCs. Previously in our group, good manufacturing practice (GMP) compliant serum-free and animal-free (xeno-free) workflows for the expansion of ASCs were developed and assessed. We have established ASC lines grown in FBS, human serum and in this novel serum and xeno free medium. We performed mixed lymphocyte reactions to determine the immunogenic and immunosuppressive properties of ASCs in these three different mediums. We have also investigated the change in CD marker profiles of the cells grown in FBS and human serum containing media and in novel serum and xeno free medium and during the passaging of the cells. As a conclusion, cell culture medium has strong effect on immunological properties of ASCs. Moreover, culturing conditions and passaging have effect on CD marker profile of ASCs. These differences in cell behavior according to choice of medium shout for safety and efficacy assessment of ASCs cultured in these novel conditions before clinical use.

42.P17
A novel micro-macroporous scaffold for adipose tissue reconstruction using adipose-derived stem cells (ADSCs)

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Introduction: Changes in tissue contour from loss of subcutaneous adipose tissue due to burns, tumour resection or congenital abnormalities severely impact on patients. Current reconstructive strategies yield unpredictable and often unsatisfactory results. Autologous ADSCs support de novo tissue formation, and we report the fabrication of a novel gelatin scaffold suitable for the delivery of ADSCs.
Methods: Gelatin solution (7% w/v) was added to moulds containing packed alginate beads and cross-linked with gluteraldehyde (25% w/v). Beads were removed from the gelatin matrix using 3% w/v trisodium citrate. ADSCs from human adipose tissue were seeded directly into the scaffold or delivered incorporated into fibrin hydrogels. ADSCs were cultured within scaffolds for 14 days prior to differentiation for a further 14 days.
Results: Cryosections showed that leaching of the beads from the gelatin matrix produced macropores with dimensions similar to the bead template (2 mm) and that the scaffold walls were microporous. H&E staining revealed ADSCs had infiltrated the macropores when seeded directly into the scaffold and when incorporated into fibrin hydrogels. Oil Red O staining confirmed differentiation of cells along the adipose lineage. ConclusionThe novel, templated scaffolds support the infiltration, proliferation and differentiation of ADSCs. Scaffolds can be moulded to any shape, making them suitable for ADSCs delivery to reconstruct soft tissue lost following trauma.

42.P18
The effects of biophysical and biochemical environment on preadipocyte differentiation

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Introduction: Adipose tissue is key for clinical applications such as breast reconstruction after cancer. Adipocyte differentiation can be induced chemically, but the physical properties of the cell environment can influence differentiation. This study aims to investigate the effect of matrix stiffness on adipocyte differentiation.
Materials and methods: 3T3-L1 cells were cultured on polyacrylamide gels of varying elastic modulus in normal or adipogenic media. RNA was extracted from cells at day one and three to assess expression of adipogenic markers by rt-PCR.
Results: Cells showed maximal spreading on gels with moduli above 4.1 kPa, but on those moduli below 4.1 kPa they showed a more spherical phenotype, forming tissue-like aggregates within 27 h of seeding. In standard medium, maximal expression of adipogenesis markers was seen in the mid range of matrix stiffness, around 4–14 kPa. There was a complex pattern of interaction between matrix stiffness and medium type, with adipogenic medium generally increasing expression of markers of early adipogenesis but not uniformly, and showing significant interaction with matrix stiffness.
Conclusions: Matrix stiffness influences adipocyte differentiation with matrices around the stiffness of endogenous adipose tissue the most favourable, but there is unpredictable interaction with chemical signals of adipogenesis that will need to be better understood for the optimal design of matrices for adipose tissue engineering.

42.P19
Local inhibition of adipogenesis in 3D coculture of adipose-derived stem cells and microvascular endothelial cells

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The interplay between adipogenesis and vascularization plays a key role in the development of engineered adipose tissue. However, the cross-talk of the cell types involved is still largely unknown. Therefore, the objective of this study was to investigate adipogenesis in 3D coculture spheroids made from adipose-derived stem cells (ASC) and microvascular endothelial cells (MVEC) in comparison to ASC monoculture spheroids. 3D coculture spheroids from human ASC and human MVEC were produced in 96-well plates using the liquid overlay technique. Employing an ASC: MVEC ratio of 1 : 1, MVEC assembled either in clusters or in network-like structures (confocal microscopy, immunohistochemistry). After adipogenic induction, ASC within coculture spheroids accumulated less triglycerides than in corresponding monocultures (histology, triglyceride quantification). Well in agreement, the vast majority of adipogenic marker genes (e.g., aP2, FATP1, adiponectin, adipsin) were expressed less strongly in the cocultures on day 2 and day 9 after induction (TaqMan array after ASC separation by MACS). Strikingly, MVEC were demonstrated to locally inhibit adipogenesis of ASC in close proximity of the MVEC (histology, immunohistochemistry). The established coculture appears suitable to further investigate the molecular cross-talk of the two cell types. Furthermore, the coculture spheroids may serve as preformed building blocks possibly favoring vascularization in approaches to adipose tissue engineering.

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42.P20
Controlled release of dexamethasone enhances long-term retention of adipose tissue

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Tissue defects from trauma, tumor resection or congenital malformations require soft tissue repair. While current reconstructive procedures involve movement of soft tissue from other areas of the body, there remains an unmet need for new modalities that are less invasive and more precise. The aim of this study was to develop a controlled delivery system of adipogenic factors, namely insulin and dexamethasone (dex), within the fat graft. Both insulin and dex were encapsulated in poly(lactic-co-glycolic acid), (PLGA) microspheres (MS) and mixed with lipoaspirate to induce adipogenesis *in vivo*. Loaded-MS were mixed with human lipoaspirate and injected subcutaneously into an athymic mouse model. Groups included both low and high doses of dex and insulin per 1 ml of human fat as well as combination groups. A control group with five animals was also added to analyze empty PLGA microspheres in 1 ml of human lipoaspirate. Samples were analyzed grossly and histologically after 3, 6, 12 and 24 weeks *in vivo*. Mass and volume were measured, with the MS-containing samples demonstrating an increased mass and volume displacement with an increased MS content. Histological analysis indicated there is increased vascularization within the insulin/dex MS-containing samples. In summary, this study demonstrates that the controlled delivery of adipogenic factors such as dexamethasone via polymer microspheres can significantly affect tissue formation and vascularization.

42.P21
Mechanical derivation of functional myotubes from adipose-derived stem cells

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Though reduced serum or myoblast co-culture alone can differentiate adipose-derived stem cells (ASCs) into mesenchymal lineages, efficiency is usually not sufficient to restore function *in vivo*. Often when injected into fibrotic muscle, their differentiation may be misdirected by the now stiffened tissue. ASCs are shown here to not just simply reflect the qualitative stiffness sensitivity of bone-marrow-derived stem cells (BMSCs) but to exceed BMSC myogenic capacity, expressing the appropriate temporal sequence of muscle transcriptional regulators on muscle-mimicking extracellular matrix in a focal adhesion-dependent manner. ASCs formed multi-nucleated myotubes with a continuous cytoskeleton that was not due to misdirected cell division; microtubule depolymerization severed myotubes, but after washout, ASCs re-fused at a rate similar to pretreated values. BMSCs never underwent stiffness-mediated fusion. ASC-derived myotubes, when replated onto non-permissive stiff matrix, maintain their fused state. Together these data imply enhanced mechanosensitivity for ASCs, making them a better therapeutic cell source for fibrotic muscle.

42.P22
Transition of adipose derived stem cells into contractile smooth-muscle-cells for bladder engineering

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Tissue engineering using smooth muscle cells (SMCs) may provide alternative treatments for diseases with smooth muscle pathologies such as bladder voiding dysfunction, urinary incontinence and erectile dysfunction. An alternative cell source would be adipose derived stem cells (ADSCs), which can be differentiated into different cell types. However, ADSC differentiated to SMCs may loose their contractile phenotype followed by a switch to a synthetic form. Since little is known with regard to the baseline functional characteristics of differentiated SMCs, we have investigated the gene and protein expression and cell contractility during induction of ADSCs into SMCs. Rat ADSCs were induced towards SMCs using induction medium. Real- time PCR demonstrated gradual increase of calponin, MyH11 and smoothelin during 1–4 weeks and decrease after 5 and 6 weeks. FACS data showed an increase of alfa-smooth muscle and calponin but decrease of smoothelin and MyH11 after 3 weeks. The same was observed using immunocytology smoothelin the contractile protein was reduced after 5 and 6 weeks. WB revealed increase of smoothelin, calponin and MyH11 protein during 1–4 weeks and reduction after 5 to 6 weeks. The contraction assay showed a maximal contraction after 2–4 weeks and response to carbochol whereas undifferentiated ADSCs did not contract.The induction time of 3 weeks seems sufficient to differentiate the cells into SMCs with a contractile phenotype suitable for bladder engineering.

42.P23
Biocompatibility of human adipose stem cells with beta-tricalcium phosphate substitutes: influence of fibronectin coating on osteogenic differentiation process

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Introduction: Bone tissue engineering requires cells with regenerative capacity, biocompatible scaffolds and osteoinductive matrix.
Objective: This study aims to evaluate the biocompatibility and osteogenic differentiation of adipose stem cells (ADSCs) on beta tricalcium phosphate scaffolds with/without coating with fibronectin (FN).
Methods: 1×105 ADSCs cultured with proliferative (PM) or osteogenic medium (OM) were seeded on 500 mg of KeraOs® (KO) granules (Keramat, Spain) with/without FN coating (5 $\mu g/cm^2$). Cell viability was detected by Alamar blue at 3, 10, 14 and 21 days. Cell morphology was observed by SEM. Osteogenic differentiation was evaluated by alizarin red staining; ALP assay and real-time PCR array to quantify a panel of 84 osteogenesis-related genes.
Result: ADSCs can attach and grown on KO and KO-FN granules. These results were confirmed by SEM. The assays revealed that KO-FN granules are able to induce the highest ALP activity and increased mineralized matrix in PM and OM at 21 days compared with KO. The PCR-array showed the activation of 45 and 54/84 genes in ADSCs culture on KO and KO-FN respectively. Overexpression of highly osteogenesis-related genes such as BMP1, BMP2, BMP4, RUNX2, SMAD1, ALPL, BGLAB or COL12A1 and downregulation of BMP3 and TWIST was measured. Conclusion: ADSCs are biocompatible with KO. The coating with FN induces an increase in ALP activity, mineralized matrix and the strong expression of a greater number of genes related with osteogenic process

42.P24
Osteogenic medium is superior to growth factors in differentiation of human adipose stem cells towards bone-forming cells in 3D culture

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Bone regeneration by autologous stem cell transplantation is a promising approach to treat large bone defects. Human adipose stem cells

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(hASCs) are easily available cells having the potential to differentiate into mesenchymal lineages, including bone-forming cells. Recently, hASC-based applications have been successfully used to treat bone defects in clinical practice by our group. Commercially available bioactive glass scaffolds (BioRestore, Inion Ltd, Tampere, Finland) and biphasic calcium phosphate granules (BoneCeramic, Straumann AG, Basel, Switzerland) were compared in this study as carriers for hASCs. For efficient osteo-induction, bone morphogenetic proteins (BMP-2 and BMP-7), vascular endothelial growth factor (VEGF), and a novel, optimized osteogenic medium were compared in combination with the biomaterials. Both biomaterials supported the cell viability and proliferation during 2 weeks of culture. The most significant osteogenic differentiation of hASCs was achieved by BoneCeramic combined with osteogenic medium. On the contrary, combining growth factors with either biomaterial did not increase osteogenic differentiation or proliferation when compared to control. Moreover, BMP-7 consistently inhibited proliferation and osteogenic differentiation of hASCs seeded on either biomaterial. To conclude, our results demonstrate that osteogenic medium enhances hASC differentiation towards bone-forming cells in 3D culture more efficiently than growth factors.

42.P25
The impact of bioactive glass, beta tricalcium phosphate and BMP-growth inducing factors on osteogenic differentiation of human adipose stem cells

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Human adipose stem cells hASCs are an attractive and abundant source of multipotent progenitor cells. Currently, optimized osteogenic differentiation methods for their usage in clinical applications are under intensive investigation. In this study, the combinatorial input of biomaterials: bioactive glass (BAG), beta tricalcium phosphate (beta-TCP) and growth inducing factors: bone morphogenetic protein (BMP)-2, BMP-7 on osteogenic differentiation of ASCs was investigated. Cell attachment, viability and proliferation was analyzed by Live/Dead staining and qDNA measurements. Osteogenic differentiation was determined by alkaline phosphate (ALP) stainings and expression of osteogenic marker genes. Our results revealed providential attachment and viability of ASCs on both biomaterials. However, BAG significantly induced cell growth in control medium (CM) and osteogenic medium (OM) compared to beta-TCP. ALP activity measurements indicated that BAG supports osteogenic differentiation in CM more than beta-TCP. Nevertheless, ALP activity was higher on beta-TCP cultured cells in OM. Surprisingly, BMP-2 and -7 supplementation had a negative impact on early cell proliferation and ALP activity of hASC independently of the biomaterial, but clearly elevated osteopontin expression of BAG cultured cells. Our data suggest that BAG enhances proliferation and early osteogenic differentiation of hASC *in vitro* and thus, may be a clinically feasible for bone tissue engineering.

42.P26
Fibrin-encapsulated, genetically modified adipose-derived stem cells for use in tissue repair

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Transplantation of stem cells engineered to express Bone Morphogenetic Protein-2 (BMP-2) remains an attractive approach for bone repair as it provides both BMP and osteoprogenitors. Adipose-derived stem cells (ADSCs) are of particular interest as they are obtained with lower donor-site morbidity and at greater yields than in bone marrow. This study aimed to develop an expedited method, using gene transfer, for preparing ADSC-loaded fibrin gels to serve as cell and BMP-2 delivery systems.hADSC suspensions were mixed with adenovirus carrying hBMP-2 or GFP cDNA (2500 viral particles per cell). Transduction was carried out using a centrifugation method (2000 g for 15 min). The supernatant was removed and the cell pellets resuspended in media. Subsequently, transduced cells were transferred to fibrinogen which then polymerized into a fibrin gel encapsulating the cells. Cell viability and transgene expression were evaluated over a 3-week study. Our centrifugation method resulted in at least 70% transduction efficiency. Cell viability and GFP expression within the scaffolds was maintained for at least 3 weeks. Ad.BMP-2 transduced cells produced ~170 ng BMP-2/24 h/106 cells at day 8, then BMP-2 secretion progressively decreased to basal levels. These data suggested that ADSCs can be expeditiously engineered and encapsulated in fibrin gels to deliver high quantities of BMP-2. *In vivo* studies are underway to evaluate the potential of this system for healing rat segmental defects.

42.P27
Induction of insulin-producing cells from human adipose tissue-derived stem cells by Pdx-1

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Pancreatic and duodenal homeobox gene 1 (Pdx-1) plays a key role in normal pancreas development and is required for maintaining the normal function of islets. In this study, we examined whether human adipose tissue-derived stem cells(hASCs) could differentiate into insulin-producing cells by exogenously expressed Pdx-1. hASCs were infected with recombinant adenovirus encoding the mouse Pdx-1 gene and differentiated under high-glucose conditions. Insulin transcript levels and the expression of key transcription factors required for pancreatic development were significantly increased by exogenous Pdx-1 overexpression. In addition to transcripts for transcription factors involved in pancreatic development, transcripts for the GLP-1 receptor, glucokinase and glucose transporter, which are required for maintaining the function of pancreatic β-cells, were observed only in Pdx-1-induced hASCs. Pdx-1-induced hASCs exhibited insulin secretion in response to glucose challenge *in vitro*. When Pdx-1-induced hASCs were transplanted into streptozotocin (STZ)-induced diabetic mice, they reduced blood glucose levels, although they did not restore normoglycemia. These results demonstrate that the expression of exogenous Pdx-1 is sufficient to induce pancreatic differentiation *in vitro*, but does not induce the fully functional, mature insulin-producing cells that are required for restoring normoglycemia *in vivo*.

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42.P28
Preclinical evaluation of adipose-derived mesenchymal stem cell efficacy in grafted burn wound healing

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Goal: To compare the efficacy of allogenic mesenchymal stem cells (MSCs) isolated from adipose tissue (AT) under burned vs. unburned skin. We hypothesize that burned skin AT-derived MSCs promote grafted burn wound healing.
Method: Third degree burns were induced in four squares (5 × 5cm each) on the right dorsum of the sheep. After 24 h, eschar was excised and four skin graft patches (2 × 2cm each) harvested from the contralateral side and were centrally placed in each of the squares. The grafted sites were treated with 7 million AT-derived MSCs harvested from area under burned, adjacent and distal to burn skin in a different sheep with third degree skin burn. The healing was evaluated at 7, 14, and 21 days. MSCs were also tested in mesh graft burn wound.
Results: The AT-derived MCSs differentiated *in vitro* into osteoblasts, chondroblasts and adipocytes. Unlike the control site, the size of treated grafts similarly increased to an average of 2.3 × 2.3 cm, and 2.8 × 2.8 cm at 7, and 14 days. The wound epithelialization was 38.7% (burned), 34.8% (adjacent) and 36.9% (distal) versus 20% (untreated) at day 14, and 82.3%, 94.1% and 93.8% vs. 65% at day 21. Histologically ~95% and 74% of wound not covered with patch in treated and untreated sites, respectively were re-epithelialized. High-resolution ultrasound revealed regular epithelization of treated meshed graft. Conclusion: MSCs isolated from 'burned' fat had an equal potency as the cells isolated from fat adjacent or distal to the burn.

42.P29
Adipose tissue engineering for a breast reconstruction

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The current surgical treatment for the breast reconstruction is performed by a medical prosthesis or an autologous transplantation. However, since they have still several shortcomings should be solved, the cure by the regenerative approach is highly expected. In this paper, the adipose tissue regeneration with the decellularized matrix was reported. Rat lungs were decellularized by SDS solution perfused by bioreactor. They were then seeded with adipose cell line 3T3-L1 cells by injection of the cell suspension. The constructs were proliferated for 3 days, differentiated for 3 days, and matured for 7 days in a circulatory culture system. They were then implanted into nude mice subcutaneously in the back. The decellularized tissue was completely cell free and only collagen-like fibers were remained. The maturation of injected adipocytes in the construct was confirmed by the Oil-Red O staining. From the implantation study, the seeded cells were well remained and matured in the constructs. These results suggest that the acellular lung may be useful as a scaffold for the adipose tissue engineering.

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43. Adipose-derived Cells for Regenerative Medicine: Clinical Overview (in coop. IFATS)

43.01
Keynote: The adipose stem cell IP landscape

L Powers
Bethesda, USA

No abstract available.

43.02
Keynote: Therapeutic applications of adipose tissue and its progenitor cells

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Many features of adipose stem/progenitor cells, such as physiological functions, have been clarified in the past decade. The progenitors play important roles in physiological turnover, hyperplasia and atrophy of adipose tissue; as well as incidental remodelings, such as post-injury repair. A variety of adipose remodeling such as those after ischemia, ischemia-reperfusion injury, mechanical injury, and lipografting were examined in a cellular level. Under severe ischemic conditions, adipocytes die soon, but ASCs can stay viable up to 3 days. ASCs are activated by various factors derived from dying cells and disrupted extracellular matrix and contribute to subsequent regenerating processes. Using adipose tissue or/and ASCs, we can promote angiogenesis or adipogenesis, boost vascularization of irradiated tissue, promote healing of chronic ulcer or diabetic ulcer. We can also clinically boost effects of lipografting for a variety of tissue augmentation and reconstruction, and for tissue revitalization of stem cell-poor tissues, such as irradiated tissue, chronically inflammatory fibrous tissue, and hypertrophic scar contracture. We need careful design of microenvironment activating ASCs, cell delivery protocol to avoid unexpected behavior and induce maximal potential of ASCs, and selection of target diseases for successful clinical applications.

43.03
Keynote: A phase I clinical trial for maxillary bone augmentation with adipose stem cells and calcium phosphate scaffolds

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For patients with maxillary atrophy, autologous bone implantation into the maxillary sinus is the 'golden standard' to achieve sufficient bone volume for dental implants. However, autografting has disadvantages, such as limited graft availability, patient discomfort, and donor-site morbidity. Synthetic bone substitutes are used as an alternative strategy, but only allow osteoconduction, since viable osteogenic cells are lacking. In this phase I trial, osteoinductive implants [calcium phosphate (CaP) carriers seeded with the stromal vascular fraction of adipose tissue] are generated in an intra-operative procedure within hours, thereby avoiding costly GMP stem cell expansions and a second intervention. Where possible, a 'split mouth design' (with only CaP scaffold at the contralateral control side) was applied to allow efficacy evaluation. Adverse events (AE) are monitored, and clinical, X-ray, and Cone-beam CT data are collected at regular intervals during follow-up. After 6 months biopsies are obtained during dental implant placement, and evaluated for bone formation by histomorphometry and μCT. Currently (May 1), we have included 10 patients. No AEs have occurred sofar. Biopsies have now been collected from five patients, which are currently being analyzed with μCT and histomorphometry. Preliminary outcomes show some unexpected but promising efficacy data. The full outcomes of these five patients will likely be evaluated before the TERMIS meeting.

43.04
Differentiation of adipose-derived mesenchymal cells to endocrine pancreatic lineage

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Loss of insulin-producing cells occurs in type 1 and type 2 diabetes but lack of donor pancreata limits cell replacement therapy. This paper assesses the differentiation of adipose-derived mesenchymal cells (AMCs) into readily available replacement insulin-producing cells. Chromatin immunoprecipitation analysis of multiple tissues from adult mice identified visceral adipose tissue to be most similar to pancreas. AMCs were isolated from Pdx1-GFP and MIP-GFP transgenic mice, where GFP expression is driven by pancreatic and duodenal homeobox 1 gene promoter (Pdx1) or mouse insulin-1 promoter (MIP). We optimized the differentiation to endocrine pancreatic lineage by quantitative assessment of GFP accumulation (promoter activity). This data was used to differentiate human AMCs to insulin-producing cells. Human AMCs were isolated and cultured from visceral pancreatic and subcutaneous abdominal fat. These were characterized for stem cell and pancreatic markers using confocal microscopy, flow cytometry and TaqMan-based real-time qPCR. Expression of GFP in transgenic mouse AMCs subjected to this differentiation protocol demonstrated commitment to endocrine pancreatic lineage. We have shown that human AMCs will migrate into islet-like cell aggregates when subjected to differentiation protocol, which induced significant increases in islet hormone transcripts *in vitro*. Transplantation studies into a mouse model of type 1 diabetes will confirm their potential for cell replacement therapy.

43.05
Safety and efficacy of adipose engineered tissue in breast reconstruction a translational experience

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The development of translational experience in Plastic Surgery allows to engineer a functional tissue dedicated to reconstruct soft tissue

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defects followed to breast surgical oncology or integrate implant surgery in staged breast reconstruction. The procedure is one-step and based on intraoperative SVF isolation followed by a fat enhancement process. The final adipose engineered tissue is SVF-enhanced and biologic/living scaffold supported, represented by mature adipocites. In the Breast Unit of the Azienda Ospedaliera-Universitaria Careggi, since December 2007 to December 2011, 105 breast reconstruction patients have been treated with enriched fat and obsereved with a 26.4 months median follow up. Clinically, enriched fat techniques obtain an high graft retention rate and long term stability in hostile sites like scars or radiated areas. Result evaluation was supported by instrumental examination as ultrasounds and MRI. Breast reconstruction treats oncologic patients and the safety issue is prioritary. In the 105 patients breast cancer local reccurences and metastasis were evaluated. A case control study was also performed comparing different fat grafting techniques in selected groups of nipple areola sparing mastectomy reconstruction patients. The patient follow up showed any evidence of an higher local recurrence and metastasis rate than the expected.

43.P01
Evaluation of a novel, rapid method to catch and release therapeutic numbers of viable adult stem cells from adipose

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Isolating therapeutic doses (>106) of adult stem cells (ASCs) is difficult. Routine methods centred on small antibody labelled magnetic particles do not work well in primary tissues without extensive pretreatment and can become internalised causing phenotypic alteration or immuno-compromisation. In this study we evaluated a new larger particle to isolate ASCs, which allows cell release without retention of isolation artefacts. Adipose was isolated from 10 week old rats and collagenase digested. Capture particles (50–100 μm diameter, Autology Health Ltd, UK) populated with anti-CD90 antibody were combined with adipose (15 min, 4 °C). Following washing, the particles were incubated with release buffer (15 min, RT) to elute any captured cells. Typically 5–10% of the cells in rat white adipose were $CD90^+$ cells, which did not vary as a function of anatomical location, ($n = 7$). Particles allowed selective capture of $CD90^+$ cells. The large particles depleted up to 80% of ASCs from primary adipose without extensive pre-treatments. The phenotype of the isolated cells was confirmed by positive RT-PCR for the CD90 transcript. Using the release buffer captured cells could be recovered and introduced into culture without internalisation of capture particles. This study presents a novel cell selection strategy for ASCs. The technique can process large volumes of minimally processed crude tissue allowing capture and release of therapeutic numbers of ASCs, whilst avoiding internalisation.

43.P02
'Cellular therapy' through lipostructuring; the role of adipose-derived adult stem cells in regenerative surgery processes

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Introduction: Adipose tissue grafting (lipostructuring) is regularly used for volume corrections due to congenital malformations and/or syndromes, burns, trauma and after oncologic and/or aesthetical procedures.
Materials: Thirty nine patients, January 2006 to December 2010, underwent lipostructuring according to Coleman technique (12F, 7M, range 13–58, mean age 36 years) for pathologic scars, congenital malformation and volume deficiency of the face (8 cases), breasts (15), thorax (2), abdomen (4), upper and inferior limbs (10). Always short term profilaxis. Range hospital stay was 3–5 days. Follow up performed at 3, 6 and 12 months after the operation with pinch and punch tests.
Results: Adipose-Derived Adult Stem Cells (ADSC) presence in adipose tissue makes of lipofilling a 'cellular therapy': the stem cell identification with histology, citologic and ultrastructural caracterization, immuno-histochemical identification confirmed the potential of this surgical option. Long-term results highlighted how a regular healing process followed the lipofilling with encouraging results in term of skin trofism, disappearance of symptoms like pain, parestesy, disestesy, allowing the resolution of volume losses with an aesthetic improvement.
Conclusion: The identification of ADSC in grafted adipose tissue clarified why skin trophism and healing processes improve dramatically, compared to other surgical procedures; the ADSC seem to play a fundamental role in 'regenerative surgery' procedure.

43.P03
Enhanced engraftment of a cell enriched matrix derived from lipoaspirate using a custom processing system with extrusion

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Autologous lipoaspirate is an attractive option for graft material when performing cosmetic or reconstructive procedures. Research indicates that clinical outcomes may be improved by increasing the inherent neoangiogenic and regenerative potential of the autograft through centrifugation to remove oil and aqueous fluids from the graft (Coleman 1995, Kurita et al 2008). This process increases the short term retention of mass in the autograft. However, it does not change the histological character of the graft. In contrast, fat grafts that are supplemented with adipose-derived regenerative cells demonstrate better long term retention, and improved vascularization (Zhu et al 2010).Yoshimura's lab (Eto et al 2012) demonstrated the importance of cell regeneration for the survival of transplanted adipose grafts and replacement of dying adipocytes. To enhance graft survival we have developed a mechanical method for producing an autologous fat graft enriched in regenerative cells. Extrusion of lipoaspirate multiple times in combination with high speed centrifugation disrupts mature adipocytes and enriches for preadipocytes and other regenerative cells. The number of viable cells per gram of graft is increased 3-fold and the number of plastic-adherent cells per gram is increased 4 fold. The enhanced graft in nude mice shows much more thorough vascularization and interstitial collagen deposition at 4 weeks. This appears to eliminate the central dead zone observed in control samples.

43.P04
Combinative approach for clinical-grade generation of mesenchymal stem cells under xeno-free conditions

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Typically mesenchymal stem cells (MSCs) are cultured in media containing bovine serum (FBS)-inconsistent, xenogenic component and potential source of pathogens, ipso facto, decreasing the clinical benefit of MSCs. Therefore it is necessary to implement xeno-free conditions from the moment of isolation, throughout maintenance of MSCs in culture until clinical application. Until now, most xeno-free culture condi-

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tions allowed efficient expansion, but not isolation of MSCs.In the present study, we performed the isolation and expansion of adipose derived mesenchymal stem cells (ADSCs) under nine different conditions. Each of either FBS-, human serum (HS)-containing medium or a chemically defined medium (StemPro MSC SFM) was tested on standard or one of either amine or vitronectin (VTN) peptide functionalized surfaces.The combination of StemPro media and VTN surface provides a far superior ADSCs attachment, morphology and growth compared to those cultured in any of the other eight conditions. ADSCs cultured under these conditions showed an average 3-fold higher expansion rate than ADSCs cultured in FBS-supplemented medium on TCT. The higher proliferation rate was confirmed by a BrdU assay. Furthermore, characteristic surface marker expression, osteogenic and adipogenic differentiation potential of ADSC was maintained. Summarised, we report the most up-to-date method of isolation and expansion of ADSC for the production of clinical-grade stem cells. Grant No. POIG.01.01.02-00-022/09

43.P05
A new method for safe isolation and maintenance of human adipose stem cells in xeno-free conditions

C Escobedo-Lucea, A Sanz-García, YR Lou, C Gandía-Ventura, M Yliperttula and JM García-Verdugo
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Adult stem cells which can be obtained from adipose tissue (hASC) are important tools for tissue engineering for various reasons: adipose tissue is abundant, hASC are easily isolated and differteniated to multiple lineages, and do not trigger an immune response. Most research and clinical protocols using hASC contain reagents derived from xenogenic sources. Thus, we aimed to design a protocol to isolate and maintain the properties of hASC without animal reagents.
Materials and methods: The hASC were isolated from samples obtained from liposuction. Cultures were maintained in media with human serum (HS). TrypLE Select was used to detach and expand the cells. Ultrastructural and molecular characterization was performed on hASC maintained for one, three and five passages. To evaluate the multipotent capacity of hASC under xeno-free conditions differentiation to adipose and cartilage was induced. Microarray analysis was performed to validate hASC expression pattern and exclude genetic anomalies.
Results: Our xeno-free culture conditions maintain hASC morphology, proliferation and stemness. Additionally, HAS, Tryple and HS had no adverse effects on hASC differentiation, chromosomal stability. or biochemical pathways.
Conclusions: We have demonstrated hat it is possible to isolate and maintain hASC in the absence of animal reagents while preserving crucial culture parameters during long term culture. Hence, we have developed a necessary tool for the improvement of clinical therapies.

43.P06
Burn induced alterations in adipose derived stem cells

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Introduction: The use of autologous adipose-derived stem cells (ADSCs) to improve wound healing in severely burned patients is a new field receiving much attention. We have recently demonstrated that a chronic inflammatory response is propagated within the adipose tissue at the same time that the lipolytic response is heightened. As inflammation and stress signaling have been implicated in DNA damaging processes, we compared ADSCs from burned and non-burned animals to determine whether burn-induced alterations occurred.
Methods: Adipose tissue samples from Sprague Dawley rats sustaining a 60% total body surface area burn ($n = 3$) were harvested and were compared to samples from three non-injured control animals. ADSCs were isolated using standard methods. Differentiation potential to yield was confirmed in all cell isolates. Cells from several different passages were measured. Telomere length was determined using Roche's TeloTAGGG Telomere Length Assay kit.
Results: There was a direct correlation between burn injury and telomere shortening that was unrelated to passage number.
Conclusion: These data indicate that the ADSCs must be well-characterized to determine their utility for tissue replacement and wound healing following a severe burn injury.

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44. Mesenchymal Stem & General Cell Therapy: Current Status and Future Developments (in coop. ISCT)

44.01
Cell type-associated differences in migration, survival and immunogenicity following grafting in CNS tissue.

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Cell transplantation has been suggested to display several neuro-protective and/or -regenerative effects in animal models of central nervous system (CNS) trauma. However, while most studies report on clinical observations, currently little is known regarding the actual fate of the cell populations grafted and whether or how the brain's innate immune system, mainly directed by activated microglia and astrocytes, interacts with autologous cellular implants. Here we grafted well-characterised neural stem cell, mouse embryonic fibroblast, dendritic cell, bone marrow mononuclear cell and splenocyte populations, isolated from C57BL/6-eGFP transgenic mice, below the capsula externa of healthy C57BL/6 or cuprizone-treated C57BL/6 mice. Two weeks post-grafting, an extensive histological analysis was performed in order: (i) to quantify cell graft localisation, migration, survival and toxicity, and (ii) to characterize endogenous CNS immune responses against the different cell grafts. Obtained results indicate dependent on the cell type grafted: (i) a different degree of cell graft migration, survival and toxicity, and (ii) a different endogenous immune response. Based on these observations, we warrant that further research should be undertaken to understand - and eventually control - cell graft induced tissue damage and activation of the brain's innate immune system. The latter will be inevitable before cell grafting in the CNS can be performed successfully in clinical settings.

44.02
Keynote: *In vitro* analysis of the transdifferentiation of adipogenic differentiated mesenchymal stem cells towards the osteogenic and chondrogenic lineage via dedifferentiation

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Human mesenchymal stem cells (MSC) are self-renewing multipotent cells. It is supposed that they become unipotent on terminal differentiation. But in transdifferentiation, the differentiated cells of a restricted unipotet stage are able to form other cell types. To address the question of how the differentiated cells with limited potency reprogram into other cell types, we turned MSC to adipogenic differentiated cells and converted them to osteogenic and chondrogenic lineages via direct cell conversion, dedifferentiation and single cell conversion. To screen the differentiated cells for potency and ways of conversion into other cell types, we applied FACS analysis, histologic and immunohistochemic staining, qPCR (PPARG, FABP4 for adipogenesis; RUNX2, SPP1 for osteogenesis; COL2A1, SOX9 for chondrogenesis) and microarray gene expression analysis. We found that the upregulation of DHCR24, MAP2K6, SESN3, RGS2, CCPG1 and GAS2L3 influences the cell cycle towards an arrested state with restricted potency, while downregulation of these genes accelerates the cell progression to dedifferentiate and resume their potency. Similarly the downregulation of DST, KAT2B, MACF1, MLL5, MSH2, RB1, SMAD3 and ZAK is associated with cell arrest to decrease the cell potency, while upregulation motivates cell dedifferentiation to increase their differentiation capacity. In conclusion our results show that dedifferentiation is involved as an intermediate step in the transdifferentiation of cells.

44.03
Influence of bioadhesive coatings on cells migration from polylactide microcarriers for cell therapy

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One of the most challenging limitations in cell therapy is poor cell survival upon transplantation. Biomaterial carriers can dramatically increase anchorage-dependent cells viability and engraftment in host tissues, by providing mechanical support and homing. However, many cell therapy strategies require cells to migrate at specific sites throughout target tissues to express their therapeutic activity, i.e. in several ischemic and tumor tissues. Carriers can be engineered to retain seeded cells, but also to act as reservoirs, from which cells can migrate, possibly in a temporally-controlled manner. Herein, the effect of functionalization of polylactide microcarriers (MCs) on Mesenchymal Stromal Cells viability, phenotype, release and migratory potential is studied. MCs with controlled size and high surface area were prepared via a novel toxic-chemicals-free method, as we recently described. Collagen and RGD-peptides were either covalently grafted or physisorbed on MCs surface. While stable covalent modifications promote better cell adhesion and higher proliferation compared to physisorption, functionalized MCs also affected the cells migratory behaviour in response to cytokines involved in cell recruitment, like SDF-1α and VEGF. Less stable coatings (physisorbed) showed sensibly higher number of migrating cells and faster release than covalent collagen/RGD coatings. These are key findings to design biomaterials for efficient and controlled cell delivery to damaged tissues.

44.04
Cell-matrix interface: ISO 13022 a new international standard for the management of risk for cell-based products

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Currently the International Organization for Standardization (ISO) is developing a new standard document which will address the requirements for risk management for medical products based on viable human cells. The standard ISO 13022 specifies a procedure to identify the hazards and hazardous situations associated with such products, to estimate and evaluate the resulting risks, to control these risks, and to monitor the effectiveness of that control. Furthermore, it outlines the decision process for the residual risk acceptability, taking into account the balance of residual risk, and expected medical benefit as compared to available alternatives. It will cover viable human materials of autolo-

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gous origin as well as allogeneic human material.The document is intended to provide requirements and guidance on risk management related to the hazards typical of medical products manufactured utilizing viable human materials such as:a) contamination by bacteria, moulds or yeasts and parasites,b) contamination by viruses,c) contamination by agents causing Transmissible Spongiform Encephalopathies (TSE),d) contaminating material responsible for undesired pyrogenic, immunological or toxicological reactions,e) decomposition of the product and degradation products caused by inadequate handling including procurement, packaging, storage, transport and application, andf) complications resulting from the mix up of human raw materials.

44.P01
Are endogenous subventricular zone neural stem/progenitor cells responsible for the remyelination of the corpus callosum in a mouse model of multiple sclerosis? an *in-vivo* bioluminescence and magnetic resonance imaging study.

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The cuprizone (CPZ) mouse model allows *in vivo* follow-up of de- and remyelination in the corpus callosum (CC) but very little is known regarding the actual origin of remyelinating oligodendrocytes. As cell proliferation was previously observed in the subventricular zone (SVZ) during CPZ intoxication, we set out to investigate whether SVZ neural stem cells (NSC) contribute to remyelination of the CC. To this end, starting on the day of *in situ* NSC labeling, C57BL6/j mice were fed a 0.2% cuprizone diet during a 4 week period and then left to recover on a normal diet for 8 weeks. Two labeling strategies were employed: (i) NSC were labeled with micron-sized iron oxide particles (MPIO) and then followed up by means of MRI at week 4, 6 and 12 post-injection. (ii) NSC were labelled using a eGFP-Luciferase lentiviral vector (LV) and then followed up weekly by means of BLI for 12 weeks. Both MRI and BLI allowed the *in vivo* follow-up of NSC migration and did not reveal any impairment in NSC migration from the SVZ towards the olfactory bulb. MRI, but not BLI, indicated the presence of labelled cells in the CC but histological validation demonstrated that these MPIOs were mainly present within astrocytes and microglia. Histological examination of the LV transfected animals showed only few eGFP positive cells in the CC which were found to be transfected astrocytes near the injection tract. Therefore our results suggest that SVZ NSCs do not contribute to CC remyelination in the CPZ model.

44.P02
Evaluation of the influence of two different hydroxyethyl starch solutions (6% HES 450/0.7 and 6% HES 200/0.5) in the processing of cord blood

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Introduction: Long-term cord blood banking requires storage space in liquid nitrogen which is limited and costly. For this reason there have to be established techniques for volume reduction of CB units.
Material and methods: Our standard CB processing procedure consists of two steps : HES sedimentation for higher RBC depletion at first and separation with the SEPAX System (Biosafe) afterwards. The aim was to analyse the influence of two different hydroxyethyl starch (HES) solutions on TNC recovery and viability of CD34 positive cells. Data were collected between January 2006 and March 2011 from our routinely processed CB Units. In this period the HES 450/0.7 was used in the processing of 394 CB units against 835 CB units produced with HES 200/0.5. HES 450/0.7 was added with a concentration of 20%, HES 200/0.5 with a concentration of 40%. TNC counting was performed with Sysmex XE-2100, viability of CD34 positive cells was evaluated according to ISHAGE protocol with 7-AAD.
Results: The mean TNC recovery (TNC count before and after processing) with HES 450/0.7 was 91,6%, with HES 200/0.5 80,6%. The difference is significant ($p < 0.00$). The mean viability of CD34 positive cells was in both groups above 98%.
Conclusion: TNC recovery can be essentially improved by applying HES with higher molecular weight and a concentration of 20%. The use of different HES solutions has no effect on the viability of CD34 positive cells.

44.P03
Influence of leptin on osteogenic differentiation of human marrow stromal cells (hMSC) and modulation of BMP-2-mediated osteoinduction

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Aim: The progress in molecular genetics provided the concept of a whole-body-physiology. Bone metabolism is one important system that is closely linked to other systems by hormones such as leptin. The aim of this study is to determine the reaction of human marrow stromal cells (hMSCs) to leptin in 2D and 3D culture.
Methods: The hMSCs were incubated with leptin (1000 ng/ml), BMP-2(100 ng/ml) or both for 21 days. In further groups, hMSCs were pre-stimulated for 7 days using osteoinductive media and subsequently leptin-treated for 21 days. Cell metabolic activity was measured weekly. At 14 and 21 days, alkaline phosphatase activity and expression of bone-specific genes (RT-PCR) was analysed.
Results: Leptin receptor gene expression showed potential sensitivity of hMSCs to leptin. Metabolic activity was not significantly influenced by leptin. BMP-2 proved to be a strong osteogenic growth factor. Interestingly, there was an additive osteogenic effect by combining BMP-2 and leptin under certain culture conditions. Whereas leptin on its own had little impact on non-stimulated hMSCs, pre-stimulated cells appeared more susceptible for leptin-mediated effects.
Conclusion: These results suggest osteoinductive effects of leptin especially in pre-stimulated hMSCs. Combining leptin and BMP-2 might further enhance osteoinduction. This needs to be confirmed in long-term culture under different conditions to clarify applicability of leptin related to *in vitro* generation of bioartificial bone.

44.P04
Cryoprotection of human mesenchymal stem cells from repeated freezing/thawing-induced damages by carboxylated poly-L-lysine

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Here, we investigated the cryoprotective effects of carboxylated poly-L-lysine (COOH-PLL) on human mesenchymal stem cells (hMSCs) subjected to repeated freezing/thawing (rFT)-induced damages. COOH-PLL was synthesized by reacting PLL with succinic anhydride to convert amino groups into carboxyl groups. hMSCs were frozen in culture media containing COOH-PLL without serum. For comparison, media containing 10% dimethylsulfoxide (DMSO) without serum were prepared. Freezing was carried out in a deep-freezer with a cooling rate

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of 1 °C/min down to -80 °C. After freezing for 2 h, cryovials of either COOH-PLL or DMSO freezing media were quickly thawed in a water bath at 37 °C and then placed at room temperature for 10 min. For rFT, this process was five times repeated every 2 h. Finally, the cryovials were stored in a liquid N2 (-196 °C) tank. After 4 week of storage, all vials were removed from liquid N2 and thawed quickly in a water bath. The cells were refreshed with complete media and then cultivated for up to 2 week. After cultivation, proliferation and morphology were determined by cck-8 assay and fluorescence microscopy, respectively. For evaluating osteogenic differentiation, alkaline phosphatase activity and Alizarin Red S staining were performed. COOH-PLL with antifreeze protein properties could protect hMSCs from injury induced by rFT and would be effectively used for cell therapy and regenerative medicine.

44.P05
Tonsil-derived mesenchymal stem cells: Evaluation of biological, immunological, and genetic factors for successful banking

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Introduction: Tonsillectomy is the most common surgical procedure in pediatric patients. The removed tonsil tissue is a kind of waste tissue. The aims of this study are evaluation of the feasibility and characteristics of mesenchymal stem cells from human palatine tonsils (T-MSC).
Materials and methods: Eighty tissues from nine tonsillectomies patients under 10 year-old were collected. Mononuclear cells were isolated and cultured. T-MSCs expanded during 4 weeks were cryopreserved in -200 °C. After 10 days cryopreservation, T-MSCs were thawed in 37 °C water bath. T-MSCs before or after cryopresevation-and-thawing were evaluated several basic characteristics; colony forming unit-fibroblast features, MSC-specific surface antigen profiles, inhibition of alloreactive T cell proliferation, mesodermal and endodermal differentiation potentials. The short tandem repeat (STR) analysis was performed in using genomic DNA randomly isolated from three subjects.
Results: Both fresh and cryopreserved-and-thawed T-MSCs showed similar high proliferation capacity and expressed primitive cell surface markers. In addition to mesodermal differentiation, fresh and cryopreserved/thawed cells also underwent endodermal differentiation. Both cells significantly decreased PMA-induced T cell proliferation. T-MSC from three independent donors formed chimerism in STR analysis.
Conclusion: The results demonstrate that T-MSCs are a potentially good source for MSC banking.

44.P06:
Proliferation and osteogenic differentiation pattern of menstrual blood derived stem cells in presence of different products of platelet concentrates

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Aim: To establish a safe and efficient protocol for development of menstrual blood derived stem cells (MenSCs) into osteoblasts, substitution effect of fetal bovine serum (FBS) with human platelet products was evaluated during osteogenic differentiation of MenSCs.
Methods: After characterization, osteogenic differentiation potential of MenSCs was evaluated in inducing media fortified by fetal bovine serum (FBS), platelet rich plasma (PRP), platelet poor plasma (PPP), platelet gel supernatant (PGS) or platelet lysate (PL). Parallel experiments were carried out to determine mineralization, alkaline phosphatase and osteocalcin levels of cultured cells in different conditions.
Results: FBS was more efficient than other media supplements in supporting MenSCs outgrowth. The proliferation rate of MenSCs in presence of FBS was about threefold greater than that of PPP ($p < 0\ 001$). However, mineralization as judged by Alizarin red staining was strongly more positive in cells differentiated in presence of PL than that fortified with other groups. These findings were confirmed with enzyme linked immunosorbent assay showing greater production of alkaline phosphatase and osteocalcin in culture media of differentiated cells under PL compared with those in another media supplements. Conclusion: FBS substitution by PL substantially improves osteogenic differentiation capacity of MenSCs. It represents a major novel step toward safe and applied stem cell therapy of bone diseases.

44.P07
Analysis of mesenchymal stem cell surface markers after osteogenic differentiation

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Detection of cell surface antigens CD166, CD105, CD90, CD73 and CD29 is a commonly used method to identify mesenchymal stem cells (MSC) for tissue engineering. To verify the specificity of those markers, non-mesenchymal cells were analyzed by FACS. Surprisingly, human bone derived cells and MG63 cells were positive for all MSC-specific markers in at least 95% while negative for CD45 and CD31. Human fibroblasts were highly positive for CD90 and CD29 but displayed lower, yet clearly detectable levels of CD166, CD105 and CD73. Next, human bone marrow mesenchymal stem cells (BMSC) and adipose-derived stem cells (ASC) obtained from four donors were subjected to osteogenesis in two different culture media. After 21 days, BMSCs showed extensive mineralization while maintaining more than 80% of CD166-, CD90-, CD73- and CD29-positive cells or 66–93% CD105-positive cells. ASCs displayed only weak Alizarin Red staining at day 21. However, using Real-time PCR analysis, an increase in expression of Runx2, collagen I, alkaline phosphatase, and osteonectin genes was observed. More than 85% of all analyzed ASC were positive for CD166, CD90, CD73 and CD29 and 65–97%-positive for CD105. In conclusion, the analyzed surface markers may not be specific for MSC and may be maintained at early stages during differentiation of BMSC and ASC. The relationship between MSCs marker expression and osteogenesis will be further investigated at later time points in culture. Grant No. POIG.01.01.02-00-022/09

44.P08
Self-assembled hydrogels enhance bone marrow derived-mesenchymal stem cell distribution and retention following transplantation to the intact rat striatum

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Poor transplant survival hinders the advancement of *ex-vivo* cell based therapies for neurodegenerative diseases. We have shown previously that naïve mesenchymal stem cells (MSCs) survive poorly following transplantation to the rat brain (~65% remaining 4 days post-transplant). We hypothesise that delivery of the cells within a hydrogel will yield improved survival in an ectopic environment by providing a physical adherent substrate as well as a protective barrier to the host response. To test this hypothesis, rats received intra-striatal transplants of 30 000 transgenic GFP-MSCs ($n = 4$) or 30 000 GFP-MSCs delivered within a cross-linked collagen type I hydrogel ($n = 4$). Four days post-transplant, 40 μM brain sections were visualised by fluorescence microscopy, and stained for microglial activation (OX42) and reactivity of astrocytes (GFAP). Applying Cavalieri's principle, we found that cells

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delivered within the hydrogel exhibited enhanced survival, occupying a greater transplant volume compared to control MSCs. Cells appeared homogenously dispersed within the gel which also facilitated focal retention of the cells within a distinct transplant site compared to control MSCs. Hydrogel mediated MSC delivery may therefore hold significant translatable therapeutic potential by enhancing cell survival, graft volume and graft positioning without elevating the host response.

44.P09
Methods for multilineage differentiation of human bone marrow-derived stem cells

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Introductions: Historically, there are various treatment options for osteoarthritis, such as drug and surgery. In recent years, human bone marrow-derived mesenchymal stem cells for bone tissue engineering have been widely used on clinical treatment. However, there are some limitations on the differential capability of bone marrow-derived stem cells. The aim of this study is to utilize tissue engineering methods on therapeutic applications.
Materials and methods: Human bone marrow was collected from total joint replacement surgery, and the mesenchymal stem cells were harvested via Ficoll-Paque gradient separation. The pre-plate technique was modified and treated to isolate various populations of human bone marrow-derived mesenchymal stem cells. In order to measure the plurepotency, we tried to add differentiation inducing agents for ectoderm, mesoderm and endoderm differentiation.
Results: The surface markers (CD29/CD34/CD44/CD45/CD73/CD90/CD184) and transcription factors (Oct-4/Nanog) were measured using flow cytometer. After differentiation induction, the data were analyzed qualitatively with immune-cytochemistry. Furthermore, the quantitative real-time PCR assay showed the gene expression for each population.
Conclusions: In summary, we successfully isolated pluripotent stem cell population from human bone marrow by Ficoll-Paque gradient separation and pre-plate technique. We hope these pluripotent stem cells could be introduced into new therapeutic applications.

44.P10
High efficiency transduction of green fluorescent protein in human mesenchymal stem cells mediated by lentiviral vector and *in vitro* multilineage differentiation

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Human mesenchymal stem cells (hMSC) are pluripotent cells of fibroblastoid morphology and plasticity to various cell lineages such as chondrocytes, osteocytes and adipocytes. There is increasing evidence of the benefits of cell therapy based on hMSC. New technologies in gene transfer using lentiviral vectors have opened the possibility to improve the function and performance of hMSC by the constitutive expression of transgenes of interest. In this work we standardized hMSC transduction of bone marrow obtained from human healthy donors by using a lentiviral vector expressing the gene for green fluorescent protein (GFP). hMSCs were infected with lentiviral particules resulting in transduction efficiency above 96% as verified by flow cytometry. The hMSC-GFP expressed CD105, CD90 and CD73, typical mesenchymal markers and maintained their potential to differentiate into osteoblasts, adipocytes and chondrocytes. GFP expression persisted over the whole culture and differentiation period. Thus, the use of the hMSC constitutively expresing GFP will allow to clarify biological and functional aspects of the MSC *in vitro* and *in vivo* and further improve potential therapeutical applications in clinical trials.
Keywords: mesenchymal stem cells, green fluorescent protein, hMSC-GFP$^+$ lentivirus, transduction.

44.P11
Immunomodulatory effects of human fetal cartilage-derived progenitor cells (FCPCs)

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Recently, FCPCs have been described as a new source for tissue engineering and cell therapy. Previous studies have shown that FCPCs exhibits cell proliferation and differentiation abilities similar to those of MSCs. Many of their characteristics, however, are yet to be identified. In this study, we investigated immunological properties of FCPCs using activated human lymphocytes (PBLs). FCPCs and PBLs were obtained from human fetal limbs cartilage and human, respectively. FCPCs were γ-irradiated to mixing with lymphocytes, and PBLs were activated with ConA. To determine the effect of FCPCs on the proliferation of PBLs, BrdU assay and cell cycle analysis using PI staining were performed after co-culturing them for 4 days. The levels of IFN-γ and IL-10 released were also measured on days 1, 4, and 6 of co-culture by. When FCPCs were incubated with PBLs at 1 : 10, 1 : 100, and 1 : 1000 ratios, the proliferation of PBLs was significantly inhibited and their cell cycle was arrested in the G2 phase. When cells were stimulated with ConA, the production of IL-10 was increased both in PBLs and FCPCs but that of IFN-γ was increased only in PBLs. Co-culture of PBLs with FCPCs for 1, 4, and 6 days resulted in the decrease of IFN-γ production by PBLs, whereas the production of IL-10 did not show significant change. These results suggest that FCPCs may act as an immunomodulator and could be used to reduce hypersensitive response of recipients to tissue engineered implants and cell therapies.

44.P12
Isolation of mesenchymal stromal cells from solid tissues: cell features after digestion versus migration

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In terms of a successful and economic clinical use of mesenchymal stromal cells (MSC), an effective isolation technique is required. Currently, collagenase digestion is most widely-used for MSC isolation from solid tissues. However, isolation of the cells by taking advantage of their capability to migrate has also been described. Therefore the aim of this study was to compare cell yields as well as cell features of MSC isolated by both techniques. Equine tissue samples with different stiffness were harvested. For cell isolation by digestion, minced tissue pieces were incubated in collagenase I solution, followed by centrifugation and cell seeding. For cell isolation by migration, tissue pieces were placed onto culture plates. Isolated MSC were then subjected to proliferation, migration and three-lineage differentiation assays as well as tendon marker expression analysis. The MSC yield after the first cell harvest

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was significantly higher in digested tissue samples, even though less primary culture time was needed. However, further analysis of cell characteristics revealed that the isolation method had no major influence. Yet, interestingly, gene expression of the tendon marker Scleraxis was significantly higher in MSC isolated by digestion. Digestion and migration are feasible methods for MSC isolation from solid tissues. If rapid MSC recovery is required, collagenase digestion appears to be the preferable method.

44.P13
Safety evaluation of cultured chondrocytes by comparative genomic hybridization analysis

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Objective: The authors fabricated chondrocyte sheets by co-culturing chondrocytes and synovial cells and conducted a clinical study of joint repair according to the Guidelines on Human Stem Cell Research, approved by the Ministry of Health, Labour and Welfare Japan, Comparative genomic hybridization (CGH) analysis was performed for safety evaluation on cultured chondrocytes.
Methods: CGH analysis, which is used to detect cancer mainly caused by the accumulation of genetic abnormalities, was performed to confirm transformation after multiple passages of chondrocytes. We studied a total of 13 samples of cartilaginous tissues obtained from total knee arthroplasty, anterior cruciate ligament reconstruction, high tibial osteotomy, or surgery for polydactyly. Cells were isolated from the samples by an enzymatic process and then cultured until the 6th passage. The DNA extracted from the passaged cells was subjected to CGH analysis.
Results: CGH analysis revealed no genomic copy number abnormality at the 4th and 6th passages.
Discussion: Although no mutations and abnormalities were detected during the passage of chondrocyte under this setting, false-positive results were obtained under a different set of criteria. Additional experiments (e.g., dye swap), performed to confirm the results, revealed no mutations and had actually false positive.
Conclusions: CGH analysis revealed no genomic copy number abnormalities even after multiple passages of cultured chondrocytes.

44.P14
Effect of environmental hydrogen peroxide in isolator on biological properties of mesenchymal stem cells

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In cell and tissue processing for clinical application, aseptic area in the clean room would be required with strict managements to prevent any contamination, resulting in huge maintenance cost. Innovative system using isolator and decontamination techniques has been attracting attention for the processing. On this system, vaporized hydrogen peroxide (H_2O_2) is an agent reducing bacteria and spores for bio-decontamination of isolator. H_2O_2 vapor converts into water and oxygen using a catalyst during the ventilation process. Meanwhile, it is well known that the oxidative stress via H_2O_2 induces harmful effects on cultured cells. To concern the effects of residual H_2O_2 in interior environment of isolator on biological properties of mesenchymal stem cells (MSC), cell suspension was exposed to environmental H_2O_2 and then cell viability and morphology were evaluated. Two ml of rat MSC suspension was prepared in ϕ35 mm culture dishes. The dish lid was opened in isolator during the ventilation process. The viability of cells and amount of dissolved H_2O_2 in each suspension at a certain exposure period were measured. Cell morphology with and without exposure to the H_2O_2 did not show any significant difference, whereas the cells with high exposure to H_2O_2 showed low viability. The results raised the possibility that residual H_2O_2 in isolator dissolved into culture medium to affect cell survival, indicating that H_2O_2 vapor decontamination needs sufficient ventilation to reduce H_2O_2.

44.P15
Efficient formation of cell spheroids using polymer nanofiber

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Spheroid culture has been used for suspension cultures of anchorage-dependent cells. In this study, we developed a new method for the suspension cultures of anchorage-dependent animal cells using polymer nanofibers. Poly(lactic-co-glycolic acid) nanofibers (785 nm in average fiber-diameter, 88 μm in average fiber-length) fabricated by the electrospinning method were added to each suspension culture of human embryonic kidney 293 cells and human dermal fibroblasts. As compared to no addition of nanofibers to the suspension cultures, nanofibers enhanced cell spheroid formation, thereby reducing cell death resulting from a lack of cell adhesion. Efficient formation of spheroids in the presence of polymer nanofibers may be useful for the suspension cultures of anchorage-dependent cells.

44.P16
Production of hemoglobin-containing micro-gel beads as oxygen carriers for cell culture

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After successful clinical application of the engineered skin and cornea, engineering of thick and functional tissues is the next emerging issue to be challenged. The tissues of important organs such as heart, liver and kidney have specific three dimensional structures with significant thickness. In such tissues, capillary vasculatures are developed very much, and oxygen is sufficiently supplied to the peripheral tissues via the blood with many RBCs, which can deliver oxygen very effectively owing to the function of hemoglobin (Hb). On the other hand, oxygen is supplied only via the dissolved oxygen in the culture medium in the case of conventional cell culture *in vitro*. For this reason, lack of oxygen inside is always a major problem in culturing of thick three-dimensional tissues *in vitro*. Therefore, the development of the effective oxygen carrier, some alternatives to RBCs, is essential for *in vitro* culture system for thick tissues. Then, considering to such necessity of the development of artificial oxygen carrier, we tried to produce Hb-containing micro-gel beads. In this research, we used ink-jet technology which can produce micro-sized and uniform-sized droplets. We could successfully obtain Hb-containing alginate micro-gel beads, which have almost similar sizes and similar Hb concentration to those of human RBCs. If Hb can be fixed effectively, the produced Hb-containing alginate micro-gel beads have good potentials to be one of the useful artificial oxygen carriers.

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44.P17
Oxygen consumption in T-47D cells immobilized in alginate

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The metabolic consequences of entrapping cells in hydrogels require further study. In this work, T-47D mammary carcinoma cells were entrapped in alginate at a concentration of about 2.5 105 cells/ml in well-defined constructs, which were covered with growth medium and incubated at 37 °C. Oxygen concentration was monitored using a fiber-optic microsensor and oxygen meter (Presens GmbH, Regensburg, Germany). Cell density and viability were determined by haemocytometer counting and live/dead confocal imaging of vibratome sliced gel sections. Viability declined with time and depended on location within the gels, at day 7 it was about 30% in the biostructure interior. Pericellular oxygen concentration fell below physiological levels within 20 h, likely explaining the loss in viability. The simplicity of the system allowed calculation of cellular consumption rates based on the rate of oxygen concentration change (disregarding influx by diffusion). 3–6 h into the experiments consumption rates were 60 ± 10 fmol/cell h, about 25% of that reported for exponentially growing T-47D cells in monolayer. In the alginate, however, no proliferation was observed. As the gel construct may be varied with respect to several parameters, including presence of metabolic factors, structure micro-elasticity, cell types and more, it allows careful determination of oxygen metabolism dependencies in a 3D micro-environment.

44.P18
Down-regulation of cellular metabolism improves cell survival under hypoxic conditions

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Building a clinically relevant sized tissue/organ requires maintenance of viable cells until host vasculature is established and integrated into the implanted constructs. However, delay in vasculogenesis results in premature cell death due to the inadequate supply of oxygen. One potential solution is to maintain cell viability by downregulating cellular metabolism until host vascularization is established. Adenosine is reported to increase during hypoxia and functions as a modulator of ion-channel arrest. This results in a decrease in ATP consumption and thus, oxygen demand. In this study we attempted to promote cell survival under hypoxic conditions by exploiting this property of adenosine. Muscle cells were cultured under three different conditions: (i) normoxia, (ii) 0.1% hypoxic conditions without adenosine, or (iii) with adenosine. Metabolic activity of cells grown in normoxic conditions increased linearly with respect to time. Hypoxic cells not treated with adenosine showed an eventual decline. However, when treated with adenosine, cells under hypoxic conditions maintained a steady state of metabolic activity and resumed their normal activity when they were returned to normoxic conditions. These results demonstrate that cell viability can be maintained by downregulating cellular metabolism under hypoxic conditions. This concept represents a novel method for increasing cell survival in cell-based therapeutic approaches.

44.P19
Temperature-regulated affinity interaction between cells and immobilized antibodies on thermoresponsive surfaces

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Antibody-immobilized, poly(N-isopropylacrylamide) (PIPAAm)-grafted cell culture surfaces were developed for enhancing cell adhesion and regulating affinity interaction between cells and surface-immobilized antibodies by temperature changes. It's noted that CD90 is expressed many type of cells, including T cells, thymocytes, neurons, endothelial cells, fibroblasts, and adipose-derived stem cells. Here, anti CD90 antibody-immobilized thermoresponsive cell culture surfaces were prepared by following methods: (i) direct immobilization on carboxylated PIPAAm-grafted surface, (ii) immobilization via poly(ethylene glycol) (PEG) spacer, and (iii) affinity binding between biotin and streptavidin. The number of adhered floating cells (Ty-82) was greater on the affinity binding surface relative to other antibody-immobilized surfaces. Neonatal normal human dermal fibroblasts (NHDFs) were also adhered on the surfaces, and formed the monolayer. By reducing the temperature to 20 °C, NHDFs were spontaneously detached as a single cell sheet. Through flow cytometeric analyses, recovered NHDFs from the directly and PEG spacer immobilized surface had no antibody on the surfaces of cellular membrane, while those from the affinity immobilized surface had both antibody and streptavidin. By optimizing the immobilization methods of antibodies onto the surfaces, this surface technology have a potential to give the application of weak adherent cells such as primary cells for cell sheet-based therapies.

44.P20
Development of selection criteria for data mining tools to analyse microarray of stem cell populations

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Genetic studies such as microarray chips generate a large volume of data which must be properly analysed to obtain the biological significance of the resulting list of genes. Data mining techniques are useful to identify and characterize the role of these genes by construction of maps reflecting expression or function. However, the choice of a suitable tool for a given experimental dataset is not straightforward. To establish the most adequate analysis tool based on the characteristics of data, gene lists were studied using different tools. Microarray studies were performed with human adipose stem cells (hASC's). We analysed different databases of Agilent gene expression microarrays from hASC's employing different bioinformatics software. Background correction was applied by the subtraction method described in LIMMA. Array data were normalized through a non-parametric quantile normalization assuming similar distribution of the signal for all arrays. For differential expression analysis, we treated our data using the LIMMA of bioconductor parametric method, to obtain stable values of differential expression, even with few replicas. To classify the biological functions associated with our differential expression gene lists, we compared some tools for functional analysis of biological enrichment including DAVID and FatiGO. Our observations suggest that software can yield distinct results depending on sample size and functional genes of interest.

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44.P21
Free-radical scavenging cerium oxide nanoparticle hydrogels for cell encapsulation

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Cellular encapsulation is a promising strategy for shielding foreign cells from direct immune attack; however, it fails to prevent inflammation or indirect immune activation, which exposes embedded cells to oxidative stress. Cerium oxide nanoparticles, or nanoceria, are highly unique nanoparticles with the capacity to mimic free-radical scavenging enzymes in a ubiquitous and self-renewing manner. We sought to localize these nanoparticles within a biomaterial. In this study, we incorporated nanoceria within alginate hydrogels and evaluated their catalytic activity as well as their capacity to protect co-encapsulated cells from free radical damage. Nanoceria was synthesized and evaluated via FT-IR, DLS, and superoxide reduction. Nanoceria (0.01–1 mM) retained its catalytic activity within alginate microbeads, where it was found to effectively scavenge superoxide and degrade H_2O_2 in the surrounding milieu. No cytotoxicity was found at <1.0 mM nanoceria. Nanoceria-alginate microbeads provided statistically significant protection (50% higher viability) of co-encapsulated MIN6 cells from superoxide-mediated cell death. Live/dead imaging qualitatively verified these results. Our data illustrates a novel, self-renewing, ubiquitous free-radical scavenging biomaterial with the demonstrated capacity to protect co-encapsulated cells from free-radical damage. It is envisioned that these materials would be highly beneficial for cell-based implants, particularly pancreatic beta cells.

44.P22
Annexin A2 as a regulator of the cellular senescence and apoptosis through p21

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Introduction: Stressors even at sublethal level cause Stress induced premature senescence (SIPS).Cells undergoing SIPS exhibit properties similar to cells in replicative senescence (RS).Mesenchymal stem cells (hMSCs) are multipotent progenitors which self-renew and differentiate into multiple lineages. Furthermore, they contribute to the homeostatic maintenance of many organs and tissues. The study identified ANXA2 and further probed whether it is associated with the cellular senescence and apoptosis through p21.

Materials and methods: SIPS was confirmed using a range of different analytical methods. Five differentially expressed spots were detected in the 2-DE map, which were identified as ANXA2, myosin light MLC2, ECH1,PSMA1 and a mutant β-actin by ESI-Q-TOF MS/MS in hMSCs. ANXA2 was confirmed up-regulated in SIPS cells. Further experiments have been done for its role in cellular senescence and apoptosis in normal fibroblasts (MRC-5 or WI-38).
Results: H_2O_2-induced overexpressed ANXA2 has shown to directly interact with CAV1 and down-regulation of ANXA2 induced caspase-mediated apoptosis through regulation of p21 which is a major inhibitor of p53-dependent apoptosis.
Conclusion: ANXA2 is associated with the cellular senescence and apoptosis through regulation of p21 expression.

44.P23
Analysis of proteomes related to cellular senescence by calorie restriction in mesenchymal stem cells

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Introduction: Mesenchymal stem cells (MSC) are one of the most promising stem cell types because of the ability to self-renew and differentiate into multiple tissues. However MSCs loose their multipotency and proliferation capability with age. Previous studies have reported that calorie restriction (CR) increases proliferation of MSCs and decreases apoptosis. Therefore, in this study, we examined the effect of low glucose on human bone marrow derived MSCs (hBM-MSCs).
Materials and methods: Proliferation under low glucose (LG, 1.4 mM) condition was compared with that under normal glucose (NG, 5.5 mM) condition. Moreover comparative studies of population doubling, β-galactosidase activity, ROS generation, and differentiation capacity in NG and LG conditions were performed. In addition protein expression patterns were investigated between NG and LG conditions using 2-DE.
Results: CR doesn't seem to have a significant effect on cell proliferation, ROS generation, and adipogenic differentiation of MSCs. Interestingly, however, a lower senescence associated β-galactosidase activity was observed under CR than under the NG condition and the differentiation potential into osteocytes was maintained under CR. In addition, three up-regulated and seven down-regulated proteins were found under the glucose restriction condition via 2-DE analysis.
Conclusion: These results imply that differentially-expressed proteins under the LG condition may provide more information on aging and differentiation.

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45. Targeting the Stem Cell Niche for Tissue Engineering

45.01
Keynote: Deconstructing and reconstructing stem cell niches

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Proper tissue maintenance and regeneration relies on intricate spatial and temporal control ofbiochemical and biophysical niche cues, instructing stem cells to acquire particular fates, for example remaining quiescent or undergoing self-renewal divisions. Despite rapid progress in the identification of relevant niche proteins and signaling pathways using powerful in vivo models, the behavior of many stem cell types can only be poorly controlled in vitro. To address this bottleneck, we have been developing biomaterial-based technologies to display stem cell regulatory signals in a precise and near-physiological fashion, serving as powerful artificial microenvironments to probe and manipulate stem cell fate.In this talk I will discuss some our recent efforts to develop two- and three-dimensional microarrayed artificial niches based on a combination of biomolecular hydrogel engineeringand microfabrication. These platforms allow key biochemical and biophysical characteristics ofstem cell niches to be mimicked and the physiological complexity deconstructed into a smaller, experimentally amenable number of distinct signaling interactions. The systematic deconstruction of stem cell niches may serve as a broadly applicable paradigm to accelerate the transition of stemcell biology to the clinic.

45.02
Keynote: Engineering homing and differentiation of mesenchymal progenitor cells during bone healing

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Clinically employed osteoinductive treatment regimen make use of bone morphogenetic protein (BMP) concentrations exceeding the physiological growth factor concentration by 1000-fold. The rather low bioactivity of the applied growth factors, which might be due to inadequate delivery modalities or negative feedback signals has been tackled by sustained, low-dose release of growth factors from biologically derived or engineered synthetic materials. In this talk, strategies to foster bone regeneration by improving the availability and responsiveness of mesenchymal progenitor cell (MPC) at the treatment site will be discussed. 3D in vitro migration as well as in vivo recruitment of osteogenic cells from murine calvarial origin in response to cell-instructive microenvironments will be presented. We believe that novel in vitro migration models using prospectively isolated, osteogenic cells in combination with in vivo validation will provide a promising strategy to screen for novel MPC recruitment promoting factors. Such findings should inspire the design of next generation cell-instructive materials by relying on the modulation of naturally occurring processes during tissue development and regeneration.

45.03
VEGF induced CXCL12/CXCR4 axis in the recruitment of mesenchymal stem cells (MSCs) by osteogenic differentiated bone marrow stromal cells (O-BMSCs)

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Cell-cell interaction is believed to play important roles in the bone healing process. However, it is still unclear how transplanted cells behave and interact with host cells in cell-based therapy, especially in the site of bone defect healing. The purpose of this study was to investigate the interactions between osteogenic differentiated bone marrow stromal cells (O-BMSCs) and mesenchymal stem cells (MSCs) in vitro and in vivo and the underlying mechanism of vascular endothelial growth factor (VEGF) induced CXCL12/CXCR4 axis in MSCs recruitment and differentiation. Our data suggested that VEGF secretion increased dramatically in BMSCs after osteogenic differentiation and that the secretion of VEGF promoted migration of MSCs via the activation of CXCL12/CXCR4 axis. For the in vivo study, type I collagen scaffolds carrying O-BMSCs were implanted into skull defects in SCID mice. In situ hybridization demonstrated that O-BMSCs recruited host MSCs in osteogenic process at orthotopic site. H&E and immunohistochemical staining revealed that remarkable new bone formation was correlated with the strong CXCL12/CXCR4 expression in the new bone matrix and blocking VEGF with neutralizing antibody resulted in significant decrease of MSCs recruitment and new bone formation. This study demonstrated that VEGF secreted by O-BMSCs plays a vital role in the MSCs recruitment via CXCL12/CXCR4 axis during osteogenesis.

45.04
Mesenchymal Stem Cells (MSC) induce the homing of endogenous stem/progenitor cells through the activation of alternatively activated macrophages in an ectopic bone formation model

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Introduction: MSC are effective therapeutic agents in a variety of clinical situations of tissue injury acting as cellular modulators. The in vivo therapeutic effects of MSC depend on their anti-inflammatory potential, as well as on their capacity to stimulate functional mobilization of host cells. Using an ectopic model of bone regeneration, we demonstrated that MSC activate endogenous mechanisms leading to new tissue formation by host cells. The aim of the project is to identify the molecular mechanisms leading to the recruitment process.
Materials and methods: Combinations of wild-type (WT) MSC/scaffold were implanted in syngenic WT micethat were lethally irradiated and reconstituted with a Green Fluorescent Protein-positive (GFP+) bone marrow (chimeric mice). Implants were extracted at different times and endogenous cells, harvested through enzymatic digestions, were characterized.
Results: Implanted MSC are able to mobilize inflammatory macrophages that progressively acquire an alternative activation profile. Macrophage polarization promotes angiogenesis and tissue repair skewing the secretion of molecules involved in the regulation of cell migration.

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Polarized macrophages were effective at inducing endothelial progenitor cells and pericytes migration within the scaffold, leading to the development of the engineered tissue.
Conclusion: The activation of endogenous stem cells from either the blood or a tissue-specific niche is a promising approach for therapeutic success.

45.05
Building bone marrow: engineering biophysical regulation of hematopoietic stem cell fate decisions

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The stem cell niche provides extrinsic signals that regulate fate. The prototypical mammalian niche is the hematopoietic stem cell (HSC) niche in the bone marrow (BM). It contains cells, the extracellular matrix (ECM), and ECM-bound or soluble biomolecules that present chemotactic, durotactic, and paratactic cues as well paracrine and juxtacrine signals to drive HSC fate. We have developed a microfluidic platform to create 3D microgels containing counter gradients of cell and matrix signals to quantify the effects of cell-matrix and cell-cell cues on HSC fate. At each point along the gradient, the region-specific hydrogel presents a defined constellation of cues. This platform enables real-time, in situ analyses of HSC activity via 2-photon imaging; discrete regions can be removed via a custom extraction system for analysis via conventional molecular biology approaches. We have created BM mimics as type I collagen microgels containing opposing gradients of HSCs vs. BM stromal cells or mesenchymal stem cells. We have shown a significant influence of matrix properties (stiffness, ligand content) on HSC morphology, viability, and bioactivity. We have also shown that co-culture of HSCs with putative niche cells significantly impacts proliferation and early lineage specification. Critically, many of these effects can be abrogated by increasing microgel density to reduce biomolecule transport, suggesting paracrine signaling plays a functionally significant role in HSC fate.

45.P01
3D electrospun PLA nanofibers favours neurovascular niche regeneration in vivo

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To develop neural tissue engineering strategies useful for repairing damaged neural pathways after Central Nervous system injury it is essential to control and optimise the neurovascular niche. The aim of this study is to develop an artificial implant made of Poly lactic acid 70/30 fibers to promote nerve regeneration and angiogenesis after a lesion in cerebral cortex. Random and aligned nanofibers produced by electrospining, were used for seeding embryonic neurons and post natal glial cells from mice cerebral cortex during 5 days in vitro and also to implant in new born mice cerebral cortex using the stereotaxic apparatus. Cells and cortices were analyzed by Immunocytochemistry. Both types of electrospun fibers supported neural cells growth, but only in aligned fibers neural cells could invade the 3D scaffold. Cells changed their morphology mimicking the progenitor neural cells as the ones seen during development, expressing the immature glial markers Nestin and Pax6 and the neural progenitor markers Tbr2. In the in vivo models, PLDLA fibers did not seem to have elicited a foreign body reaction. It was also discovered that glial cells, neurons and blood vessels could penetrate into nanofibers after 2 months post implantation following the direction of aligned fibers. To conclude, PLDLA fibers induce an immature phenotype of neural cells necessary to promote a good environment to regenerate the neurovascular niche helping neuronal migration after a lesion.

45.P02
Carbohydrate based scaffolds for mesenchymal stem cell culture

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Tissue regeneration requires biogedredable natural polymers for manufacturing scaffolds. In addition to forming three dimentional media they also offer improved biocompatibility. This study aims at designing porous carbohydrate based natural scaffolds. The surfaces of alginate gels are usually not suitable for cell attacment, however, increasing the porosity in these gels may provide efficient micro environment for cell growth. Here, glucose and carboxy cellulose is combined with alginate as an attempt to improve cell culturing properties. Gels were prepared in different combinations of alginate, glucose and carboxymethyl cellulose by using 1 M $CaCl_2$. Gels were analysed by scanning electrone microscopy. They were kept in 0.09% NaCl and the swelling abilities were analysed by measuring the increase in weight. Cytotoxicity was detected by growing human mesenchimal stem cells on gels and measuring cell viability be MTT. Human mesenchimal stem cells were also grown on these scaffolds to test cell proliferation, in dMEM tissue culture medium containing 10% FBS, 5% CO_2 and antibiotics. Multiway variance analysis is applied to the results for testing their accuracy.It is concluded that, the swelling behaviour and microscopic appearence indicates that gels have a highly porous structure. They have no toxic effect on mesenchimal stem cells. Furthermore, they increased the proliferation of these cells by providing a large surface area due to their porous structures.

45.P03
VEGF and BMP-2 are the key regulators in bone regeneration by promoting stem cell homing and differentiation

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VEGF and BMP-2 have been widely used to stimulate angiogenesis and new bone formation, with bone defects repaired by scaffolds releasing these dual factors without cell seeding. In this study, the goal was to determine whether these two factors played roles as cell homing molecules for mesenchymal stem cells (MSCs) in bone tissue regeneration and to provide new insight into the mechanism of function. In vitro, we used a transwell chemotaxis model to examine the homing capacity of VEGF and BMP-2 on bone marrow-derived mesenchymal stem cells (BMMSCs). More stem cells were recruited in the presence of VEGF or both VEGF and BMP-2. In vivo, more stem cells were found homing to silk scaffolds with the growth factors, and more endothelial cells in the two groups with VEGF and more osteogenic cells in the two groups with BMP-2. Importantly, osteogenic cells at different differential stages were observed. At four weeks, VEGF enhanced angiogenesis of the tissue engineered complex. At twelve weeks, observations based on sequential fluorescence labeling and Micro-CT revealed that the bone formation process was accelerated in both quantity and quality when BMP-2 was used. These findings support our hypothesis that localized release of VEGF and BMP-2 promote bone regeneration, in part, by facilitating the homing of endogenous stem cells and then directing the differentiation of these cells into endothelial and osteogenic lineages.

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45.P04
Decellularization of bone marrow and its evaluation as a hematopoietic stem cell niche

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Recent studies revealed that hematopoietic stem cells (HSCs) home to the microenvironment (HSC niche), and induce hematopoiesis. In the HSC niche, the maintenance and proliferation, differentiation of HSCs are regulated. Niche is consisted of extracellular matrix (ECM) and supporting cells such as mesenchymal stem cell (MSC), osteoblast and CXCL12-abundant reticular cell (CAR cell). It is well known that HSCs are regulated by biological substances secreted from various supporting cells. However, the role of ECM is not yet known in detail. In this study, we constructed an artificial niche using decellularization method in order to fine out the role of ECM. The decellularized bone marrow is expected to provide the 3-D structural template which preserves the original niche structure. The decellularization was performed by using detergents methods and high-hydrostatic pressure (HHP) method. The decellularized bone marrow was implanted to the C57BL/6 mice to investigate whether the decellularized bone marrow would function as hematopoietic stem cell niche template for blood forming. Although decellularized bone marrow was implanted subcutaneously, we found out that the decellularized bone marrow became red bone marrow after 4 weeks. Therefore, it is demonstrated that the decellularized bone marrow provides a specific microenvironment for hematopoiesis.

45.P05
Human dermal fibroblasts for TERM- a story of phenotype plasticity

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Several years ago we began experimenting with human dermal fibroblasts, attempting to change their phenotype. The idea was to standardize a minimally invasive way of obtaining autologous cells for use in repair of multiple tissue types. We investigated the use of medium-based differentiation of fibroblasts, based on similar methods that were desribed for adult stem cells. Work with primary and clonal fibroblasts led to a publication showing that dermal fibroblasts could indeed be coaxed toward three mesenchymal lineages: adipogenic, chondrogenic and osteogenic, based on protein markers and tissue staining. A broad range of publications has emerged from labs around the world on the use of cells from the dermal compartment to obtain autologous cells that can be differentiated in vitro. Further experiments brought us into the realm of vascular tissue engineering, and we have obtained cells reminiscent of endothelial cells. We are now moving in two directions: approaching clinically relevant studies on the use of the differentiated fibroblasts, including in vivo experiments and functional studies; and also exploring the transcriptome and the genetic regulation underlying the phenotype plasticity of fibroblasts. Briefly, these developments and key findings will be presented.

45.P06
Effects of interactive mechanical and biochemical niche signaling on stem cell differentiation in 3D

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Stem cells hold great promise as autologous cell sources for tissue repair. A long-standing bottleneck in stem cell biology and tissue engineering field is the lack of understanding of how the complex niche signals regulate stem cell fate in 3D. While stem cell niche is a multi-factorial environment, most previous studies focus on studying the effects of individual type of microenvironmental cue on stem cell fate regulation. We hypothesize that biochemical and mechanical cues of microenvironment interact in a non-linear manner in regulating stem cell osteogenesis in 3D. The goal of this study is to develop novel 3D combinatorial hydrogels with various biochemical and mechanical properties to facilitate rapid analysis of interactive niche signaling on stem cell osteogenesis in 3D. Using a modular design approach, we have developed an interpenetrating network with two distinct crosslinking mechanisms for the biochemical and mechanical building blocks. Our data suggests scaffold biochemical and mechanical signals synergize only at specific concentration and combinations to promote bone differentiation. Leading group led to over 150-fold increase in expression of mature bone marker osteocalcin, and blebbistatin treatment decreased the non-muscle myosin IIB up-regulation and the enhanced bone differentiation in 3D. The technology platform is very versatile and can be adapted to optimize niche cues for directed differentiation of any type of stem cells towards any lineages.

45.P07
Mononuclear cells enhance cell migration out of human articular cartilage

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The belief that adult articular cartilage lacks progenitor cells has been challenged with the hypothesis that a progenitor cell population might reside in the superficial zone of cartilage. This study aimed to characterize the articular cartilage cell population and to compare two different cell isolation methods. In addition, a real-time cell analyzer was used to monitor cell migration from human cartilage tissue revealing the novel finding that peripheral blood mononucleated cells (PBMC) act as a chemoattractant for cartilage-derived cells. Human tissue was obtained from patients undergoing total joint replacement with full ethical consent. The cell surface antigen phenotype and protein expression of the primary cell lines were analysed with flow-cytometry and western-blot. The xCELLigence (Roche) was employed to assess the migration potential of chondrocytes. Cells derived from a three hour digestion were 96.5% positive for CD90 (Thy-1), however, the cell population migrating out from cartilage explants only 0.025%. In addition, digested and migratory cell populations had markedly different protein expression profiles. The results show that the addition of mononuclear cells increased the cell migration rate by 40%. In order to develop new therapies for the osteochondral niche it is essential to understand the underpinning biological mechanisms that control the fate of progenitor and other cell types homing to the tissue repair site.

45.P08
A method for multicellular spheroid engineering

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Forming multicellular spheroid (MCS) is an essential method to maintain cell functions because it can mimic three-dimensional environment. Integrating dissimilar cells and other materials in MCSs may induce more cell characteristics. However it is difficult to form MCSs with these cells/particles because of their different cohesive properties. In this study, we report a new method that overcomes this difficulty and demonstrate MCS engineering. This method of aggregating heterogeneous cells/particles was based on the swelling property of a culture medium containing 3% methylcellulose (MC medium). Injection of several microlitres of normal medium containing suspended cells into the MC medium resulted in absorption of the normal medium and

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aggregation of the cells within 30 min. We confirmed that this method could be used to aggregate dissimilar cells and multiple-sized polystyrene beads (0.1–100 μm diameter). When we used pre-formed MCSs (up to 100 μm diameter) and single cells, multicore-shell structures were obtained. The combination of alginate gel beads (20 μm diameter) with single cells was effective in the formation of network-like distribution of cells in MCSs. We also found that cultivation of MCSs comprising dissimilar cells led to spontaneous migration inducing specific patterns in the MCSs. These results show that MCS engineering has a potential to reconstitute tissue/organ-like structures, which are adaptable to regenerative medicine and pharmaceutical screening.

45.P09
Effect of surface-immobilized extracellular matrices on the proliferation of neural progenitor cells derived from induced pluripotent stem cells

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Introduction: Induced pluripotent stem (iPS) cells have a capability of self-renewal and differentiation into multiple cell types. Neural progenitor cells (NPCs) derived from iPS cells have been considered as one of the potential sources for the cell transplantation therapy of central nervous disorders. A next challenge may be to establish a culture method for preparing NPCs in a large quantity. In this study, we investigated the effect of surface-immobilized extracellular matrices (ECMs) on the proliferation of NPCs derived from iPS cells.
Methods: iPS cells were differentiated into NPCs by the serum-free floating culture method [1]. Then NPCs were cultured for 3 days on an ECM array that displayed collagen I, collagen IV, gelatin, laminin-1, laminin-5, Matrigel, fibronectin, vitronecitn, and ProNectin F to compare the efficiency of NPCs proliferation.
Results: The array-based screening showed that NPCs derived from mouse iPS cells efficiently proliferated on a substrate with immobilized laminin-1, fibronectin, and vitronectin. Based on this result, a laminin-1-immobilized substrate was tested using NPCs derived from human iPS cells. Our results showed that the human NPCs also proliferated selectively on this substrate without impairment of multipotential differentiation capability.
Conclusion: When immobilized on a substrate, laminin-1 is effective for the selective expansion of NPCs derived from mouse and human iPS cells.
Reference:
1. Okada Y., et al.

45.P10
Characterisation of the proliferative properties of three types of adult stem cells

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Background: Adult stem cells exist in niches surrounded by somatic cells and extracellular matrix. Analysing the proliferation of stem cells after tissue extraction may shed light on how the artificial cell culture environment influences their behaviour.
Methods: The proliferation rates of stem cells derived from tendons, bone marrow, and cancellous bone were compared as well as the effects of passaging and defrosting.
Results: When first seeded after isolation, marrow stem cells attached to tissue culture plastic quickly, whilst tendon stem cells and bone stem cells attached more slowly (1, 8 and 11 h respectively). Marrow stem cells had the slowest doubling time followed by tendon stem cells and bone-derived stem cells (61, 42 and 37 h respectively). Proliferation of bone stem cells increased with passage, whilst tendon and marrow stem cell proliferation slowed. Freezing and defrosting tendon and bone stem cells slowed attachment, but increased the overall proliferation rate.
Discussion: Bone stem cells proliferate faster in vitro than marrow and tendon stem cells and this difference increases with passage. The relationship between proliferative potential and phenotypic marker expression is currently under study and will be invaluable in therapeutic stem cell production.
Acknowledgments: The research is funded by the European Union Seventh Framework Programme (FP7/2007-2013) under grant no. 251385 (Tendon Regeneration).

45.P11
Using design of experiments (DOE) to identify crucial factors in hematopoietic niches for the expansion of CD34+ cells from the umbilical cord blood

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A deep insight into the niches within different organs where haematopoiesis takes place helps to identify large number of cues implicated in the process. The complexity of the system regulating the process and the crosstalk between the various regulatory molecules mandates the application of a methodology capable of not only identifying the crucial components but also elucidating clearly the interactions between them. Herein we use the design of experiments (DOE) approach to identify, characterize and optimize the ex vivo expansion of hematopoietic stem cells (HSCs) from the umbilical cord blood in a serum-free culture system. We have selected a novel combination of early acting cytokines, morphogenes, hormones, hormone-like proteins, and some cell signalling pathways to identify the important key players and to study the interaction between them. The present results show the feasibility of using DOE approach to define not only the critical factors required for the expansion of HSCs, but also to determine the number of experiments required and the optimum combination levels of novel factors that need to be tested in order to expand the cells of interest. This study demonstrates the advantages of the DOE and its significance as an efficient tool to analyze complex systems comprising the HSCs niche to develop an optimized ex vivo HSC culture system for clinical applications.

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46. From Cells to Secretomes in Regenerative Medicine

46.01
Keynote: Paracrine factors- non-invasive alternative to cell transplantation?

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Advances in therapy have led to a significant decline in mortality in patients with acute myocardial infarction. Unfortunately, this decrease in mortality is paralleled by an increase in the incidence of heart failure in patients surviving with significant myocardial damage. The development of heart failure after myocardial infarction is determined by the size of the infarcted area, the wound healing response that occurs in the first weeks after the event, and chronic left ventricular remodeling. Reperfusion therapy can effectively limit infarct size in patients presenting early after symptom onset. Moreover, several strategies can be pursued to attenuate chronic remodeling. So far, no specific therapies are available to target the wound healing process and prevent its complications, infarct expansion and aneurysm formation, which ultimately determine heart failure development. Only recently, it has been shown in randomized-controlled clinical trials that an intracoronary infusion of autologous bone marrow cells during the wound healing phase can promote functional improvements in patients recovering from a myocardial infarction. These beneficial effects are thought to be mediated, to a large extent, by paracrine factors that are released from the transplanted bone marrow cells. The paracrine concept implies that individual paracrine factors might be identified and developed as therapeutic agents for patients with myocardial infarction. We will provide examples that this may indeed feasible and that systemic treatment with individual paracrine factors may ultimately provide a non-invasive alternative to cell transplantation.

46.02
Keynote: Mesenchymal stem cell derived secretomes: cardiac regeneration and cytoprotection *in vitro and vivo*

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Transdifferentiation into cardiomyocytes (CMC) and into vascular lineage cells has been originally proposed as principal mechanism underlying adult stem cell (ACS) therapeutic action. More recently, permanent engraftment and transdifferentiation of transplanted ASC have not been confirmed. Furthermore, so far it has not been possible to reproducibly induce a functional cardiac phenotype in bone marrow-derived (BM) stem cells in vitro using physiological growth factors or non-toxic chemical compounds. These negative results have questioned the plasticity of BM stem cells. Therefore, it has been proposed that the functional benefits observed after BM stem cell transfer in animal models of cardiac injury might be related to secretion of soluble factors (SF) that, acting in a paracrine fashion, protect the heart. The most compelling data pertain to BM mesenchymal stem cells. The mechanisms mediating the effects of paracrine factors are many. SF may favor in particular neovascularization, cytoprotection and endogenous cardiac regeneration. Furthermore, the post infarction inflammatory and fibrogenic process, CMC contractility, and cardiac metabolism may also be influenced in a paracrine fashion. The demonstration that stem cells secrete therapeutic factors provides a potential breakthrough in that, rather than administering cells, it may be possible to administer specific factors produced by these cells for cardiac therapy.

46.03
Keynote: Mesenchymal stem cells as 'medicinal' cells: induction of endogenous bone repair

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The use of adult stem cells in clinics to enhance tissue regeneration is under debate for safety, efficiency and cost-effectiveness issues. Bone marrow-derived mesenchymal stem cells (MSC) are considered effective therapeutic agents for tissue repair, not just for their multipotent differentiation capacity, but also for their interesting role as cellular modulators. MSC therapeutic effects in vivo are often associated with functional mobilization of host cells into injured tissues rather than with a direct differentiation into the tissue cell types. Taking advantage of an ectopic bone formation model in immuno-competent mice, we showed that the capacity of implanted MSC to activate endogenous regenerative mechanisms is critically dependent on the conditions of their ex vivo culturing prior to transplantation. In particular, the presence of a factor such as FGF-2 in the culture medium during MSC expansion critically influences their subsequent in vivo ability to activate endogenous regenerative mechanisms. We also gathered evidence that such MSC function depends on paracrine factors. In our model, in response to the implanted MSC, host cells, such as inflammatory (M1)and alternatively activated (M2) macrophages as well as endothelial progenitor cells and pluripotent mesenchymal cells were mobilized within the lesion site.The cell interaction, the nature, and the compartmental origin of the migrated host specific cell populations playing a role in the bone regeneration process will be discussed.

46.04
Using the secretome of mesenchymal progenitors of the umbilical cord as a modulator of neural and glial survival, viability and differentiation

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Although it is hypothesized that mesenchymal stem cells' secretome plays a major role in CNS regeneration, little is known on the mechanisms that regulate these actions. In the present work we aimed to assess if the secretome of a population of MSCs isolated from the WJ of the UC (HUCPVCs) was able to modulate neural/glial survival differentiation and proliferation in in vitro and in vivo models. Two approaches were used: (1) primary cultures of neurons and glial cells, were incubated with conditioned media (CM) from HUCPVCs and (2) HUCPVCs or their CM were injected in the dentate gyrus of male Wistar rats. In vitro results revealed that HUCPVCs CM increased cell proliferation in neuronal and glial cells cultures, as well as the survival of astrocytes, oligodendrocytes and neurons. In vivo experiments revealed that animals injected with HUCPVCs, had an increased endogenous cell proliferation, and disclosed higher levels of FGF-2, BDNF and NGF, as indicated by qRT-PCR. On the other hand, in the animals injected with

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CM, the effect caused in the DG was more evident for astrocytes and neuronal cell densities. The CM was also able to induce the differentiation of resident neural precursor towards the neuronal lineages. With this work it was possible to show that HUCPVCs secretome is able to modulate in vitro and in vivo neural/glial cell survival proliferation and differentiation, a fact that may facilitate the future applications of these cells in future CNS related therapies.

46.05
Human placenta mesenchymal cells after intravenous transplantation into rats with experimental ischemic stroke undergo homing in brain neurogenic zones and enhance neurogenesis

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We used magnetic resonance imaging (MRI), fluorescent microscopy, and immunohistochemistry to study homing and further fate of human placental MSC after intravenous transplantation into rats with experimental stroke imitated by transient middle cerebral artery occlusion (MCAO). MSC isolated from human placenta and labeled in vitro with fluorescent magnetic microparticles were infused i.v. 24 h after the operation. The fate of transplanted cells was monitored in vivo by MRI and post mortem on histological slices. MSC transplantation significantly reduced the volume of the ischemic lesion evaluated by MRI. Some transplanted cells invaded brain and after 2–3 weeks concentrated around the infarction area and in brain neurogenic zones - the dentate gyrus (DG) of hippocampus and subventricular zone (SVZ). Six weeks after operation most of them could not be identified suggesting that they either perished or lost the label. A tiny proportion of MSC invading brain survived and expressed glial or neural markers. However, differentiating human cells failed to acquire astrocyte- or neuron-like morphology. Human MSC transplantation further stimulated proliferation of rat cells in SVZ, already enhanced by ischemia, and their migration towards the infarction site. Favourable effects of human MSC transplantation in rat MCAO model are likely to be at least partly associated with their targeted migration to brain neurogenic zones and activation of endogenous neural and glial precursors.

46.P01
The influence of culture conditions on the growth factor and cytokine expression of human bone marrow-derived mesenchymal stromal cells

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Human mesenchymal stromal cells (hMSC) are of enormous interest for various clinical applications. Besides the capacity to differentiate into various tissues, the beneficial effect after direct or systemic application is reported to be a paracrine effect. The aim of this study was to investigate which influence the different culture conditions (medium, serum, passage number, long-term storage) have on the growth factor and cytokine expression of hMSC. Human MSC were isolated from bone marrow and expanded in DMEM or Panserin 401 supplemented with either 10% or 2% FBS. In the low serum culture conditions bFGF, EGF, PDGF-BB and dexamethasone were additional supplemented as previously described by our group. In this investigation two different sera were applied. RNA was isolated after passage 1, 3 and 5 from naive hMSC and from cultivated hMSC after long-term storage. Quantitative RT-PCR was performed to investigate the different growth factor and cytokine expression. The growth factor and cytokine expression of each donor MSC was altered by all different culture conditions (medium, serum, passage, long-term storage). Interestingly, no obvious trend could be determined. The expression of the investigated marker of each individual donor demonstrated enormous differences. The culture conditions of hMSC have an enormous impact on the individual growth factor and cytokine expression. However, there is no obvious trend detectable.

46.P02
Periodontal tissue regeneration with the stem cells cultured conditioned media

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Introduction: Tissue regeneration using mesenchymal stem cell (MSC) suffers from some problems such as high capital investment, expensive cell culture, complicated safety and quality management issues regarding cell handling. Recent studies of MSC transplantation revealed that the implanted MSC did not survive for a long time and MSC secreted many growth factors and chemokines during their cultivation. We hypothesized that the conditioned media from MSC had osteogenic and angiogenic potentials and investigated the effects of conditioned media from MSC on periodontal tissue regeneration.
Material and methods: CM from human BMMSC (hBMMSC-CM) were collected during their cultivation. Canine BMMSC (cBMMSC) and rat BMMSC (rBMMSC) cultured in hBMMSC-CM were assessed from the point of proliferation, migration and osteogenic activity. Periodontal bone defects were prepared in the canine and rat models and the collagen sponges or β-TCP with or without hBMMSC-CM were implanted to the defects. Each defect was evaluated radiologically and histologically.
Results: Proliferation and migration of cBMMSCs and rBMMSCs oncreased significantly in hBMMSC-CM. In vivo, bone and blood vessels regenerated by hBMMSC-CM were more prominent than the others.
Conclusions: The stem cell cultured conditioned media will be the alternative therapy in periodontal tissue regeneration.

46.P03
Trophic capability of adipose-derived stromal vascular fraction cells for regenerative medicine

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Regenerative properties and utilities of adipose-derived stromal vascular fraction (SVF) cells are being increasingly realized. A number of factors, including lipoaspiration, tissue location, collagenase, and isolation techniques and apparatuses, can potentially affect the resultant SVF cells' composition and properties. Our study supports the widely recognized trophic and differentiation capabilities of SVF cells using a cell-based technological platform. SVF cells were isolated from human adipose tissue using the fully automated Cell Isolation System™ (Tissue Genesis, Inc.). SVF cells were directly plated in 60-mm dishes and grown to 90% confluency (day 0). Conditioned medium was collected at days 2 (n = 15) and 4 (n = 15) to measure secreted angiogenic and anti-apoptotic factors. SVF cells demonstrated continuous secretion of vascular endothelial growth factor (2578 ± 726 to 4768 ± 1485 pg/10^6 cells), hepatocyte growth factor (793 ± 459 to 2762 ± 1108 pg/10^6 cells), and tissue inhibitor of metalloproteinases-1 (3543 ± 1485 to 5727 ± 2619 pg/10^6 cells) and -2 (311 ± 93 to 624 ± 161 pg/10^6 cells) over days 2 and 4 ($P < 0.0001$). Stromal

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cell-derived factor-1 secretion was not sustained after day 2. DNA and RNA were also isolated and are currently being evaluated for concomitant gene expression and markers associated with pluripotency. Thus far, we demonstrate sustained intrinsic secretion of trophic factors by SVF cells over time, further implicating their integral role in regenerative medicine.

46.P04
Human amniotic membrane derived soluble factors: an immunomodulatory cocktail

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Mesenchymal stromal cells (MSC), obtained from different sources, have generated enormous interest for their potential application in regenerative medicine due to their stem cell potential and immunological features. Despite many studies have provided support for the immunoregulatory role of MSC, the exact mechanisms whereby this immunomodulation occurs still remain to be fully elucidated. In this study we aimed to identify the immunomodulatory soluble factors released in culture by human amniotic mesenchymal tissue cells (hAMTC) and amnion membrane fragments (hAM). Conditioned medium (CM) from hAMTC or hAM cultures was harvested at day 5. CM was subsequently subjected to temperature changes, fractionation or deproteinization. Moreover we specifically inhibited the activity of IDO (idoleamine 2,3-dioxygenase) and the production of PGs (prostaglandins) during CM production. We demonstrate that CM from both hAMTC and hAM were able to inhibit in vitro lymphocyte proliferation. This inhibitory effect seemed to be mediated by low molecular-weight, non-protein, thermo-stable compounds. We showed that anti-TGF-β, IL-10 and HGF, and the IDO activity inhibitor were all unable to revert the anti-proliferative effect of CM. On the contrary, the addition of COX inhibitors and anti-IL-6 induced a partial reversal of this effect. In this study we have shown that CM-hAMTC and CM-hAM effect partially involves the activity of PGs and IL-6, but not that of IDO, NO, TGF-β, IL-10 or HGF.

46.P05
Secretome from mononuclear cells confers immunosuppression in a murine autoimmune myocarditis model

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Although auto-immunity is thought to play a major role in the pathogenesis of myocarditis, "classical" immunosuppression has not been effective in treating patients suffering a post-infectious viral myocarditis. However, a modification of the autoimmune response could possibly lead to better results. We have recently shown that a high dose application of paracrine factors obtained from mononuclear cells (MNC) modulates the inflammatory response following myocardial ischemia. In this subsequent study, we sought to determine immunosuppressive features of MNC secretome in a $CD4^+$ cell dependent model of murine myocarditis. Cell culture supernatants derived from murine MNC were injected intraperitoneally after induction of autoimmune myocarditis with a cardiac myosin peptide homologue. The inflammatory response was determined by histopathological evaluations and by ELISA. Impact of MNC secretome on proliferation and cell viability of T- cells was measured by FACS and histone release assays. Treatment of EAM mice with a single high dose of MNC secretome resulted in an attenuation of myocardial infiltrate (myocarditis score 2.7 ± 0.4 vs 0.01 ± 0.01; $P = 0.002$). We further evaluated the effect of MNC secretome on JURKAT cell line and purified human $CD4^+$ cells. Coincubation of MNC secretome with T-cells led to a caspase-9 dependent induction of apoptosis. Our data give first evidence that secretome obtained from MNC possess immunosuppressive features in an autoimmune myocarditis model.

46.P06
Conditioned media from bone marrow derived mesenchymal stem cells and adipose derived stem cells enhanced bone regeneration in rat calvarial bone defects.

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Recently, it was reported that stem cells secrete many growth factors and cytokines during their cultivation and that could affect on the cellular characteristics and behavior. This study investigated the effect of conditioned media from bone marrow derived mesenchymal stem cells (BMMSC-CM) and adipose derived stem cells (ADSC-CM) on bone regeneration in vitro, and in vivo.BMMSC-CM and ADSC-CM enhanced the migration, proliferation and expression of osteogenetic marker genes of rat MSC (rMSC). MSC-CM included several cytokines such as VEGF. They have angiogenic potential which is almost equal to VEGF. In vivo, a prepared bone defect of a rat calvarial model was implanted in four different rat groups using one of the following graft materials: BMMSC-CM/Collagen, ADSC-CM/Collagen, PBS/Collagen, and defect only. Micro-CT and histological analysis indicated that the conditioned media groups had a greater area of newly regenerated bone compared with the other groups ($P < 0.05$). In vivo imaging and immunohistochemical staining showed that migration of rMSCs to the bone defect in the conditioned media groups were greater than in the other groups. These results demonstrated that BMMSC-CM and ADSC-CM can regenerate bone through mobilization of endogenous stem cells and angiogenesis. The use of conditioned media from stem cells for bone regeneration will be a unique concept that utilizes paracrine factors of stem cells without cell transplantation.

46.P07
Preliminary and first-in-human clinical study of novel bone regenerative medicine using the conditioned media from bone marrow derived mesenchymal stem cells

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The growth factors and cytokines contained in the conditioned media from bone marrow derived mesenchymal stem cells (MSC-CM) have several effects on cell behavior. Our previous studies revealed that MSC-CM enhanced bone regeneration through cell mobilization, angiogenesis and osteogenesis in vitro and in vivo. The aim of this study is to evaluate the safety and the effects of MSC-CM in clinical use for bone regeneration. Commercially available human mesenchymal stem cells were cultured in basal media and MSC-CM was prepared. Sixteen patients (Twenty-six sites) who were diagnosed to need bone augmentation procedure because of periodontitis, tooth extraction or application of dental implants were treated with mixture of MSC-CM/β-TCP or MSC-CM/collagen sponge after informed consents. The clini-

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cal and radiographic assessments were performed during the follow-up period (at least 6 months). Histological assessments were also performed in some cases. No systemic and local complications were reported throughout the study. Radiographic assessment revealed early and effective bone regeneration in seventeen sites (65.4%). Histologic evaluation also showed early bone regeneration. Only one site showed the resorption of the grafted material because of infection. This is a preliminary and first-in-human clinical study of bone regeneration using MSC-CM. MSC-CM seemed to have a great osteogenic potential for the regenerative medicine of bone.

46.P08
Conditioned media obtained from human outer root sheath follicular keratinocyte culture increases the hair-inducing capacity of cultured dermal cells

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The neogenesis of hair follicle through follicular cell implantation is a key for the cell-based therapy for hair loss and is believed to greatly depend on the ability to reproducibly expand hair-inductive dermal cells in vitro. However, hair-inductive capacity of these cells is lost during subculture. Recent studies showed that hair-inductive potential of cultured dermal cells can be maintained by the addition of conditioned media obtained from epidermal keratinocyte culture. In this study, we investigated whether treatment of human outer root sheath follicular keratinocyte conditioned media (FKCM) activates signaling pathways that contribute to the maintenance of hair-inducing capacity (trichogenicity) and increases the trichogenicity of cultured dermal cells. RT-PCR analysis showed that expression of alkaline phosphatase (ALP), a marker of the enhanced trichogenicity, was increased in the presence of FKCM. Immunoblot showed that FKCM treatment activated BMP and β-catenin signaling pathways and induced more hair follicles compared with control cells in hair reconstitution assays. Altogether, our data demonstrates that factors that promote trichogenicity of dermal cells are contained in human FKCM and suggest that FKCM is of use for hair regeneration research and may be applied for cell-based therapy for hair loss.

46.P09
Real-time quantification of autologous artificial connective tissue (AACT) secreted factors using a surface plasmon resonance biosensor (SPR)

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Introduction: Evaluation of proteins secreted by fibroblasts comprising skin substitutes show that they are secreted in a way that promotes wound healing. Yet, assessment of proteins secreted by oral fibroblasts forming part of oral substitutes is scarce. We developed oral autologous artificial connective tissue (AACT) and its profile of secreted factors at the moment of grafting was done with an antibody-array system. Although the array allowed the quantification of secreted factors, this was a relative quantification. Based on biosensor technology here we established a real time method to quantify AACT-fibroblast secreted proteins. This direct single step analysis is sensitive, fast and specific and requires no sample labeling.
Methods: For quantification a Surface Plasmon Resonance (SPR) biosensor (BiaCore®) was used. A surface chip CM5 was used to immobilize anti- fibroblast secreted factor monoclonal and polyclonal antibodies used to capture the analytes. The activation of the surface chip was made via-chemical coupling. The best electrostatic interaction between antibodies and the chip surface was determined using Sodium Acetate buffer. Thereafter, factors specific for each immobilized antibody were injected at different concentrations to obtain the standard curves where FI of secreted factors were interpolated for quantification.
Results: Three sequential pre-concentration assays allowed obtaining the specific conditions of pre-concentration, immobilization.

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47. Mechanism on Stem Cell Action (in coop. REMEDIC)

47.01
Keynote: Progenitor and non-progenitor functions of MSCs: mechanisms and contexts

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Cells referred to a MSCs (skeletal stem cells) play a dual set of functions, both of which are revealed by in vivo transplantation assays: in organs generated by in vivo transplantation, they represent self-renewing and multipotent progenitors of all skeletal tissues, and at the same time the organizers of non-skeletal host tissues (hematopoietic and vascular). This dual function makes MSCs unique among all cells that are currently called stem cells. Both arms of this dual function can be harnessed for therapies, some of which are traditionally envisioned (regeneration of bone and cartilage) while others emerge as the non-progenitor functions of MSCs are more clearly recognized. The 'niche' effect exerted by MSCs on hematopoietic stem cells can now be accurately modeled in vivo with human cells, and dissected in some detail via ad hoc systems for in vivo transplantation that eliminate the need for exogenous scaffolding, and murine models. These systems reveal, through experimental modeling, unexpected links between the progenitor and non-progenitor functions of MSCs, and within the lineage system. While progress is made in understanding the stem cell biology of a unique system (with features that single it out from other well known systems such as hematopoiesis or epithelia), the predominant approach to translation mostly focuses on non-progenitor functions, but drifts away from the notion of stem cells or regenerative medicine. Intravenous infusion of (rapidly cleared) M

47.02
Keynote: Skeletal tissue engineering: development of combination products

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The clinical impact of (stem) cell based combination products is still limited. The complexity of 'living' implants requires more work including the development of robust manufacturing methods. Also, in depth studies are needed on the mechanisms by which these implants may contribute to tissue repair. In an effort to optimize bone healing, we have been exploring implants using periosteal cell populations. We have found that these cells not only contribute through their osteo/chondrogenic potential, but that they also display pro-angiogenic features and may contribute to tissue remodeling by affecting osteoclastogenesis. We have found that the survival and function of periosteal cells relies on timely interactions with blood vessels from the nearby muscle, which re-emphasizes the importance of the local environment. We have further investigated the rational selection of calcium phosphate (CaP) containing materials, based on biomaterial properties, and evaluation of their bone formation capacity. We found that the cell-material combinations behave quite differently in vivo, despite apparent in vitro similarities. Microarray studies with a variety of analysis approaches such as principal component analysis, gene clustering and osteogenic network analysis provided input on the factors/pathways critical in driving the in vivo bone formation processes. In conclusion, more rational approaches for tissue repair will hopefully lead to more predictable clinical outcomes.

47.03
Keynote: Immunomodulation by mesenchymal stem cells

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Mesenchymal Stromal Cells (MSCs) are non-hematopoietic progenitor cells found in the bone marrow and many other tissues. In vitro and in vivo, the cells differentiate into adipocytes, chondrocytes and osteocytes after appropriate induction. Both undifferentiated and MSCs induced to differentiate, have immune-modulatory properites and promote peripheral tolerance. In vitro and in vivo in experimental animal models, MSCs suppress alloreactive donor anti-host T-cell responses. MSCs also prevent the maturation of monocytes to first immature dendritic cells (DCs) and next mature myeloid DCs that support T-cell alloresponses. Instead, MSCs re-polarise pro-inflammatory DCs into tolerogenic IL-10^{+} DCs that together with other effects promote T-cell anergy and Treg induction. Interferon induces MSC to produce indeolamine 2,3 dioxygenase, prostaglandin E2 and other factors that are believed to mediate these effects. Many questions remain to be answered before MSCs can be established as an immunomodulatory treatment. Efficacy of the cells needs to be established in clinical trials. This is particularly true since no efficacy marker has been established that predicts the clinical outcome of patients treated with MSCs. So far, data indicates low infusional toxicity. Response rates in the literature indicate that MSCs are a promising tool for immunomodulation.

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48. Gene Transfer Approaches in Tissue Engineering

48.01
Keynote: Combinatorial development of biomaterials and synthetic siRNA delivery systems

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High throughput, combinatorial approaches have revolutionized small molecule drug discovery. Here we describe our work on high throughput methods for developing and characterizing biomaterials, and in particular siRNA delivery systems. Libraries of nanoparticles, degradable polymers and lipid-like materials have been synthesized, formulated and screened for their ability to deliver siRNA, both in vitro and in vivo. A number of siRNA delivery formulations have been developed with in vivo efficacy, and show potential therapeutic application for the treatment of genetic disease, viral infection, and cancer.

48.02
Keynote: Ultrasound-mediated gene delivery: Potential and current limitations

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Ultrasound-mediated gene transfer is emerging as a practical alternative to other gene transfer modalities. Using ultrasound as a stimulus for gene transfer/delivery is attractive from a variety of perspectives including its non-invasive nature and its ability to stimulate biological events with a high degree of both three dimensional spatial and temporal control. In exploiting ultrasound for the purposes of gene delivery, these attributes offer unique benefits enabling the extracorporeal stimulation of gene transfer in a variety of environments. Although ultrasound alone can be employed to stimulate gene transfer, it is significantly enhanced in the presence of exogenously-added microbubbles. Comprising a variety of shell-based materials and gas cores, these agents afford an extra level of control from a gene delivery perspective. In this presentation the potential advantages and current limitations associated with the use of ultrasound as a gene transfer/delivery stimulus will be reviewed.

48.03
A simple and efficient 3D cyclised 'Knot' polymer structure for nonviral gene delivery

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Since the polyamidoamine dendrimer (PAMAM) demonstrated that its 3D hyperbranched structure could facilitate effective transfection, much effort has been turned to the preparation of branched versions of common transfection agents. Herein, we report the design and synthesis of new 3D 'Single Cyclized' polymeric gene vectors with well-defined compositions and functionalities via a one-step synthesis from readily available multi-vinyl monomers. The cyclization agent ethylene glycol dimethacrylate was used at 10% of monomer mixture, usually impossible due to insoluble gel formation were it not for the use of the highly controllable Deactivation Enhanced – ATRP synthesis method. This allowed single growing chains with PEG and a cationic monomer to crosslink within themselves forming a new structure for a transfection agent. This structure of cyclizing chains offers a different pattern of interaction between the polymer and plasmid DNA, and leads to a general profile of higher transfection capability (over 6 cell types) than SuperFect® (partially degraded PAMAM). By adjusting the monomer ratio of PEG, lower toxicity is also achieved, thus rendering the cyclised knot a more attractive alternative to the PAMAM dendrimer.
Acknowledgement: SFI, Strategic Research Cluster (SRC) (grant number 07/SRC/B1163).

48.04
Non-viral sonoporation gene therapy for orthotopic bone regeneration

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Sonoporative gene transfer, a minimally invasive non-viral gene transfer method, has been shown to trigger expression of plasmid vectors in vivo. The combination of Bone Morphogenetic Protein 2 and Bone Morphogenetic Protein 7 in a co-expression strategy has been proven highly osteoinductive. Therefore, the aim of this study was to design and test BMP2/BMP7 co-expression plasmids for their regenerative potential in in vivo models. BMP2 and BMP7 were cloned into a constitutive expression plasmid and an inducible TetON system for co-expression. Induction of osteogeneic differentiation was tested in vitro in C2C12 cells with an osteocalcin specific reporter system and standard methods. Subsequently, the plasmids were administered in an ectopic mouse model and 3 days post fracture in a rat femur non-union model, using sonoporation. Animals received five treatments on five subsequent days. Controls received luciferase plasmid, which allowed monitoring of gene transfer efficacy & localization via bioluminescence imaging. All animals were imaged using an in vivo μCT on days 28 and 56. Bioluminescence showed strong luciferase expression after sonoporation. μCT images showed ectopic bone formation in muscle and enhanced bone volume in critical size femur defects. Therefore, we conclude, that sonoporative gene transfer is an effective non-viral method to mediate transient transgene expression in ectopic and orthotopic settings and to improve bone regeneration in orthotopic settings.

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48.05
Efficacy of TissueGene-C (TG-C), a cell mediated gene therapy, in patients with osteoarthritis: A phase IIa clinical study

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Kolon Life Sciences, Korea; Samsung Medical Center, Korea; TissueGene Inc, Korea; Seoul National University Hospital, Korea

TG-C is a gene-modified cellular therapy for the treatment of knee osteoarthritis (OA). TG-C contains normal (hChonJ) and transduced (hChonJb#7) human chondrocytes transduced with a TGF-β1 gene-containing retroviral vector at a 3:1 ratio. In a Phase I study no severe adverse events (SAEs) and dose limiting toxicity were observed up to the dose level of $3 \times 10e^7$ cells/knee. The current study was a randomized, single blind phase IIa trial conducted to determine both the safety and efficacy of TG-C in patients with OA. Participants ($n = 27$) with a confirmed diagnosis of OA by MRI were randomized to either $0.6 \times 10e^7$ cells/knee (low dose, $n = 13$) or $1.8 \times 10e^7$ cells/knee (high dose, $n = 14$) of TG-C treatment.TG-C significantly improved IKDC, WOMAC, and 100 mm VAS scores. Low and high doses of TG-C treatment showed significant improvement in total IKDC score by 33% (from 44.9 ± 7.8 to 59.93 ± 10.5, $P < 0.001$) and by 25% (from 49.9 2 ± 9.5 to 62.48 ± 9.7, $P < 0.001$), WOMAC scores by 45% (from 31.92 ± 8.4 to 17.43 ± 8.2, $P < 0.001$) and by 41% (from 30.00 ± 9.5 to 17.79 ± 9.7), and 100 mm VAS scores from by 47% (51.07 ± 13.4 to 26.50 ± 14) and by 45% (from 50.77 ± 13.3 to 28.14 ± 11.2), respectively, at 6 month post treatment. No serious adverse events (SAEs) were observed in either treatment group. Overall, the current Phase IIa study indicated that TG-C improved three evaluation criteria for pain, sports activities, and quality of daily life in patients with OA without producing any serious adverse effects.

48.P01
Surface-modified microbubbles and their use in ultrasound-mediated gene transfer

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It has been strongly suggested that ultrasound presents a very attractive means of facilitating non-invasive, site-specific gene transfer. In an ultrasonic field microbubbles enhance the formation of transient pores in cell membranes (sonoporation) and this can be used to facilitate intracellular delivery of macromolecules such as nucleic acids. It has previously been shown that the spatial proximity between the microbubble, the cell surface and the nucleic acid, in the presence of an ultrasonic field, plays an important role in promoting sonoporation events and achieving efficient gene transfer. Here we describe the effect of cationic surface charge and the presence of biotin on ultrasound-mediated gene transfer into RIF1 target cells, in vitro. Microbubble reagents (neutral, cationic and biotinylated cationic surface) were provided by SONIDEL Ltd. The plasmid pCMV-Luc was used as a reporter of gene transfer and luciferase expression was detected using bioluminescent imaging. Ultrasound treatment was performed using a SONIDEL SP100 sonoporator (1 MHz). The results demonstrate that cationic charge and the presence of biotin on the surface of the microbubbles significantly enhance the interaction of these reagents with plasmid DNA and the cell membrane. This, in turn, promotes ultrasound-mediated gene transfer and the data suggest that this approach might find application in areas such as non-invasively promoting gene transfer for the purposes of tissue regeneration.

48.P02
Ultrasound mediated gene transfer for therapeutic angiogenesis-proof of concept with luciferase

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Peripheral vascular diseases are often accompanied by delayed wound healing. Therefore local delivery of growth factors, e.g.Vascular Endothelial Growth Factor-A (VEGF-A) and Platelet Derived Growth Factor-B (PDGF-B) offers a promising therapeutic possibility. Ultrasound mediated non-viral gene therapy (Sonoporation) is an alternative simple and minimal invasive method for temporary gene expression. The aim of the study was to optimize a Sonoporation protocol to improve angiogenesis in a murine ischemic hind limb model. Non-viral plasmids were injected intramusculary into mouse hind limb muscle and percutaneously sonoporated. Transfection efficacy and expression kinetics using different concentrations of luciferase encoding plasmid (20, 80 and 120 μg + microbubbles) were investigated. No microbubbles and sonoporation served as controls. Bioluminescence signals were measured to evaluate optimized DNA amounts. Sonoporation was effective irrespective of dosing. However, differences were obtained when comparing plasmids + microbubbles + sonoporation versus controls. In controls, luminescence signals could only be detected up to 3–7 days. In contrast, plasmid transfection via sonoporation assisted by microbubbles showed luminescence signals up to 28 days. In vivo transfection with sonoporation is effective when plasmids are combined with microbubbles. The maximum observed protein expression is 28 days. This time frame is optimal to induce angiogenesis by VEGF-A and PDGF plasmid therapy.

48.P03
The use of multicellular tumor spheroids (MCTS) as in vitro model for electrogene therapy optimization

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Cell membrane can be transiently permeabilized under application of electric pulses. This process is actually clinically used to introduce small anticancer drugs into solid tumors as well as gene in tissues (Rols,. Curr Gene Ther, 2010). To be effective, electrogene therapy (EGT) requires the optimization of electrical parameters of the applied voltage such as electric field strength, pulse width and pulse frequency. Because MCTS display nutrient, oxygen and signal gradients, cell-cell contacts as well as cell-extracellular matrix interactions, we propose to use them to in vitro optimize EGT electrical parameters (Chopinet et al.,. Int J Pharm, 2011). Electrotransfection was performed at different intensities (from 300 to 600 V/cm) by applying 4 to 10 pulses of 5 ms duration at 1 Hz frequency. A secure and efficient electrogene transfert lays on a compromise between cell viability and gene expression. GFP-gene expression and cell viability were directly detected with a fluorescence microscope in fresh MCTS made of human HCT-116 cells and quantified by flow cytometry after enzymatic dissociation. Preliminary results show that 500 V/cm electric fields, 6 to 8 pulses of 5 ms duration at 1 Hz frequency induce a good compromise between cell viability and gene expression in MCTS. Our aim is now to confirm and test EGT conditions onto a more complex MCTS model including stromal and endothelial cells to better mimic in vivo tumor microenvironment.

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48.P04
A unique bioluminescent prostate cancer mouse model for the evaluation of stem-cell based gene therapy

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Most prostate cancer (PCa) related deaths result from metastases. Bone marrow derived mesenchymal stem cells (BMSC) show promise as therapeutic gene transfer vehicles due to their ability to home to tumour sites anywhere in the body while evading degradation by the immune system. BMSCs can be genetically modified with therapeutic gene(s) ex vivo to deliver its therapeutic payload on re-introduction into the donor. Understanding BMSC behaviour in the context of the tumour environment in a living body is essential for the successful development of novel cell therapies. This study describes the use of a unique, syngeneic C57BL/6 model of PCa. Sequential Lumina II bioluminescence full body imaging (BLI; Caliper/ Thermo Scientific) was used to quantify: therapeutic gene and/or firefly luciferase modified BMSC (BMSC-fl) homing to PCa; renilla luciferase modified RM1 (RM1-rl) pseudometastases growth in albino C57BL/6 male mice. BMSC therapeutic genes included in this study are the yeast cytosine deaminase and uracil phosphoribosyltransferase fusion suicide gene or interleukin-24. RM1-rl cells injected subcutaneously or intravenously formed solid and measurable subcutaneous tumours and lung pseudometastases respectively. We showed light reactions produced by BMSC-fl substrate D-luciferin and RM1-rl substrate coelentrazine were transient and did not cross-react in vitro or in vivo. BLI and survival studies confirming therapeutic BMSC killing of PCa in vivo will be discussed.

48.P05
Development of disulfide-modified hyaluronan graft polyethyleneimine nanocarrier for gene transfection

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In this study, a novel thiolated hyaluronic acid (HA)- polyethylenimine (PEI) copolymer with environmental sensitive functional group was synthesized, characterized and examined as a potential non-viral gene vector. The physical and chemical properties of fabricated gene carrier were analyzed via 1H NMR and FT-IR for the demonstration of cross-linking of HA with PEI. TNBS assay and Ellman's reagent were conducted for the verification of disulfide bond (s-s) formation. The particle size and morphology were investigated by dynamic light scattering, zeta potential and transmission electron microscopy, respectively. The ability of HA-ss-PEI to complex with plasmid DNA was observed by gel electrophoresis. Fluorescent microscopy and ELISA spectroscopy was utilized to examine transfection efficiency and protein expression level of therapeutic pDNA. From the results, a novel environmental sensitive gene delivery nanocarrier has been successfully synthesized. HA-ss-PEI (+23.9 mV) complexed with negatively charged plasmid DNA can be achieved via electrostatic attraction to form a stable spherical nanoparticle of 100 nm in diameter. The nanocarrier could successfully complex with pDNA in the N/P ratio above 3 and transfect stem cells to produce specific protein for therapeutic purposes. In brief, the environmental sensitive HA-ss-PEI nanoparticles can be used as a promising non-viral gene carrier for therapeutic gene therapy in biomedical applications.

48.P06
Towards inhibition of fibrotic encapsulation of implants using siRNA nanoparticles

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Introduction: We aim to use siRNA to inhibit undesired fibrotic encapsulation of implants by down-regulating cellular collagen secretion. Inhibition of fibrotic encapsulation can, among other things, be important for accurate performance of implanted biosensors.
Materials and methods: To overcome extracellular and intracellular barriers that restrict therapeutic application of siRNA, we utilize chitosan as a biomaterial carrier. Subjects of our work are electrostatic binding of siRNA to the biomaterial and characterisation of the resulting nanoparticles using photon correlation spectroscopy. Furthermore, in cell culture we analyse particle uptake, subsequent gene knockdown and collagen synthesis.
Results: Fibrosis is characterised by enhanced synthesis and unorganized aggregation of collagen. In cell culture experiments we are able to effectively down-regulate a gene important for collagen secretion and to modify collagen secretion. Usage of chitosan in certain ratio to siRNA allows the formation of small particles with size in the nanometer range and positive zeta potential. Moreover, these particles are efficiently taken up into fibroblasts and produce a significant reduction of the targeted gene.
Conclusion: Our results highlight the potential of chitosan/siRNA nanoparticles to inhibit a fundamental fibrotic pathway. Our aim for the future is to use siRNA nanoparticles to biologically functionalise implants for different medical applications.

48.P07
Potential of bioengineered cell sheets prepared with lentivirally transduced adipose tissue-derived stem/stromal cells for hemophilia B therapy

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Hemophilia B is an inherited bleeding disorder caused by the deficiency of Factor IX (FIX), a functional clotting factor. Gene transduced cells are considered to be a useful tool in establishing cell-based gene therapies for diseases with monogenic plasma protein deficiencies. Patient-derived adipose tissue-derived stem/stromal cells (ADSCs) are an attractive cell sources. This study explored the potential of mouse ADSCs (mADSCs) for human FIX (hFIX) gene transduction with a self-inactivating lentivirus simian immunodeficiency virus (SIV) vector in vitro and their ability to produce and secrete biologically active hFIX. The optimal SIV-vector dose for mADSCs transduction was determined by their transduction efficiency and the production level of hFIX. Although the hFIX production levels showed a slight decrease in the first week, sustained production levels were observed for 4 weeks. For establishing a tissue engineering-based treatment modality with hFIX transduced ADSCs, a method to create a contiguous cell sheet format was investigated. Transplantation of hFIX-transduced ADSC sheets was found to

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be successfully engrafted in the subcutaneous space, resulting in a detection of hFIX in the recipient's plasma. The present study showed that a combination of SIV-mediated gene transduction and cell sheet tissue engineering technology was able to establish a valuable cell and gene-based therapy utilizing autologous ADSCs for individuals with hemophilia B.

48.P08
Cellular and genetic medicines advancing the treatment of prostate cancer

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While early prostate cancer (PCa) can be cured with surgery, metastatic disease is incurable. Suicide gene therapy (SGT) is feasible for the prostate which is a non-essential organ for life. SGT involves the transfer of a non-mammalian suicide gene directly into the prostate rendering PCa sensitive to killing by systemically administered non-toxic prodrug. SGT shows several advantages: prodrug activation in the prostate avoids systemic toxicity; the toxin readily diffuses across cell membranes killing local bystander PCa cells that do not express the suicide gene; stimulation of immune cells providing PCa metastases killing (distant bystander effect). We are currently exploring two novel SGT delivery methods. The first utilises a sheep atadenovirus delivery of a bacterial purine nucleoside phosphorylase gene and prodrug fludarabine phosphate (FP253; PCTAU03/00381). FP253 has been registered for a Phase I clinical trial (NCT00625430/ClinicalTrials.gov). We are now successfully engineering bone marrow-derived stem cells (BMSC) to deliver yeast cytosine deaminase uracil phosphoribosyltransferase fusion gene to metastatic PCa in the presence of prodrug 5-fluorocytosine. BMSC have attracted much attention as cellular gene vehicles due to their: ability to seek out cancer in the body; autologous or allogeneic transplantation as they overcome issues of host immunity and the ability to avoid degradation by the immune system, a major limitation of most current gene delivery methods.

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49. Gene Transfer Approaches in Tissue Engineering II and Gene Activated Matrices

49.01
Keynote: Healing bones with gene activated matrices and other genetic strategies

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It is widely appreciated that gene transfer offers much potential as a means of promoting bone healing, but there is no consensus as to which gene delivery strategies are best suited for individual applications. Four different approaches are represented in the literature. Two are in vivo and two are ex vivo. The in vivo strategies include the use of Gene Activated Matrices and the direct injection of vectors into the site where bone needs to be generated. Traditional ex vivo methods require the harvest and expansion of autologous cells, which are genetically modified and returned to the individual. Expedited ex vivo methods aim to simplify this by accomplishing the tissue harvest, genetically modification and reimplantation in a single sitting. Both viral and non-viral vectors have been used for these purposes, with successes noted in many small animal studies and a few large animal studies. Despite the promising pre-clinical data, no human clinical trials have yet started.

49.02
Controlling drug release spatially and temporally in scaffolds

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Most organs and body parts consist of multiple cell types arranged in discrete spatial geometries. The generation of each of these cell types is in turn controlled by numerous factors, such as proteins and RNA, which are active during specific timed phases. Successful engineering of complex tissues requires the development of methods that recapitulates this spatial and temporal information. We pursue this by functionalizing porous tissue engineering scaffolds with drug delivery systems. By changing various parameters in the scaffolds and delivery systems it is possible to construct composites that deliver these drugs in specific areas at specific time points. To gain temporal control, we have, for example, devised different encapsulation and incorporation techniques that allow proteins to be released from scaffolds at different rates. Furthermore, we have investigated how properties such as scaffold surface area, hydrophilicity and drug bonding influence the release of adsorbed protein and RNA from scaffolds. For imparting spatial information we have developed scaffolds that can retain different RNA containing nanoparticles in discrete locations and through theseguide the development of different tissue types in those locations. We also demonstrate how it is possible, using co-printing of implant and drugs, to replicate the structure seen on a CT-scan in a scaffold containing different nanoparticles in areas with soft and hard tissue.

49.03
Development of a gene-activated matrix for enhanced bone tissue regeneration combining dual angiogenic and osteogenic non-viral gene delivery

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Gene-activated matrices (GAMs) have shown potential in localised gene delivery resulting in bone tissue regeneration. The potential of using a collagen-nanohydroxyapatite (Coll-nHa) scaffold developed in our laboratory as a GAM suitable for bone tissue engineering is explored within. Furthermore, the possibility of combining two non-viral vectors containing an angiogenic (angio gene) and an osteogenic gene (osteo gene) in the GAM is investigated. (Due to I.P. constraints these vectors and genes cannot be disclosed until September.) Firstly, the transfection parameters of two vectors were optimised using green fluorescent protein and luciferase in monolayer MSCs and assessed using flow cytometry and a LumiFlex GLuc Assay kit respectively. Monolayer osteogenesis and angiogenesis assays were performed with each vector and gene. Vector A was observed to be suitable for delivery of the angio gene while Vector B was apt for osteo gene transfection. Coll-nHA scaffolds were loaded with vector-gene complexes as follows: Vector A both genes; Vector B both genes; Vector 1-Angio Gene/Vector B-Osteo Gene (Mix GAM). These GAMs were seeded with MSCs and cultured in osteogenic media. Calcium quantification, μCT analysis and alizarin red staining revealed that the Mix GAM had superior osteogenic capabilities compared to controls and all other GAMs. Incorporating two different vectors allows us to harness the synergism of an osteogenic and an angiogenic gene to mimic natural bone healing.

49.04
Gene silencing of chordin in combination with supplementation of BMP-2 has positive effects on osteogenic differentiation of human adipose tissue derived stem cells (hADSC)

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Introduction: Although BMP-2 is known to potently induce osteogenic differentiation of human mesenchymal stem cells, strong individual differences have been reported. In part, this is due to internal antagonists against BMP-2 e.g. noggin and chordin, secreted by differentiating cells. We hypothesized that osteogenic effects of BMP-2 can be improved by transient non-viral gene silencing of chordin.
Methods: hADSCs were isolated from lipoaspirates of different donors. Cells of passage 4 to 5 were transfected with siRNA against chordin and noggin using a commercial liposomal transfection agent. Subsequently, cells received BMP-2 in a dose of 100 ng/ml. Osteogenic differentiation of hADSC was determined by normalized alkaline phosphase (ALP) activity and matrix mineralization.
Results: ALP activity of hADSC treated with siRNA against chordin showed increased ALP activity for every donor tested. In contrast,

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silencing of noggin increased ALP in some donors only. In combination with BMP-2, silencing of either chordin or noggin showed strongly improved ALP activity compared to the scambled control group that was also supplemented with BMP-2. Mineralization was observed to start earlier in groups that received siRNA against chordin or noggin and showed increased amounts of incorporated Calcium on day 16 compared to the control groups. Improved effects of chordin compared to noggin silencing may be due to an up-regulation of BMP-2 expression in the chordin group only.

49.05
Fibrin mediated proangiogenic and secretory control gene therapy for compromised wound healing

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Diabetic patients are susceptible to chronic ulcers especially on lower extremities. Despite rigorous treatment, success has been modest in reducing the rate of amputations. Hyperglycemia is an important predisposing factor. Here, the objective was to unleash the pathological disarray at molecular level by studying the effects of hyperglycemia on wounded keratinocytes and subsequently to develop a controlled delivery system capable of delivering multiple therapeutic genes. The microarray data analysis on the wounded keratinocytes under hyperglycemic vs normoglycemic culture conditions revealed a profound differential gene regulation, with a number of up- and down-regulated genes. The secretory control molecule Rab18, found to be significantly downregulated, was chosen as therapeutic gene, considering hyper-secretion of pro-inflammatory cytokines and proteolytic enzymes in diabetic wound healing. eNOS was chosen as proangiogenic gene, considering reduced angiogenesis in diabetic wound healing. With eNOS in fibrin gel and Rab18 in fibrin microspheres embedded in fibrin gel, the Rab18-eNOS loaded fibrin-in-fibrin system was investigated in alloxan induced hyperglycemic rabbit ear ulcer model. Rab18-eNOS treated group showed significantly higher percent wound closure at day 14 post-wounding with reduced inflammatory cell infiltrate and more functional angiogenesis. Thus, fibrin mediated delivery of Rab18-eNOS is a promising therapy towards normalization of diabetic wound healing.

49.P01
Gene transfection using PLL-PRP conjugates binding to protein based physical hydrogels

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Recently, the neural stem cell transplantation to the diseased site is focused as a novel therapy for neural disease, such as Huntington's, and Parkinson's. The viability of the transplanted cells is one of important key factors to succeed in the treatment. We have proposed that two-component protein-based hydrogel system that can self-assemble under constant physiological conditions helps cell viability during transplantation procedures by protecting the cells from shear stress during injection. In the present study, we have proposed that the addition of plasmid DNA, which encodes functional proteins such as neural growth factor, to this hydrogel system enhances neural regeneration and have synthesized poly-L-lysine (PLL) conjugated with proline-rich peptide (PRP) that binds to one of the two components (WW domains) of the hydrogel. The ϵ-amino moiety of PLL was modified with PRP through disulfide binding. The modification ratio of PRP to PLL was varied from 1% to 5%. The obtained PLL-PRP conjugates were mixed with plasmid DNA or polypeptide having WW domains. The complex formation of PLL-PRP and plasmid DNA was confirmed by an agarose gel electrophoresis. Also, the complex of PLL-PRP and plasmid DNA could transfect to various cells. The binding of PLL-PRP and WW polypeptide was also achieved. From these results, it suggests that PLL-PRP could be applicable to this two components hydrogel system as gene carrier.

49.P02
Biodegradable and cationic polymer coupled with minicircle COL7A1 for the corrective gene therapy of recessive dystrophic epidermolysis bullosa

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Introduction: Recessive dystrophic epidermolysis bullosa is an inherited disease characterized by severe blistering of the skin after mild trauma caused by a missing anchoring protein called collagen type VII (gene: COL7A1). Here we aim to provide corrective gene therapy of RDEB using a cationic polymer to reintroduce the COL7A1 sequence (in the form of miniature plasmid known as minicircle, MC-COL7A1) into skin cells by topical application.
Experimental methods: Polymer synthesis: The polymer was synthesised by DE-ATRP and characterised by chromatography (GPC) and proton NMR. Organotypic skin culture and transfection: Fibroblasts and keratinocytes (keratinocyte growth medium II) (Promocell) from RDEB patients were embedded or seeded in a fibrin gel-matrix, respectively. After 28 days in culture at the air-liquid interface, the skin equivalents were transfected with the polymer/MC-COL7A1 polyplexes and analysed for protein expression 48 h later. Analysis: Collagen VII expression in skin equivalents was analysed using immunofluorescence and western blot. Monoclonal LH7.2 antibody (Sigma) specific for collagen VII was used in all cases.
Results and Conclusion: Cells treated with polymer/MC-COL7A1 polyplexes showed collagen type VII expression levels similar to those in normal human keratinocytes and fibroblasts. The results suggest direct treatment of RDEB using a topically applied polymer/MC-COL7A1 polyplexes is possible and has a potential therapeutic application.

49.P03
Utilizing chitosan nanoparticles for the production of gene-activated matrices for bone regeneration

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Chitosan is a natural cationic polymer which shows promise as a gene delivery vector as it is biocompatible, biodegradable and capable of intercellular delivery of nucleic acids. Mesenchymal stem cells (MSCs) are notoriously difficult to transfect and toxicity is a major issue associated with commonly used non-viral vectors such as polyethylenimine (PEI). The objective of this study is to develop a method of transfecting mesenchymal stem cells (MSCs) with a reporter gene, followed by ther-

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apeutic genes, using chitosan nanoparticles before applying this technique onto a collagen-glycosaminoglycan scaffold, thereby producing a gene-activated matrix for bone regeneration. Chitosan nanoparticles were formed by ionic gelation and characterized for size and zeta potential followed by assessment of complexation efficiency by gel mobility shift assay. MSCs were transfected with chitosan-pDNA nanoparticles using PEI-pDNA polyplexes as a positive control. Transfection efficiency was monitored by fluorescence microscopy and flow cytometry. Efficiency of chitosan as a gene delivery vehicle is influenced by a huge number of parameters. Results from this study have led to the development of a method for formulating nanoparticles of diameter 100–120 nm, a positive zeta potential and excellent complexation efficiency making them suitable for transfection. Ongoing work involves applying this MSC transfection system for the development of a gene-activated matrix for bone regeneration.

49.P04
Current approaches of bone tissue engineering

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Introduction: The problem of bone defects repair is highly relevant to surgical practice, but is not resolved. For this reason we develop tissue engineering approaches for creating effective osteoplastic materials.
Materials and methods: The first approach is based on cell technology. We combined a scaffold (demineralized bone matrix) with multipotent mesenchymal stromal cells (MMSC) and evaluated in vitro. This bone graft was transplanted into the defect of rabbit leg and cranial bones. The second approach is based on gene technology. We combined a scaffold (collagen/hydroxyapatite) with DNA-plasmid containing VEGF/GFP genes. Produced gene-therapy osteoplastic material was investigated in vitro (cultured with MMSC and endotheliocytes on Matrigel) and in vivo (transplanted into cranial bone defects of rabbits).
Results: We've developed and patented effective technologies for creating cell-based and gene-therapeutic osteoplastic materials. In vitro we've found that plasmids of gene-therapeutic osteoplastic material leave the structure of a scaffold and transfer into MMSC, were the genes of plasmids are expressed. Edotheliocytes form capillary-like structures on Matrigel. Cell-based and gene-therapeutic osteoplastic materials both show efficiency for repair bone defects in vivo.
Conclusion: The result of our research shows that both tissue engineering strategies are effective, but most promising gene approach as it isn't associated with costly personalized cellular service.

49.P05
Bioactive implants: RNA interference as a tool to direct stem cell differentiation

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Exogenous short interfering RNAs (siRNAs) or microRNA enable the specific control of cellular protein production which has many potential applications in implant and tissue engineering technology including directing the differentiation of stem cells into a specific lineage by targeting e.g. a transcription factor or a repressor of differentiation. To functionalize scaffolds with siRNA and/or microRNA, interphasing the scaffold with the nucleic acid in way that ensures RNA stability and effective uptake and function in stem cells is needed. We have previously reported that adherent nanoparticles containing a number of siRNAs and coupled to nanostructured scaffolds, allowed spatial retention of the siRNAs within nanopores until their cellular delivery, enhanced lineage-specific differentiation of skeletal (mesenchymal) stem cells (MSCs) into osteogenic and adipogenic lineage (Andersen et al; 2010. Mol Ther. 18:2018–27). To further enhance the differentiation efficiency, we are currently employing a similar approach and including miRNA and miRNA inhibitors. We have identified novel miRNAs that control the differentiation induction of osteogenic and adipogenic differentiation by miRNA profiling of MSC using next generation sequencing. These candidate miRNAs -coated implants are being tested for their ability to enhance osteogenic and adipogenetic differentiation of MSC and exploring the possibilities of spatially localized differentiation effects.

49.P06
Intracellular regulation of ES/iPS cells on E-Cad-Fc engineered matrix facilitated by bio-functionalized nanoparticles of carbonate apatite

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Gene therapy through intracellular delivery of a functional gene to embryonic stem (ES) and induced pluripotent stem (iPS) cells which have the potential to be differentiated to a specific cell type represents a new and versatile source of cell replacement in regenerative medicine. Therefore, a unique pH sensitive nanoparticles of carbonate apatite has been successfully fabricated by embedding fibronectin on the carrier for specific recognition to cell surface integrin of ES cell cultured on the E-Cad-Fc which is artificial ECM with a fusion protein of E-cadherin extracellular domain and IgG Fc region in maintaining pluripotency of ES cells without colony formation which may accelerate transfection efficiency due to homogenous single cell state providing every ES cell for nanoparticle interaction and subsequent gene transfection. Moreover, intrinsic fascinating properties of carbonate apatite—ability of preventing crystal growth for efficient endocytosis and fast dissolution rate in endosomal acidic compartments to facilitate DNA/siRNA release from the particles and endosomes made it suitable for DNA, siRNA and drug delivery even to ES and cancer cell lines. Therefore, we achieved highest transgene expression due to the dual effect of smart biodegradable pH sensitive carbonate apatite carrier and novel homogenous culture system for ES cell at single cell level using E-cad-Fc matrix which will guide to homogenous differentiation towards hepatocytes for regenerative medicine.

49.P07
Development of in vitro and in vivo evaluation methods for advanced biotherapeutics

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Recent years have witnessed significant innovation in the biopharmaceuticals area. The reason for this is that biopharmaceuticals can be tailored for a specific problem in a given individual. The greatest potential of pharmaceutical biotechnology lies in gene therapy. In this study, we intended to develop in vivo and in vitro efficacy test for evaluation of gene therapy product. This study was planned for 2-year projects and performed to establish foundation of evaluation for gene therapy products. First, we established gene therapy products for this study. We investigated the gene candidates for treatment of ischemic heart disease and selected VEGF and GATA-4genes for gene therapy products

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and DNA plasmid was accepted for Vector system. Second, to develop in vivo animal test model, we induced ischemic heart disease with ligation of coronary artery, LAD. We calculated EF, LVIDs and LVIDd of ischemic heart disease after 4 weeks of surgery. Infarction area and fibrosis were observed with triphenyltetrazolium chloride (TTC) and trichrome staining on histopathogy. Third, we induced functional cardiac differentiation from ESCs with Embryonic Stem Cell Test and modified the protocols to control cardiac differentiation. Characterization were performed with immunohistochemistry and RT-PCR et al. Taken together, we studied for establishment of foundation as in vivo and in vitro test for evaluation of ischemic heart disease gene therapy products.

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50. Preformed Vasculature and Induced Angiogenesis

50.01
Keynote: Angiogenesis and tissue engineering – from isolated endothelial cells to preformed vasculature and surgical angiogenesis

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Engraftment, differentiated function and long-term viability of thick three-dimensional engineered tissue constructs represent a major challenge. Addressing it requires development of vessel-like networks that will allow the survival of the construct in vitro and its integration in vivo owing to improved angiogenesis directly after implantation. This keynote lecture features recent advances in engineering of vascularized structures. Thorough understanding of endothelial cell biology, state of the art cell culture technology including co-culture with mesenchymale cells and dynamic culture conditions and embodiment of angiogenetic growth factors in polymeric scaffolds for prolonged release are prerequisites for successful in vitro vascularization strategies. Prefabrication of vascular networks in vitro can be achieved either by cell self-assembly or by use of microfabrication methods. Recent developments in surgical implantation strategies allow generation of vascularized tissue units with a defined vascular axis that can be transferred into defect sites using microsurgical techniques. In the near future, interdisciplinary approaches aiming on combination of optimized (micro)surgical implantation modalities and innovative tissue engineering strategies with a perspective of readily transplantable bioartificial tissues for large volume applications will be developed.

50.02
Role of heme oxygenase-1 in regenerative medicine

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Heme oxygenase-1 (HO-1) involvement has been demonstrated in the blood vessels formation (Dulak et al., Circulation 2008, 117: 231-241). Here we investigated the role of HO-1 in reparative processes using cell and gene therapy approach. The restoration of blood flow after hind limb ischemia was impaired in HO-1-deficient animals. Even the lack of one HO-1 allele attenuated regeneration in heterozygotic mice when combined with hyperglycemia. The lack of HO-1 impaired bone marrow-derived proangiogenic progenitor cells (PPS), affecting their resistance to oxidative stress, migration, proliferation and vascular differentiation. Nevertheless, the expression of HO-1 was not sufficient in PPCs to achieve regenerative effect after delivery of PPCs to the damaged tissues. However, the secretory properties of PPCs required HO-1 expression as evidenced by enhanced revascularization in ischemic muscles treated with conditioned media. Additionally, conditioned media from HO-1 overexpressing myoblasts accelerated revascularization in the ischemic muscles of diabetic mice. Finally, both adenoviral transfer of HO-1 as well as hypoxia-driven HO-1 expression from plasmid vector enhanced angiogenesis in diabetic wounds and in ischemic limbs, respectively. Our studies indicate that HO-1 is required to achieve full regenerative capacities. Secretome or gene therapy employing the HO-1 overexpression may enhance the regeneration of damaged tissues.

50.03
VEGF expression by human mesenchymal stem cells promotes vascularization of osteogenic grafts, but shifts bone homeostasis towards increased resorption

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Rapid vascularisation of tissue-engineered osteogenic grafts is a major obstacle in the development of regenerative medicine approaches for bone repair. Vascular Endothelial Growth Factor (VEGF) is a powerful angiogenic factor. However, VEGF has a very short half-life in vivo while its expression needs to be sustained for approximately 4 weeks in order to allow the stabilization and persistence of newly formed vessels. Therefore, we investigated a cell-based gene therapy approach to generate osteogenic grafts with an increased vascularization potential in an ectopic nude rat model in vivo, by genetically modifying human BMSC to achieve sustained rat VEGF expression. 8 weeks after in vivo implantation, the vascular density of constructs seeded with VEGF-expressing BMSC was 3-fold greater than with control cells. However, VEGF over-expression specifically caused a global reduction in the quantity of bone, which formed thin trabeculae of freshly deposited matrix. VEGF-expression did not impair MSC proliferation and differentiation potential in vitro, nor their long-term engraftment in vivo, but strongly increased the recruitment of TRAP- and Cathepsin K-positive osteoclasts. These data suggest that an equilibrium exists between VEGF-triggered angiogenesis, osteogenesis and bone resorption, so that VEGF over-expression might disrupt the balance between bone formation and resorption towards excessive degradation.We acknowledge support by the EU FP7 Project MAGISTER (CP-IP 2146).

50.04
Recellularization of decellularized human placental vascular scaffolds for bioengineering

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Introduction: Bioengineering transplantable or extracorporeal organoids may offer new therapeutic options for organ failure. Decellularized native vasculature is regarded as a suitable material for bioengineering. Human placenta is rich in native vasculature and could be used as a template for cell repopulation in tissue and organ regeneration. In this study, we have evaluated the adherence and growth of different cell types in decellularized human placental vascular scaffolds (DHPVS).
Methods: DHPVS sterilized in 0.1% peracetic acid was cut into 8 mm^3 tissue blocks. For recellularization, PDAC®, HUVEC, 293/GFP and HepaRG cells were seeded on DHPVS. Cell adherence and growth was evaluated under a fluorescent microscope and by MTS assay. Cell viability was determined by Live/Dead cell kit. Hepatocyte function was assessed using the Albumin Blue Fluorescent Kit.

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Results: All cell types studied demonstrated adherence and proliferation on DHPVS. The cells had better growth on DHPVS than in conventional 2D culture. PDAC cells and HepaRG cells showed adherence and proliferation on the scaffolds with high viability. HepaRG cultured on DHPVS were found to produce significantly more albumin than cells grown on in the absence of DHPVS ($P < 0.05$), an early indication of maintainence of cellular function on the scaffold.
Conclusions: The current study illustrates the potential for using decellularized placenta scaffolds as natural 3D bioengineering matrix for organoid development.

50.05
In vivo fabrication of a vascular bed suitable for grafting cell sheets

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An engineered thick tissue is not able to survive without functional vascular network to supply oxygen and nutrients to the cells. Polysurgery of cell sheet grafts has already overcome this barrier. In this study, we challenge to engineer a vascular bed for ectopical fabrication of multilayer cell sheets graft. The rat femoral artery and vein were used as central vessels of vascular bed. The artery and vein were incorporated in a novel silicone chamber with collagen gel to isolate them from the surrounding tissue. Two weeks after in vivo incubation of the chamber, some new capillaries from the artery and vein were formed in the chamber. Then neonatal rat cardiac cell sheet were transplanted on the vascular bed, and the chamber was resealed again. Two weeks after the cell sheet implantation, a well organized vasculature and visible cardiac pulsation was detected in the chamber. A cross sectional view of Azan staining indicated the substantial vascular connection between the vascular bed and implanted cell sheet. When saline was injected with the artery, it drained successfully from the vein, after removing the chamber from the rat. These results indicated the establishment of a perfusable vascular network in the construct, and the possibility of ectopical transplantation of the vascularized thick construct.

50.P01
Angiogenesis and cell infiltration induced by enzymatically synthesized amylose hydrogels impregnated with bFGF

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Background: Enzymatically synthesized amylose (ESA) is non-biologically produced linear polysaccharide using enzymes. ESA is obtained with any desired molecular weight and has narrow molecular weight distribution. We have already reported that carboxylated ESA exhibits heparin-like anti-coagulant activities.
Objective: To obtain functional scaffold, we synthesized novel cross-linked ESA hydrogel and assessed their ability to release bFGF, bioactivities and biodegradability.
Methods: Carboxy group was introduced to ESA by treating with succinic anhydride, and then sulfonate group was introduced to a part of the carboxy group on ESA treated with taurine. Degree of substitution (DS) of these groups was measured by potentiometric titration. Cross-linking by ethylenediamine resulted ESA hydrogels, and bFGF release profile were measured by ELISA. bFGF-impregnated ESA hydrogels were implanted subcutaneously into rat dorsal area for 2–4 weeks and assessed their biological activity and biodegradation. Acetyl group was also introduced to control biodegradability.
Results: ESA hydrogel having higher DS(COOH) suppressed initial burst of bFGF release and showed longer release profile. Acetylation of ESA hydrogel having DS(COOH) = 2.1 showed slow release of bFGF up to four weeks. Only ESA hydrogel having both sulfonate and acetyl groups remained after four-weeks implantation and showed cell infiltration and blood vessel formation in the gel.

50.P02
Transplantation of cord blood mesenchymal stem cells as spheroids enhances vascularization

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Poor survival of cells transplanted into ischemic regions is one of the problems of stem cell therapy. Cord blood mononuclear cells (CBMNCs) are an alternative source of mesenchymal stem cells (MSCs) without the disadvantages of MSCs derived from bone marrow or adipose tissue. We investigated whether the angiogenic efficacy of cord blood mesenchymal stem cells (CBMSCs) can be enhanced by grafting as spheroids in a mouse hindlimb ischemia model. Animals were divided into no-treatment, dissociated hCBMSC, and spheroid hCBMSC groups and received corresponding hCBMSC treatments. After surgery, the ischemic hindlimbs were monitored for 4 weeks, and then the ischemic hindlimb muscles were harvested for histological analysis. Apoptotic signaling, angiogenesis-related signal pathways, and blood vessel formation were investigated in vitro and/or in vivo. The transplantation of hCBMSCs as spheroids into mouse ischemic hindlimbs significantly improved the survival of the transplanted cells by suppressing apoptotic signaling while activating anti-apoptotic signaling. Furthermore, the transplantation of hCBMSCs as spheroids significantly increased the number of microvessels and SM α-actin positive vessels in the ischemic limbs of mice, and attenuated limb loss and necrosis. hCBMNC can be an alternative source of MSC and spheroid-based CBMSC delivery can be a simple and effective strategy for enhancing the therapeutic efficacy of CBMSCs.

50.P03
Vasculature of artificial scaffolds for bone tissue engineering

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Bone is a blood-vessel dependent tissue, which would not survive without blood circulation. As a matter of fact, vasculature of an artificial tissue matrix represents one of the most significant challenges in tissue engineering of vascularised types (bone is one of them). Vasculature demands two contradictory requirements on the porous structure of scaffolds. The proliferation of endothelial cells requires high surface areas; which however will block the ingrowth of vessels at a later stage. The growth of the vascular tissue requires a highly interconnective and open porous structure, which however would not provide a sufficient surface area for cells to attach and proliferate. To address the above dilemma, we have fabricated highly porous and open scaffolds from Bioglass and used a type-I collagen gel as a carrier to seed vascular endothelial cells throughout the 3D scaffold. In this design, the collagen gel supports and fosters cells for the regeneration of vascular tissues, and the highly open solid structure maintains the physical integrity of the tissue engineering construct. Our study with the above 'Scaffold-Collagen Gel' system has demonstrated that endothelia cells can proliferate throughout the 3D network, eventually growing into a vessel-like tubular tissue.

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50.P04
Stem cells clustering and therapeutic angiogenesis

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Many limitations remain of using stem cells for clinical application due to their low therapeutic efficiency. First of all, most of stem cells die within the first week after transplantation and only a small proportion of transplanted stem cells are alive in ischemic region. Therefore, for successful stem cell therapy, it is prerequisite to develop stem cells that can survive well into ischemic tissues and differentiate into vascular cells. Recently, we established a 3D stem cell masses (3DCMs) culture, demonstrating that 3DCMs formed from human adipose derived stem cells (hASCs) differentiated into vascular cells in vitro and had a proangiogenic action in hind limb ischemia models. 3DCMs were applied to augment stem cell effect for treatment of myocardial infarction induced in rat model. We demonstrated that 3DCMs injection increased the survival and retention of transplanted cells as well as their vascularization as compared to hASCs injection. The wall thickness in 3DCM group further increased as compared with hASC group. The cardiomyocyte apoptosis in 3DCM group was two times lower than that in control group. Capillary density in 3DCM group increased three times than in hASC group. We conclude that 3DCM culture promotes the efficient vascular differentiation of stem cells, and 3DCM transplantation results in vascular regeneration through the direct therapeutic effects of the injected cells.

50.P05
Prevascularization strategy in fibrin matrix

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Vascular tissue engineering aims at mimicking the complexity of the vascular network in cell based scaffolds and promoting angiogenesis in regenerating tissues. Providing sufficient blood supply for complex tissue-engineered constructs is the goal of different prevascularization strategies. The establishment of a capillary network prior to scaffold implantation has been shown to enhance tissue survival. Importantly, microvessel formation is dependent on the matrix structure the cells are seeded on. In this study, the use of polyethylene glycol (PEG) to fabricate channels for prevasularization in fibrin is evaluated. A spacer formed with PEG was embedded into a fibrin matrix. After dissolution of the spacer we seeded the resulting channel with OECs and show ingrowth of endothelial cells into the surrounding matrix. Co-culture with adipose-derived stem cells embedded in fibrin and exposure to shear stress enhanced migration and tube formation of OEC after 7 days, as evidenced by immunhistochemical stainings against endothelial cell specific marker CD31 and pericyte marker α-smooth muscle actin. PEG itself does not have an influence on migration and tube formation, as shown by appropriate controls. The present findings demonstrate that PEG appears as a suitable biomaterial used for applications in vascular tissue engineering.

50.P06
Biomaterials for the stabilization of Hypoxia Inducible Factor 1 (HIF-1) in stem cell recruitment and vascularization

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Low oxygen tension (hypoxia) in vivo plays a pivotal role in coupling angiogenesis with osteogenesis via progenitor cell recruitment and differentiation. The concept proposed in this project involves using materials-based strategies to design scaffolds which activate the hypoxia pathway for the purpose of developing and regenerating skeletal tissue. We have synthesized hypoxia-mimicking scaffolds by incorporation of metal ions such as cobalt. The control release of cobalt ions showed a role in stabilizing hypoxia inducible factors (HIF-1) from degradation by ubiquitination in mesenchymal stem cells (MSCs) and subsequent induction of vascular endothelial growth factor (VEGF) production. VEGF produced by the stem cells in the hypoxia-mimicking scaffolds not only activated the endothelial cells in neovascularization, but also recruited un-differentiated MSCs via the activation of CXCR4/CXCL12 axis. The recruited MSCs further stabilized the tube-like structure formed by the activated endothelial cells, showing well-organized vessel networks. This study provided some new thought in developing bone substitutes by stimulation of cell signalling pathways of angiogenesis and osteogenesis coupling.

50.P07
Controlled release of PDGF-BB from heparin-conjugated electrospun PCL/gelatin fibers on bioactivity and cellular infiltration

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A smart scaffolding system provides not only physical support for an engineered tissue, but also a sustained release of bioactive molecules that can stimulate cell proliferation, migration, and differentiation towards desired cell phenotypes. We developed a heparin-conjugated electrospun poly(ϵ-caprolactone) (PCL)/gelatin fibers to provide controlled delivery of platelet-derived growth factor-BB (PDGF-BB) for vascular smooth muscle cell (SMC) infiltration. We attempted to improve cell infiltration into the electrospun scaffolds by controlling the fabrication parameters. Subsequently, heparin was conjugated on the surface of the fibers using its free amine groups, and the heparin-conjugated scaffolds were immobilized with PDGF-BB using binding affinity. The biological activity of PDGF-BB from the scaffolds was assessed, which was defined as the ability of PDGF-BB to stimulate the growth of SMC. PDGF-BB that was strongly bound to the heparin-conjugated fibers was able to induce proliferation of the SMC better than PDGF-BB that had been released from non-heparinized fibers. In addition, the combination of the large pore structures in the 3.0-μm fibers and the heparin-mediated delivery of PDGF-BB induced the SMC infiltration into the scaffold via synergistic actions of the scaffold's physical and chemical properties. We demonstrate that our scaffolding system is capable of generating fully cellularized vascular constructs for potential vascular tissue engineering applications.

50.P08
Injectable system for spatio-temporally controlled delivery of hypoxia-induced angiogenic signaling

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We previously reported on two strategies for delivering hypoxia-induced signaling (HIS) in vivo, namely implanting living or non-viable hypoxic cell-matrix depots that actively produce angiogenic factors or act as carriers of factors trapped within the matrix during in vitro preconditioning, respectively. This study aims to improve on this approach by development of an injectable system for delivering cell-free matrix HIS-carriers. 3D spiral nano-porous collagen constructs, comprising an

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inner cellular and outer acellular compartment, were cultured under hypoxia (5%O2) for 3 weeks. The acellular matrix was fragmented into micro-fractions and added into a 5°C thermo-responsive type I collagen solution, which underwent a concentration-dependent sol-gel phase transition at 37°C. A range of angiogenic factors (e.g VEGF,FGF,PLGF,IL-8) were trapped within the acellular matrix, as they diffused through it. Levels of VEGF and IL-8, delivered from matrix fractions into media through collagen sol-gel, were up-regulated by day 4 of hypoxic culture, peaked at day 8, and declined towards the baseline by day 20, while FGF levels were stable over this period. Factors were bioactive after 3 months freeze storage, as shown by their ability to induce endothelial tubule formation in vitro. This system provides a minimally invasive, and repeatable, method for localized delivery of time-specific cell-free HIS factor mixtures, as a tool for inducing spatio-temporally controlled angiogenesis.

50.P09
Blood vessel engineering using a novel biomimetic compartmented tubular scaffold and human adipose mesenchymal stem cells

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There exists a substantial need for 'off-the-shelf' functional vascular grafts for use in a number of cardiovascular disorders. A biomimetically-designed tubular scaffold and an easily accessible stem cell source may help to achieve this goal. We have recently described a novel method for fabricating a compartmented tubular scaffold, composed of a macroporous media and a nanofibrous intima template (patent application TR-PT-2012/01600). Electrospun nanofibrous poly(ϵ-caprolactone) side of the construct served as the intima layer for endothelial cells, while the type I collagen/elastin media layer was fabricated by pore formation, lyophilisation and crosslinking steps for smooth muscle cells. Human adipose-derived mesenchymal stem cells (hAdMSCs) were solely considered as the stem cell source. Initially, the smooth muscle cell (SMC) differentiation protocol was optimized using combinations of TGFβ1, BMP4 and Angiotensin II inside smooth muscle growth medium (Medium 231, Gibco) which yielded cells phenotypically similar to smooth muscle cells. Secondly, hAdMSCs were differentiated in vitro into phenotypic endothelial-like cells (ECs) using the endothelial growth and differentiation media (EGM-2, Lonza). Preliminary cell-surface interactions studies were performed. Currently, we are evaluating the dynamic culture/conditioning of the hybrid tubular construct inside a real time computer controlled bioreactor system. The support of TUBITAK (111M336) is acknowledged.

50.P10
Adherent and functional properties of endothelial cells on anti-thrombotic polymers

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Introduction: There are no appropriate materials which exhibit both excellent anti-thrombotic and endothelialization to develop functional artificial blood vessels and vascular stents. We have reported that poly (2-methoxyethyl acrylate) (PMEA) and poly(tetrahydrofurfuryl acrylate) (PTHFA) show excellent anti-thrombotic properties. In this study, we report on the adhesion of endothelial cells on PMEA and PTHFA.
Materials and Methods: Test polymers were coated on polyethylene terephthalate (PET) films. Human umbilical vein endothelial cells (HUVECs) were seeded on the polymer surfaces and cultured. At several time points, we investigated the number of adherent cells and formation of focal adhesions by observing the expression of vinculins using confocal laser scanning microscopy.
Results and Discussion: On PMEA, cells adhered at the initial stage of culture, cell spreading area got larger and vinculin expression became stronger with time. On PTHFA, much more cells adhered and strong vinculin expression was already shown at an early stage. These results suggest that endothelial cells could adhere on PMEA and PTHFA with formation of strong focal adhesions.
Conclusion: PMEA and PTHFA could have both excellent anti-thrombotic and endothelialization properties. It is supposed that PMEA and PTHFA could be appropriate coating polymers for developing small-diameter artificial blood vessels, improving current artificial blood vessels and vascular stents.

50.P11
In situ forming starPEG-heparin hydrogels to evoke vascular morphogenesis and angiogenesis

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Engineered biomimetic hydrogels promoting angiogenesis are instrumental for progress in regenerative medicine. We report about a novel 3D hydrogel system incorporating integrin binding peptides, cell responsive crosslinkers and reversible conjugated growth factors to induce angiogenesis by modulation of the biophysical and biochemical cues which guide endothelial cells (ECs) in vivo. To enable cell embedding we developed a cytocompatible strategy for in situ crosslinking of star shaped poly (ethylene glycol) (starPEG)-peptide conjugates and heparin utilizing Michael-type addition chemistry. Parameters adjusted to evoke EC morphogenesis in vitro were material stiffness, adhesive ligand concentration, and growth factor presentation. Primary ECs showed a viability of ~90% when entrapped in the cell responsive starPEG-heparin hydrogels, thus proving the applicability of the crosslinking method. Within 3 days, ECs underwent pronounced morphogenesis resulting in cellular vacuolization and coalescence into lumenized tubular structures as shown by light, confocal and electron microscopy. Cell viability and morphogenesis were superior in softer gels and in the presence of vascular endothelial growth factor (VEGF165). Furthermore, hydrogels releasing VEGF165 induced angiogenesis in vivo, as confirmed by the chicken chorioallantoic membrane (CAM) assay. In sum, starPEG-heparin in situ gels offer valuable options for cell transplantation and can effectively support angiogenesis.

50.P12
Multilayer cell sheet transplantation for vascularization

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We manufactured multilayered cell sheets from bone marrow-derived mesenchymal stem cells (MSC). It was found to take 30 min for the MSC sheet to attach to ischemic heart tissue following cell sheet transplantation. The purpose of this study was to confirm vascularization and cardiac function once MSC sheets were transplanted onto the ischemic heart. Bone marrow-derived cells were seeded on temperature-responsive dishes. Once MSC reach confluence at 7 days, a monolayer of MSC was detached as the culture temperature was decreased from 37 to 20°C. Multilayered cell sheets were prepared in stacks of three monolayers. Myocardial infarction was created by the ligation of the left coronary artery in six pigs. Multilayered MSC sheets were then

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transplanted onto the ischemic heart surface. Histological analyses and echocardiography were performed after MSC sheet transplantation at 1, 4 and 8 weeks. The transplantation of MSC sheets increased the ejection fraction in this myocardial infarction model. Moreover, the blood vessels were clearly observed at 1 week, and increasing vascularisaton of the transplanted cell sheets and the infracted heart tissue were observed at 4 and 8 weeks. These results suggest that the transplantation of multilayered MSC sheets improves cardiac function. This improvement may be explained in part by the growth factor-mediated effects of the MSC sheet. Thus, the transplantation of multilayered MSCs can potentially provide a new therapeutic strategy.

50.P13
Engineering a pro-angiogenic and anti-fibrotic tissue engineering scaffold

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Inadequate vascularization is a major bottleneck which limits clinical applications of tissue engineering mainly to thin or avascular tissues. In addition, an implanted biomaterial will inevitably evoke a foreign body reaction, which in turn can lead to fibrous encapsulation of the implant and damage to surrounding tissues. Here we propose the incorporation of prolyl hydroxylase inhibitors (PHIs) into scaffolds as a means to overcome both of these problems. PHIs are a class of small molecule drugs with known pro-angiogenic and anti-fibrotic effects. In this study, 2,4-pyridinedicarboxylic acid (PDCA), an established PHI, was conjugated via amide bonds to a gelatin sponge scaffold. The PDCA is released when the amide bonds are cleaved by infiltrating cells. For in vitro testing, fibroblasts were cultured on the scaffolds for 7 days. Cytotoxicity test results showed that the PDCA-conjugated scaffolds have low cytotoxicity at all dosages tested. VEGF secreted into the medium was measured and it was found that the PDCA could increase VEGF secretion significantly, indicating a pro-angiogenic effect. Collagen output was measured by SDS-PAGE followed by densitometry, and results showed that the PDCA could dramatically reduce collagen secretion, demonstrating strong anti-fibrotic potential. These preliminary results suggest that the incorporation of PDCA into scaffolds may be a viable solution for improving vascularization and reducing fibrosis in tissue engineered constructs.

50.P14
Digital light processing of photoelastomers as customized biomaterials for vascular tissue restoration

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The aim of our research is to design new biocompatible materials for the application of narrow blood vessel replacement like coronary bypasses in the cardiovascular system. The approach is to use elastic photopolymers for artificial vascular grafts. Digital light processing (DLP) is an additive manufacturing technology (AMT) capable of realizing very complex structures offering the possibility to create cellular structures within the artificial grafts to enable ingrowth of new tissue. Photoelastomers can be customized by variation of the ratio of reactive diluents and crosslinker and the addition of thiols to the monomer formulation. The best composition of the monomer system was adjusted for the DLP regarding the photoinitiator- and absorber concentration to reach the mechanical properties of natural small caliber blood vessels [1, 2]. To characterize the mechanical properties of the materials elastic modulus (E), the tensile strength (σM) and the elongation at break (ϵB) were tested. Additionally, a special suture tear test has been developed to insure the conformity of the suture tear resistance (RT) of synthetic and native blood vessels as the vascular grafts are sewed to the natural blood vessels.

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50.P15
Re-cellularization of a biological vascularized matrix (BioVaM) for the generation of 3D artificial tissues

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Tissue engineering is a promising technique for reconstruction of failing organs. Based on its size the supply with nutrients and oxygen in constructs requires an in vitro and/or in vivo vascularization e.g. a biological vascularized matrix (BioVaM). Here we report the generation of a vascularized matrix for 3D artificial tissues. A decellularization process utilizing Triton X-100 and SDS was established to generate a matrix with preserved pedicles derived from porcine small intestine. The decellularization protocol was optimized to overcome cytotoxic effects observed with other protocols. The arterial and/or venous vessel bed was recellularized with lentiviral transfected primary porcine cells (smooth muscle cells and endothelial cells). The reseeded matrix was cultivated for 2 weeks under static conditions. After cultivation the whole construct and cryosections were analyzed via fluorescent microscopy. With the optimized decellularization procedure cytotoxic effects were eliminated. Each BioVaM was characterized with regard to the maximal injection volume of the vessel bed. Coverage of the entire arterial and venous vessel bed was achieved after 14 days of cultivation. Recellularization of the matrix was already achieved under static conditions; however further perfusion might improve functional vessel formation. Perfusable, endothelialized constructs may aid in solving the problem of nourishing cells inside 3D tissue-engineered constructs.

50.P16
Dose- and time-dependent angiogenesis by controlled delivery of matrix-bound VEGF

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Vascular endothelial growth factor (VEGF) is the key factor to induce therapeutic angiogenesis. The induction of safe and stable angiogenesis requires sustained VEGF delivery for at least 4 weeks at homogeneous microenviromental doses. Continuous release of matrix-bound growth factors is a convenient approach for clinical translation of this biological concept. Therefore, here we aim to determine the requirements to induce normal and stable angiogenesis by controlled release of transglutaminase (TG)-bound VEGF from a fibrin gel. Both VEGF dose and

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duration are a function of degradation rate, which was studied to identify the best gel composition. Then, to determine VEGF therapeutic window, different VEGF concentrations were tested combined with various concentrations of aprotinin, which inhibits gel degradation by plasmin. An aprotinin concentration of 56 μg/ml ensured both sufficient in vivo persistence and an adequate VEGF release rate. In this condition, after 9 days a VEGF concentration of 25 μg/ml triggered the switch from aberrant to predominantly normal angiogenesis. A lower VEGF dose of 5 μg/ml almost totally eliminated any aberrant angiogenesis. After 4 weeks, this condition showed only normal and well perfused capillaries. In conclusion, in vivo release of TG-VEGF from fibrin gels can be precisely tuned under optimized conditions to efficiently induce dose-dependent and stable angiogenesis.We acknowledge support by the EU FP7 Project ANGIOSCAFF (CP-IP 214402).

50.P17
In vitro vasculogenesis: Endothelial colony-forming cells based 3D model

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Endothelial colony-forming cells (ECFCs) which exhibit an endothelial phenotype, have a large proliferative potential, and functionally integrate into new vascular networks have been isolated from peripheral and umbilical cord blood. Since markers to unambiguously phenotypically characterize these cells remain elusive, their identification relies mostly on late outgrowth of colonies and cell morphology. Similarly, relatively little is known about origin, in vivo function, and angiogenic potential of the ECFCs. In vitro 3D vasculogenesis and angiogenesis models containing ECFCs, perivascular cells, extracellular matrix molecules (ECM) and growth factors would provide an important link between 2D cultures and in vivo assays. The objective of this work is to promote and evaluate the in vitro vasculogenic potential of ECFCs in 3D cultures. Their differentiation and morphogenesis potential will be followed in fibrin gels and synthetic cell-instructive PEG-based matrices containing adhesive (e.g. RGD, FN fragments), pro-angiogenic (e.g. VEGF, HGF) cues and perivascular cells. Morphogenesis assays will be performed by time-lapse microscopy and expression profiles of relevant angiogenic genes will be performed.

50.P18
Construction of ECM-growth factor integrated protein through coiled-coil structure to promote angiogenesis

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Angiogenesis is a vital physiological process for tissue regeneration and repair where the extracellular matrix (ECM) plays an important role. In our previous study, an artificial ECM (EREI2CBD) containing two repeats of elastin-derived unit (APGVGV)12, cell-adhesive sequence (RGD) and laminin-derived IKVAV sequence was engineered then fused with collagen-binding domain (CBD). EREI2CBD promoted tubular formation in collagen gel. While gowth factors are serving as stimuli in neovascularization, our laboratory constructed a bFGF-thered ECM using a coiled-coil helical interaction to increase cell proliferation. In this study, we tried to improve EREI2CBD by binding it to multi-growth factors noncovalently through designed coiled-coil heterodimer between Helix A and Helix B; immobilized form of EREI2CBD-HelixB with co-adsorbed HelixA-scVEGF121 (121-aminoacid single chain vascular endothelial growth factor) and HelixA-bFGF (basic fibroblast growth factor). Proteins were expressed in E.coli and purified using His-Tag. To confirm the activity of HelixA-scVEGF121 and HelixA-bFGF, human umbilical vein endothelial cells (HUVECs) were used. HelixA-scVEGF121 and HelixA-bFGF enhanced the growth of HUVECs. HUVECs could adhere to designed ECM that revealed strong binding to collagen type I. Noncovalent binding of designed ECM and multi-growth factors through coiled-coil structure may be a novel approach in biomaterial which may bring new contribution to the tissue-engineering field.

50.P19
Prefabrication of tissue engineered constructs – a viable strategy for neovascularisation – a model tested on rabbits

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The micro-surgical implantation of an arteriovenous vascular loop represents a reliable technology for the neovascularisation of three-dimensional cultivated cell constructs. Auricular cartilage biopsies were taken from 15 rabbits and populated in 3D cell carriers made from polycaprolactone in the form of a human ear. These cartilage cell constructs were implanted into a flap of skin and neovascularised by means of vascular loops implanted micro-surgically. They were then enhanced as 3D tissue and freely re-implanted in-situ through microsurgery. It was possible to demonstrate, on a macroscopic level, that the 3D flaps could be freely transplanted. On a micro-angiographic level it was evident that all the skin flaps and the implanted cultivated constructs were well neovascularised. There is evidence of cartilage-like tissue with the new formation of the extra-cellular matrix. It was possible to prove the new formation of collagen II in the cultivated biomaterial constructs immunohistochemically. Other tissue types or organoid structures could also be neovascularised through a microsurgically implanted arteriovenous vascular pedicle in order to ensure a sufficient supply of blood and nutrients. This is particularly interesting for cultivated tissue intended for the replacement of defects without skin flaps, for example liver cells or contractile elements. This introduces a new and and promising neovascularisation technology for the field of tissue engineering.

50.P20
Method for the fabrication of macroscopic high resolution scaffolds by the combination of inkjet-printing and laser initiated polymerization

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The circulatory system is a network of branched vessels, bridging diameters from the centimeter range to a few micrometers. The fabrication of a synthetic vessel system faces the problem that the demand for high resolution meets the necessity for macroscopic structure sizes, which generally contradict each other. A possible fabrication method with sufficient high resolution is two photon polymerization (TPP). Here, a focused laser beam is used to initiate a local hardening of a photosensitive material. Although the possible resolution is below one micrometer, process time is generally too high to generate macroscopic structures. In contrast to TPP, 3D-Inkjet printing, allows a macroscopic 3D structure with feature sizes down to 50 μm. The main advantages are high possible process speed through parallelization and the ability to generate multimaterial scaffolds, but the resolution is not sufficient for small vessels. Hence, we combine these two fabrication methods in a single machine setup, merging the high resolution TPP with the fast 3D-inkjet printing. For this application a specially designed inkjet printhead was realized, which allows temperature controlled, sterile print-

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ing. Additionally a small footprint TPP setup, adapted for process combination was built. First results demonstrate the feasibility of the combination. This setup will be used to generate a branched, high resolution vessel system, which can be used to supply an artificial skin model.

50.P21
The effect of hypoxia on the induction of angiogenic differentiation in human amniotic fluid-derived stem cells (hAFSCs)

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The survival of tissue engineered constructs post-implantation depends significantly on rapid and successful integration with the host vasculature. This study sought to create, in vitro, a functional vasculature in a collagen-GAG scaffold, designed as a biomaterial for bone regeneration prior to implantation using a unique stem cell source, hAFSCs (isolated from amniotic fluid retrieved during routine amniocentesis). We propose to achieve this by using hypoxic conditions (therefore activating Hypoxia Inducible Factor-1 pathway) to create an environment that is more appropriate for the de novo formation of vascular tissue within the seeded scaffold. In order to determine the endothelial differentiation potential of hAFSCs, the cells were seeded on Matrigel, which promotes tubule formation. hAFSCs were then cultured in hypoxic or normoxic conditions for 48 h and imaged at a range of time-points. Human Umbilical Vein Endothelial Cells (HUVECs) and human Mesenchymal Stem Cells (hMSCs) were used for comparison. Quantification of tubule forming ability revealed that cells cultured in hypoxic conditions had significantly higher total tubule length and displayed a significant delay in the degradation of tubule structures formed by all three cell types. Tubule forming ability was not as evident in hAFSCs as it was in HUVECs or hMSCs. This is most likely due to the primitive nature of hAFSCs. We conclude that hypoxia may enhance nascent vascular networks in a tissue engineering construct.

50.P22
Vascularized tissue fabrication from human cells using in vitro bioreactor

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We have developed original technology to fabricate three-dimensional (3-D) tissues by layering cell sheets. In multi-layered cell sheets, however, hypoxia, nutrient insufficiency and waste accumulation limit the final size of viable constructs. To clear this obstacle, we have developed a perfusion bioreactor system with collagen-based microchannels as a culture-bed for fabricating vascularized 3-D tissues. In this study, we stacked human cell sheets on the culture-bed and challenged to fabricate vascularized human 3-D tissues. Co-cultured cell sheets, which contain normal human dermal fibroblasts (NHDF) and GFP-expressing human umbilical vein endothelial cells (GFP-HUVEC) were harvested on a temperature-responsive culture dish. A triple-layered co-cultured cell sheets was placed on the microchannels and the construct was cultivated in the perfusion bioreactor for 5 days. Microscopic observation demonstrated that GFP-HUVEC migrated and formed capillary-like structure in the collagen gel. Tri-cultured cell sheets containing NHDF, GFP-HUVEC, and Human Aortic Smooth Muscle Cells (AoSMC) were also cultivated and the results indicated the number of migrating cells increased in the tri-cultured case rather than the co-cultured case.In conclusion, vascularized human 3-D tissues were constructed in vitro by cell sheet technology and the perfusion bioreactor system. This method will contribute to in vitro fabrication of various types of thick function.

50.P23
Enhancing implanted cell survival in *in vivo* tissue engineering

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A major issue confronting tissue engineering is the poor survival of implanted cells in vivo, due to hypoxia and loss of cell/cell attachment. Techniques that diminish the influence of these factors were investigated in rat and mouse models, where a vascular pedicle is enclosed in a plastic chamber in vivo. The pedicle sprouts capillaries within a week, but a hypoxic environment exists for 7–10 days. In minimizing hypoxic cell death two techniques have been used: (1) a delayed implantation/prevascularized rat chamber technique in which rat primary myoblasts were implanted at either 0, 4 or 7 days post pedicle/chamber construction. Capillaries increase in this model from 0 at day 0 to an extensive capillary network at day 14. DiI labeled myoblasts/mm^2 counted 7 days post implantation were positively correlated to the vascularization within the chamber ($P = 0.017$). (2) In vitro preconditioning of L6 myoblasts with DETA-NONOate (nitric oxide donor) significantly increased percent myoblast volume ($P = 0.007$) after 3 weeks in SCID mouse chambers. To counteract loss of adjacent cell attachments, the survival of liver progenitor cell (LPC) spheroids (20 000 cells/sph.) compared to the same number of single LPCs in the mouse chamber demonstrated spheroid implantation significantly increased LPC survival ($P < 0.05$) and differentiation ($P < 0.005$). Prevascularization, cell preconditioning, and multicellular-spheroid implantation offer significant potential to improve implanted cell survival.

50.P24
Dendritic VEGF blockers to control angiogenesis in tissue regeneration

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Vascular endothelial growth factor (VEGF) is the most potent angiogenic factor. However, its uncontrolled expression may lead to the development of pathological conditions including fibrocartilage development and maculopathy. This work presents the synthesis of hyperbranched poly(epsilon-lysine) dendrons exposing a VEGF blocker(seq WHLPFKC) to their uppermost molecular branches (generation 3) and their ability to inhibit HUVEC and HDMEC proliferation. Both linear and dendritic VEGF blockers were synthesised by a solid phase method using a Biotage microwave and characterised by HLPC and MS. Their in vitro anti-angiogenic effects were analysed in the presence and absence of 4 ng/ml of hVEGF165 spiking with both blockers at an equimolar concentration in the range from 0.49 to 7.84 nM after 24 and 48 h. The data show the synthesis of both peptide blockers with batches of 60 mg and purity above 70%. Blockers prevented VEGF/VEGFR2 interactions and controlled cell fate after already 24 h. The threshold of blocker action varied between the two blockers where the linear one having an effect at concentrations lower than the dendronised blocker. However, the previously established ability of the dendronised VEGF blockers to be retained in tissue engineering scaffolds

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was shown to provide a better control over a localised inhibition of angiogenesis. This work was supported by EU FP7 project Disc Regeneration, no. NMP3-LA-2008-213904.

50.P25
Improved elastomeric materials for CAD/CAM generation of vascular structures in soft tissue replacement therapies

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Computer aided design and manufacturing (CAD/CAM) find use in regenerative medicine and tissue engineering fields. Popular fields of use of include dental modeling and partial bone repairs. In comparison, application of CAD/CAM in softer tissue replacement procedures such as vascular grafting has been much slower to progress. Thus, while cardiovascular diseases continue to account for a majority of mortalities in the western world, currently used materials and manufacturing methods provide artificial vessels insufficiently stiff for narrow diameter applications. Lithographic based CAD/CAM methods overcome such limitations with reliable production of sub-millimeter scale structures; however, the majority of photoresins used for CAD/CAM cure with a restrictively high modulus for soft tissue application. To address the mechanical shortcomings of commercially utilized CAD/CAM build materials, urethane diacrylates have been implemented to give comparably softer materials. Further reduction of modulus with improved tear resistance was afforded by addition of thiol chain transfer agents. Good control of formulation and processing conditions (light intensity, exposure time, and write speed) provide narrow (1–2 mm ID) tubes with mechanical properties identical to or exceeding those of native vessels. Ester linkages within the polymer backbone are shown to assist rate of degradation for improved tissue regeneration. Cellular assays with HUVECs indicate no problems with compatibility.

50.P26
Investigation of the vascularization potential of channeled alginate scaffold

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One of the major hurdles in regeneration of a thick, functional cardiac tissue is mass transfer limitations in in vitro engineered a-vascular tissue, especially of dissolved oxygen which is limited to a diffusion distance of ~200 μm. To overcome this, we designed and fabricated by CO_2 laser a macro-porous alginate scaffold with an array of ~2.7 channels per mm^2, ~200 μm in diameter and ~400 μm wall-to-wall spacing, intended to mimic the vasculature of cardiac tissue. Cell cultivation (endothelial cells (ECs), fibroblasts) in the channeled scaffolds, in a perfusion bioreactor or under static mode, revealed that most of seeded cells concentrated near and around the channels. In particular, ECs were shown to line the channel walls. Channeled and non-channeled alginate/alginate-sulfate scaffolds supplemented with affinity-bound bFGF were subcutaneously implanted into BALB/c mice and retrieved for analysis on week 3, 4, 6 and 8 after transplantation. A mature, functional (red blood cell-containing) and denser blood vessel network was evident in the channeled scaffolds earlier and to a greater extent compared to the control non-channeled ones. In addition, the number of penetrating cells and collagen deposition were greater in the channeled scaffolds, indicating that the channels are more accessible for cell penetration than the torturous macro-porous structure. Our study suggests that the channeled alginate scaffold is appropriate for regeneration of vascularized tissues.

50.P27
Longterm cell-demanded release of TG-VEGF for the development of mature vascular networks in vivo

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Establishment of mature vessels in ischemic or engineered tissues by biomaterial engineering and growth factor delivery remains a challenge. Here, a fibrin engineering platform allowing covalent immobilization of proteins has been utilized to deliver angiogenic growth factors such as vascular endothelial growth factor (TG-VEGF). However, control over growth factor delivery from such gels has been hindered by their in vivo ephemerality. To modulate the stability of fibrin gels and the release profile of VEGF, we simultaneously incorporated TG-VEGF and a protease inhibitor, Aprotinin (TG-A). Fibrin gels that were formed with or without TG-VEGF and different amounts of TG-A were implanted in the backs of mice. The immobilization of variable amounts of TG-A resulted in a series of fibrin gels whose lifespan could be tuned from 2 to 4 weeks. Morphological and histological evaluations indicate that angiogenic response (vessel # and maturity) is greatly dependant on fibrin gel stability. As the magnitude of angiogenesis was only due to materials properties and not growth factor dosing, these findings indicate growth factor bioactivity can be influenced by delivery; by varying the material composition, growth factor release can be tailored for specific treatments. Thus, this translational work represents a significant step forward towards developing mature vascular networks for engineered tissues but might also find use in other tissue engineering and drug delivery applications.

50.P28
BioVaSc as matrix for tissue engineering of bone substitutes

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The current clinical possibilities to treat critical sized bone defects which do not heal spontaneously exhibit distict disadvantages. The tissue engineering of cell-seeded bone substitutes based on synthetic scaffolds aims at nullifying these limitations. Thus, high integrative and regenerative potential and the possibility of vascularization of the implants are prerequisites. This project seeks the formation of a vessel-rich neomatrix by combining β-tricalcium phosphates with a biomatrix made from decellularized porcine intestine (BioVaSc).β-tricalcium phosphate granules were combined with a BioVaSc that was pre-seeded with perfluoropolyether (PFPE) labeled mesenchymal stem cells (MSC) and/or microvascular endothelial cells (EC) and were cultivated in bioreactors. Growth and degree of ingrowth of the cells into the scaffolds were investigated by means of histological methods and magnetic resonance imaging (MRI).The immunohistological analysis of the cells on the BioVaSc shows a successful population of the matrix by MSCs and ECs. The cell types could be identified on the surface and

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the periphery of the β-tricalcium phosphate scaffolds by MRI. The investigations indicate a suitability of the BioVaSc for use as basis of the creation of a vessel system in bone substitutes. In further experiments, it has to be investigated how the re-populated vascular system of the BioVaSc can be effectively used in order to support vascularization in bone substitutes.

50.P29
Matrix metalloproteinase (MMP) sensitive PEG diacrylate (PEGDA) hydrogels with spatial variations in matrix properties direct vascular cell invasion

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The spatial presentation of immobilized extracellular matrix (ECM) molecules, soluble growth factors and matrix mechanical properties guide numerous physiological processes. The goal of this work was to explore the role of these gradients in directing 3D cell behavior and vascularization in tissue engineered scaffolds. Perfusion based frontal polymerization (PBFP) was used to impart PEGDA hydrogels with simultaneous gradients in elastic modulus and immobilized YRGDS adhesion ligands. Bulk polymerized hydrogels served as controls with gels rendered susceptible to cell mediated proteolysis by the inclusion of MMP sensitive domains. Gradient hydrogels exhibited an 81% decrease in elastic modulus and a 73% decrease in immobilized YRGDS, while properties of bulk control gels remained constant. Aggregates of smooth muscle and endothelial cells seeded within bulk hydrogels invaded uniformly in all directions while those seeded in gradient hydrogels invaded bidirectionally with sprout alignment observed in the directions parallel to the gradient. In PBFP hydrogels, aggregate sprout length was found to be twice as long in the direction of the gradient as compared to the perpendicular direction after three weeks in culture. This directionality was more prominent in gradient regions of increased stiffness, presentation of MMP sensitive domains and YRGDS concentration. These studies provide significant insight on the role of gradients in directing 3D cell behavior and vascularization.

50.P30
Collagen: chitosan hydrogels for stimulation of angiogenesis in a type I diabetic mouse model: potential use as a pre-vascularized ectopic site for islet transplantation

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Islet transplantation to treat type 1 diabetes (T1D) has shown varied long term success, due in part to poor blood supply, suggesting that pre-vascularization of the transplant site is needed. In the current study, we compared preformed collagen (1C) and collagen:chitosan hydrogels (2C), +/−circulating progenitor cells (CPCs), as materials to promote angiogenesis in a T1D (streptozotocin-induced) nude mouse model. CPCs were isolated from human peripheral blood mononuclear cells cultured on fibronectin for 4 days. 1C or 2C (10:1 collagen:chitosan) hydrogels +/− CPCs were crosslinked using EDC/NHS. After gelation at 37°C for 18 h, live/dead staining showed greater CPC viability in the 2C gels compared to 1C gels (79% vs. 69%, $P < 0.05$). The 2C gels were mechanically stronger than the 1C gels (0.6 vs. 0.4 kPa at 30% strain, $P < 0.05$), had more crosslinks (9.2 vs. 7.4 per μm^2, $P < 0.05$), and were degraded more slowly by 100 U of collagenase (3 h vs. 2 h). Consequently, 2C gels could be retrieved after 6 weeks of subcutaneous implantation, whereas not all 1C-CPCs gels were recovered. The 2C gels showed increased pro-angiogenic cytokines at 1 and 2 week timepoints. The 6 week explants trended toward more vWF+ cells for the 2C vs 1C gels; which correlates with the increased expression of VCAM-1 in 2C explants compared to 1C-CPCs gels at 6 weeks. The mechanical, degradation and cytokine data all suggest that the 2C gel is a better candidate for use as a pre-vascularized ectopic islet transplant site.

50.P31
Large-area multilayer microfabricated alginate methacrylate scaffold for tissue engineering

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The success of therapeutic vascularization and tissue engineering will rely on our ability to create vascular networks using clinically friendly biomaterials. The ability to influence the direction and structure in the formation of a vascular system is crucial in engineering tissue. Traditional approaches for three-dimensional scaffold fabrication are not suitable for generating large area and complex structures due to the lack of control of the architecture, microvasculature, and cell-cell interactions. The proposed photolithography platform allows us to design uniform large area, multilayers and complex tissue scaffolds with arbitrary architecture that can mimic the microarchitecture of tissues and to augment regeneration therapies. The photolithography system was developed and optimized to fabricate complex three-dimensional architecture using gelatin methacrylate biomaterial by optimizing a number of process parameters to get good nutrient diffusion and endothelial cell viability. Variation of the methacrylation degrees enabled tailoring mechanical and degradation rate properties of the scaffolds. A dynamic cell seeding method was designed to optimize the coverage of the scaffold. Scaffolds were incubated in a cell suspension and submitted to constant agitation, resulting in enhanced cell adhesion compared to conventional cell seeding.

50.P32
Bioactive acellular substance induces angiogenesis and adipogenesis in vitro and permanent regeneration of soft tissue in vivo

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Soft tissue defects and chronic wounds are a medical and economic challenge to the health care system. Soft tissue engineering seeks to fabricate replacements for soft tissue defects like burns, scars, chronic wounds, surgical defects, congenital malformations or for cosmetic use. However, the lack of vascularization in transplantable constructs is the major obstacle in tissue engineering. Current soft tissue replacements also cause severe complications, donor-site morbidity, implant volume loss, or allow only autologous transfer of cells. Therefore, no curative treatment for sustained soft tissue restoration exists. We have developed a novel substance, an acellular and xeno-free adipose tissue extract that contains a wide number of angiogenic and adipogenic factors. In vitro, adipose tissue extract is able to induce adipose stem cell differentiation and endothelial cell tubule formation. In in vivo implantation tests in rodents, when combined with hyaluronan hydrogel, adipose tissue extract is able to induce angiogenesis and adipogenesis. The extensive capillary induction appears in a week and the adipose tissue accumulation at 12 weeks. The inductive effect is shown to remain for 9 months. The adipose tissue extract is biocompatible and

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induces permanent soft tissue restoration. This substance is a potential replacement for the current soft tissue products to overcome the challenge of vascularization, and for permanent soft tissue restoration and improved wound healing.

50.P33
Vascular morphogenesis in ASC cultures is mediated by heterotypic cell-cell interactions

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Adipose-derived stem cells (ASCs) hold great promise for regenerative medicine. Recently, it has been shown that ASCs can spontaneously form endothelial vessels in 3D hydrogels. We investigated the cellular mechanism by which ASCs undergo vascular morphogenesis in 2D and 3D. ASCs were seeded at 20 000 cells/cm^2 and cultured in the presence or absence of AG1295, an inhibitor to platelet-derived growth factor (PDGF) receptor activity. ASCs self-assembled into complex 3D vascular structures. This phenomenon was only apparent when ASCs were seeded at high density and occurred through orchestrated interactions among three distinct sub-populations: CD31 positive cells (CD31$^+$), smooth muscle actin positive cells (SMA$^+$), and cells unstained for both of these markers (CD31-/SMA. These vessel-like structures arose from CD31$^+$ cells through their interactions with CD31$^?$/SMA$^?$ cells. SMA$^+$ cells proliferated and migrated toward the vessel structures. By 2 weeks, the lumen-containing CD31$^+$ vessels grew extensively and were encased within a dense multi-layered cluster of SMA$^+$ and CD31$^?$/SMA$^?$ cells. Recruitment of SMA$^+$ cells was largely due to PDGF signaling, as inhibition of PDGF receptors substantially reduced SMA$^+$ cell growth and vessel coverage. Studies with multicellular spheroids have demonstrated that ASCs are capable of assembling lumen-containing vascular structures within a 3D environment via similar mechanisms and has elucidated a novel role for PDGF in this process.

50.P34
Ephrin-mimicking matrices for the study of Eph signaling and angiogenesis

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Erythropoietin-producing human hepatocellular carcinoma (Eph) receptors and their ephrin ligands are thought to be crucial for developmental processes such as cell migration, tissue boundary formation, axon guidance and arterial or venous differentiation. However, their use in vascular engineering application has been limited due to the lack of knowledge on the exact role of ephrin signaling during morphogenesis. The aim of this study is to design ephrin-mimicking materials that can be employed to study and later specifically induce arterial and venous structures. Therefore, we developed three transglutaminase (TG)-tagged ephrin mimetic peptides (TG-EMPs) that can be incorporated into PEG gels by Factor XIII polymerization. Additionally, as the clustering of native ephrin ligands was found to be fundamental for their activity, multimerized TG-EMPs were formed by a PEG-based strategy. The activity of the mono- and multimeric TG-EMPs was determined by studying their ability to stimulate or inhibit receptor tyrosine phosphorylation of human endothelial and prostate cancer cells. Next, the effects of different TG-EMPs on the migration of these cells were determined. Based on the hypothesis that Eph-ephrin interactions might influence VEGF signaling, and consequently vascular morphogenesis, we are currently studying the influence of ephrins and TG-EMPs mediated signals on VEGF induced angiogenesis (vessel number, morphology and function) using the chicken CAM assay.

50.P35
Generation of a cell-based controlled delivery device for induction of angiogenesis

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Uncontrolled expression of Vascular Endothelial Growth Factor (VEGF) can induce aberrant angiogenesis. We developed a method to ensure sustained but controlled VEGF release by FACS-based purification of transduced adipose tissue-derived mesenchymal stromal cells (ASC), which could already induce effective vascularization in the myocardium. However, efficacy is limited by poor cell survival after injection. In this study we aim to generate an engineered ASC-based patch both to increase cell survival and as a controlled delivery device capable to promote angiogenesis in the surrounding ischemic tissue. Transduced or naïve human ASC were cultured for 5 days onto collagen scaffolds in a perfusion-based bioreactor. The in vivo intrinsic angiogenic potential of patches generated by these ASC, or cell-free scaffold was investigated subcutaneously in nude mice. A critical size empty scaffold was stitched underneath the patch to test the ability of causing vessel ingrowth into an avascular tissue. Seeding efficiency was 63 ± 5% and ASC were uniformly distributed. After 5 days in vivo, vessel ingrowth was 10-fold higher in the patches generated by VEGF-ASC compared to controls (CD31$^+$ area = 2% vs 0.2% and 0.2% for naive ASC and cell-free scaffold, respectively), but also in the empty scaffold underneath (2% vs 0.22% and 0.38%, respectively). We are currently investigating the angiogenic efficacy in relation to the VEGF dose and the proportion of transduced cells in mixed cultures.

50.P36
Engineering cell-instructive microenvironments to study angiogenesis

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Although angiogenesis is centrally involved in tissue malfunction and disease, morphogenetic processes involved in vessel formation are only beginning to be understood. To study and engineer angiogenic processes under well defined conditions, reductionistic in vitro models that recapitulate the natural milieu while allowing for systematic evaluation from single to combinatorial biological events, are needed. We exploit natural and artificial extracellular matrices, patterning techniques and/or additive layering to engineer angiogenic microenvironments that recapitulate main features of the structured natural milieu; extracellular matrix components, neighbouring cells, soluble as well as immobilized factors. Preliminary results on human umbilical vascular endothelial cells (HUVECs) in poly (ethylene glycol) (PEG) gels show the importance of the individual components involved in the efficient formation of vessel-like structures in artificial environments. Based on this know-how, we have engineered instructive environments to generate organized vascularized bone tissue-like constructs. We now evaluate the angiogenic potential of late outgrowth endothelial cells (OECs) in comparison to microvascular endothelial cells (MVEC) in 3D Fibrin and PEG-based gels in presence of bioligands and morphogens. Functional assays and expression profile of relevant angiogenic genes are being analysized.

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50.P37
Differential behaviour of hypoxia-inducible genes during neuronal aging

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Mammalian cells respond to hypoxia increasing the expression of several hypoxia-inducible genes. The master regulator of this cellular adaptation is hypoxia-inducible factor 1 (HIF-1). It has been shown that hypoxia influences several aspects of angiogenesis; however, there are few studies that examine angiogenesis-related genes, such as vascular endothelial growth factor (VEGF), transforming growth factor-β1 (TGF-β1), and adrenomedullin, in the hypoxic striatum during aging, which constitutes our goal. Adult (4 months old) and aged (24 months old) Wistar rats were submitted to hypoxia (20 min) and sacrificed after 0 h, 24 h, and 5 days of reoxygenation. Expression of VEGF, TGF-β1, and adrenomedullin was determined by RT-PCR (TaqMan assays). The number of blood vessels was evaluated after lectin immunohistochemistry using fractal dimension.HIF-1α mRNA levels were higher after the hypoxic stimulus in old rats vs. adult ones. VEGF, TGF-β1, and adrenomedullin mRNA expression increased at 0 h of reoxygenation in adult striatum, but decreased in aged rats. Finally, quantification of striatal blood vessels remained unaltered in both age groups.To conclude, although striatal HIF-1 responds stronger to hypoxia during aging, its target genes (VEGF, TGF-β1 and adrenomedullin) decrease immediately after the hypoxic insult. Conversely, HIF-1 response to hypoxia was weaker in adult striatum; however, angiogenesis-related genes significantly boosted at 0 h of reoxygenation.

50.P38
Bioactivity of the recombinant human antimicrobial peptide LL37: a key role in wound healing through vascularization

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Antimicrobial peptides (AMPs) are usually defined as small, cationic, amphipathic peptides making part of the innate immune system. Human cathelicidin AMP LL37 is overexpressed in different cells, tissues and body fluids and, in addition to its antimicrobial activity, induces chemotaxis of immune system' cells, arteriogenesis, angiogenesis and promotes wound healing. Our group developed a new, low-cost method for the expression and purification of LL37, by fusing it with the Carbohydrate Binding Module from family 3 (CBM3) from CipA from Clostridium thermocellum. The CBM3-LL37 protein is overexpressed in E. coli and retained both the antibacterial and immunophysiologic properties. Additionally, it induced proliferation, migration and formation of tubule-like structures by endothelial cells. Topical application of rLL37 also increased vascularization and re-epithelialization, thus accelerating the wound healing process, in wounds of dexamethasone-treated mice. This indicates a key role for LL37 in wound regeneration, mainly mediated by its proangiogenic activity, which is currently being studied with further detail in diabetic mice, a chronic wound model. Moreover, we are working on the development of natural polymer-based delivery systems for the topical sustained release of LL37 that can be applied to wound sites, thus promoting faster and more efficient wound regeneration. Acknowledgements: JPS and RR are supported by FCT grants SFRH/BPD/64958/2009 and SFRH/BD/27404/2006.

50.P39
Inhibitory effect of porcine cartilage-derived water-soluble biomaterial on angiogenesis

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Introduction: Articular cartilage is known as an avascular tissue and to have anti-angiogenic factors such as chondromodulin-?, endostatin and sulfated glycosaminoglycan side chains. In our previous studies, an extracellular matrix (ECM) biomaterial derived from porcine chondrocytes showed activity of angiogenesis inhibition. In this study, we produced a water-soluble form of the porcine cartilage-derived ECM biomaterial (PCP-ws) and evaluated its anti-angiogenic property.
Materials and Methods: The slices of cartilage tissues were lyophilized and comminuted into a particulate form, which was then decellularized and solubilized by pepsin/HCl. The toxicity of the PCP-ws was evaluated on young rabbit fibroblasts, chondrocytes and human umbilical vein endothelial cells (HUVEC) by MTT assay. Tube formation assay using HUVECs was performed after treating PCP-ws at 1, 2.5 and 5 mg/ml in concentration.
Results & Conclusion: The PCP-ws was successfully made from the decellulized PCP powder. In MTT assay, there was no significant difference in cell viability between the PCP-ws treated and untreated groups. In the tube formation assay, the formation of tube network of HUVECs was significantly inhibited by PCP-ws in a dose-dependent manner. Remarkably, the experimental group treated with 5 mg/ml of PCP-ws showed almost no tube-like structure.
Conclusion: These results suggest that PCP-ws have an inhibitory effect on angiogenesis in vitro and could be a useful biomaterial for many applications.

50.P40
Apolipoprotein E modifies angiogenic factors production and microRNAs expression in an isoform-dependent way

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Apolipoprotein E (ApoE), existing in human population in three major isoforms - ApoE2, ApoE3 and ApoE4, plays an important role in cholesterol transport and metabolism. Although the association between ApoE isoforms and neurodegenerative and cardiovascular diseases is well established, little is known about the effect of ApoE polymorphism on the production of angiogenic mediators, including microRNAs.Murine macrophages (RAW264.7) stably transfected with one of the human forms of either ApoE3 or ApoE4, differ in the expression of prosurvival, proangiogenic and anti-inflammatory factors – heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF). The highest expression of VEGF observed in ApoE4-expressing macrophages was HO-1 dependent as the silencing of HO-1 with siRNA decreased VEGF mRNA and protein level. Macrophages with ApoE4 have also higher expression of CXCR4 and MMP-9 as well as proangiogenic miR-146a and proinflammatory miR-155 but decreased level of VCAM, MCP-1 and miR-24 in comparison to ApoE3-expressing macrophages. Additionally, conditioned media from ApoE4-expressing macrophages

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more potently stimulated angiogenesis in vitro. ApoE4 isoform changes macrophages phenotype to more proangiogenic and proinflammatory, what can have vital implications to in vivo angiogenesis-dependent conditions like wound healing or atherosclerotic plaque formation.Supported by the Foundation for Polish Science, Parent-Bridge Programme co-financed by the Europ. Union.

50.P41
A new model of angiogenesis to test the angiogenic potential of matrix delivered factors

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Angiogenesis is pivotal in tissue engineering and regeneration. We developed a new model of angiogenesis to standardize "in vivo" testing of angiogenic substances liberated from (bio)matrices. Utilizing a silicon tube to isolate a vascular bundle, a protected niche is created and newly formed vascular structures can then be precisely attributed to the specific local conditions. In this study, standardized silicon tubes were used to sheath rodent epigastric vascular bundles. As proof of concept VEGF165 was administered in fibrin which served as matrix for spatial stability and prolonged release. Fibrin alone was tested as the vehicle group. An empty silicon tube served as a control group. The angiogenic response was evaluated by immunohistological means (von Willebrand Factor (vWF) and smooth muscle actin (sma)). This in vivo model of angiogenesis is technically easy and timely to perform. Results with VEGF165 at 200 ng/mL showed an increase in vWF positive stained vessels compared to the empty control group. In contrast, a suppression of vascular growth was noted when compared to the fibrin sealant group which seems attributable to high VEGF dosing. The vehicle (fibrin) group showed a potent angiogenic effect capable in inducing persistent vessel formation. The developed model of angiogenesis seems to be a reliable and reproducible approach to further evaluate the angiogenic potential of scaffold delivered growth factors. Study supported by EU grant Angioscaff.

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51. Cocultures as Tools for Angiogenesis

51.01
Keynote: Vessel network assembly within 3D tissues and its integration with functional vasculature

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Vascularization continues to represent a major challenge in the successful implementation of regenerative strategies. Current approaches for inducing vascularization in vivo includes pre-forming a vasculature ex vivo, and the use of a variety of strategies to stimulate vascularization in situ. Vessel network assembly within 3D tissues can be induced in-vitro by means of co- culturing of endothelial cells (EC), mesenchymal/ fibroblast cells and cells specific to the tissue of interest. This approach supports formation of endothelial vessels and promotes EC and tissue-specific cell interactions. In addition, it has been shown that in vitro prevascularization of engineered tissues can promote its survival and vascularization upon implantation and that implanted vascular networks of in vitro engineered constructs, can anastomose with host vasculature and form functional blood vessels in vivo. Very exciting and recent research questions involve the degree of vascularization in vitro required for best vascularization of tissue constructs in vivo as well as understanding the mechanisms of host-implant vessel integration and anastomosis. In addition, new co culture approaches for inducing predefined vessel structures in vitro will be discussed.

51.02
Construction of functional ex-vivo liver tissues with pericyte-incorporated microvessels using microporous membranes

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Reconstruction of functional liver tissues in vitro remains a major challenge in liver tissue engineering. In the liver, hepatic stellate cells (HSCs) locate the outer surface of microvessels to form a functional unit, termed the liver sinusoid, with endothelial cells (ECs) and hepatocytes. We previously demonstrated EC capillary formation in tri-culture where a polyethylene terephthalate microporous membrane was intercalated between ECs and hepatic organoids composed of small hepatocytes (SHs), i.e., hepatic progenitor cells, and HSCs. However, the high thickness and low porosity of the membranes limited heterotypic cell-cell interactions, which are essential to form HSC-EC hybrid structures. Here, we demonstrated the effective use of the thin and high porous poly (D,L-lactide-co-glycolide) (PLGA) microporous membranes in SH-HSC-EC tri-culture to reconstruct the HSC-incorporated sinusoidal structures in vitro. First, the formation of EC capillary-like structures was induced on Matrigel-coated PLGA microporous membranes. Next, the membranes were stacked on hepatic organoids composed of SHs and HSCs. HSCs selectively migrated to the EC capillary-like structures and were located along the outer surface of the structures with their long cytoplasmic processes. In these tissues, SHs acquired high levels of differentiated functions, compared to those without ECs. This model will provide a basis for the construction of functional, thick, vascularized liver tissues in vitro.

51.03
Adipose-derived stem cells induce vascular tube formation of outgrowth endothelial cells in a fibrin matrix

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Vascularization of engineered tissues is one of the current challenges in tissue engineering. Several strategies aim to generate a prevascularized scaffold which can be implanted at sites of injury or trauma. Endothelial cells derived from peripheral blood (outgrowth endothelial cells, OEC – acc. Fuchs et al. 2007) display promising features for vascular tissue engineering, including their autologous nature, capacity for proliferation and their ability to form mature vessels. In this study we investigated the ability of OEC to form vascular structures in co-culture with adipose-derived stem cells (ASC) in a fibrin matrix. Using microcarrier beads coated with OEC, we showed ingrowth of endothelial cells in the fibrin scaffold. Furthermore, co-cultures with ASC induced vessel formation, as evidenced by immunostaining for CD31 and alpha-smooth muscle actin. The degradation of fibrin is at least in part mediated by expression of matrix metalloproteinase-14. Moreover, we show OEC/ASC-induced vessel-like structure formation even in the absence of microcarrier beads. Our data add new insights into co-culture-induced vessel formation of outgrowth endothelial cells within a fibrin matrix representing an autologous system, which can be easily translated to clinical settings.

51.04
Delaying the addition of human mesenchymal stem cells to pre-formed endothelial cell networks results in increased vascularisation within a collagen-GAG scaffold in vivo

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To overcome the problem of graft failure due to avascular necrosis of tissue engineered (TE) constructs, one approach is to pre-vascularise a TE construct in vitro prior to implantation. This study has developed a pre-vascularised collagen-GAG (CG) scaffold using a delayed addition of human mesenchymal stem cells (MSCs) to pre-formed human endothelial cell (EC) networks. Functional assessment of these pre-engineered vessels was carried out in an immunocompromised rat subcutaneous model. Animals were sacrificed at 4 weeks post-implantation and vascular perfusion using a lead chromate-based, radiopaque contrast agent was conducted. Micro-computed tomographical angiography to observe construct vascularisation indicated successful perfusion with no leakage of the contrast agent evident. Quantitative analysis demonstrated a significant increase in vascularisation in the co-culture group compared to cell-free and EC-only groups ($P < 0.03$). Positive staining for human CD31 was observed in the cell-seeded groups; thus demonstrating successful integration of the in vitro engi-

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neered vessels with the host vasculature. This study has shown that pre-engineered vessels, formed by an EC/MSC co-culture within a CG scaffold becomes functional and results in significantly increased vascularisation in vivo. This mode of vascularisation, with the short-term culture period and efficient host integration may be used not only in the repair of bone but in the treatment of other ischemic tissues.

51.05
The role of endogenous myeloid cells in cocultures of outgrowth endothelial cells and osteogenic cells in the formation of vascular structures

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During wound healing and inflammation myeloid cells play a key role in the regulation of angiogenesis. In this context previous animal studies have shown that myeloid cells from the host invade into implants and contribute to their neovascularization (Melero-Martin 2010). In this study we provide evidence for a role for endogenous myeloid cells in the formation of vascular structures in cocultures of peripheral blood-derived outgrowth endothelial cells (OEC) and osteogenic cells. OEC from human peripheral blood and primary osteoblasts (pOB) or mesenchymal stem cells (MSC) were cocultured as described earlier (Fuchs et al. 2007, Kolbe et al. 2011). Angiogenesis was evaluated by CLSM. In addition, the expression of CD11b was assessed by immunofluorescence microscopy, flow cytometry and real time PCR. CD11b positive cells in co-cultures were closely associated with vascular structures. In addition, these myeloid cells stained for CD31, suggesting an active contribution to vascular structures. The potential origin of the myeloid cells was characterized by several methods and indicated CD11b-positive subpopulations in OEC and osteogenic cells. During the co-culture the CD11b-positive cell population increased in parallel to the increase in angiogenic structures. These data suggest a role for endogenous myeloid cells in the formation of vascular structures and indicate that the co-cultures might favour a pro-myeloid niche.

51.P01
Expansion of bone marrow angiogenic progenitor cells by high density culture

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Adult stem cells are distributed in variety of tissues and the stemness of these cells is maintained by the stem cell 'niche'. Growing evidence suggests that the cell-cell contact plays an important role in this niche. Most of the cell culture methods break the cell-cell contact by seeding cells at a relatively low density. We hypothesized that seeding cells at a high density to maintain the cell-cell contact in culture might be beneficial for stem cell expansion. To test this hypothesis, bone marrow cells from wistar rats were seeded at 2×10^5 cells/cm^2 in a 10 cm diameter culture dish. To avoid the quick exhausting of nutrients, total of 9×10^5 cells were seeded separately into six high density spots in the culture dish. Meanwhile, same amount of cells were seeded evenly in the culture dish at a regular density of 1.6×10^4 cells/cm^2 as a control. After 15 days of culture, flow cytometric analysis demonstrated that cells derived from high density culture expressed higher levels of angiogenic markers than cells from regular density culture. In addition, these cells showed strong capillary tube formation ability and a therapeutic potential in a hindlimb ischemia model, indicating that bone marrow angiogenic progenitor cell could be expanded in a high density culture without additional growth factors. The high density culture system not only provides a novel method for bone marrow angiogenic progenitor cell expansion, but also a new in vitro model for studying stem cell niche.

51.P02
Characterization of outgrowth endothelial cells derived from peripheral blood

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Vascularization of tissue constructs is one of the current challenges in tissue engineering. Peripheral blood is a readily available autologous source for endothelial progenitor cells, which can be differentiated into outgrowth endothelial cells (acc. Fuchs et al. 2007). To employ these cells in prevascularization experiments, we characterized OEC by cell biological and molecular biological methods and by functional assays. Flow cytometry showed that the cells are positive for endothelial markers CD31, Vascular Endothelial Growth Factor Receptor 2 (VEGFR-2), VE-Cadherin and CD146, whereas negative for CD14, CD45, CD36 and CD133. In addition, low passage cells are slightly positive for Tie-2 and a subpopulation also for CD34. Quantitative PCR revealed expression of CD31, VEGFR-2, VE-Cadherin, platelet derived growth factor β (PDGFβ) and von Willebrand factor (vWF). Moreover, by immunoblotting we show that OEC are positive for CD31 and vWF, reinforcing the previous findings. Furthermore, the cells took up acetylated low lensity lipoprotein and formed tube-like structures on Matrigel as well as on fibrin. Finally, upon stimulation with tumor necrosis factor α (TNFα) OEC upregulated endothelial-leukocyte adhesion molecule 1 (ELAM-1) while thrombomodulin expression decreased. No significant change was found for tissue factor, CD41 and CD61. Altogether these results prove the endothelial character of the OEC and therefore establish these cells as candidates for the use in tissue engineering purposes.

51.P03
Vasculogenesis of human dental pulp stromal cells in vitro and in vivo

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The ability to enhance dental pulp cells vasculogenesis is crucial to maintain the tooth vital. This study investigated the ability of using human dental pulp stromal cells (HDPSCs) to form new vasculature in vitro & in vivo. HDPSCs were isolated from human permanent teeth and cultured as monolayers or on 3D Matrigel scaffold in endothelial cell growth medium-2 (EGM-2) with/without 50 ng/ml of vascular endothelial growth factor (VEGF). After 24, 48 & 72 h, endothelial marker (CD31, CD34, vWF & VEGFR-2) expression was determined by qRT-PCR and immunocytochemistry. For in vivo study, the canal space of human dentine slides (3 mm) were filled with HDPSCs/Matrigel (1×10^{-6} cells/ml), which were implanted subcutaneously in nude mice. In monolayer, EGM-2 with VEGF up regulated HDPSCs expression of CD31 & VEGFR-2 compared to control while expression of CD34 and vWF remained unaffected. However on Matrigel, all four genes were up regulated, which were confirmed by Immunocytochemistry. HDPSC formed tube-like structures on 3D Matrigel in VEGF treated group only. After 3 weeks in vivo, H&E staining showed HDPSCs pre-cultured under vasculogenic conditions enhanced blood vessels formation. Immonohistochemical staining confirmed new blood vessel formation in the root canal and the cells were positive to CD34, which

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was identical to normal dental pulp tissue. In conclusion, HDPSCs have the potential for vasculogenesis & revascularization of dental pulp tissue after tooth implantation.

51.P04
Three specific antigens to isolate EPC from human liposuction material

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Human fat tissue is an attractive source to gain stem cells in high quantity because of its easy availability coupled with low morbidity. In cooperation with Miltenyi Biotec we tried to enrich the very rare endothelial progenitor cells (EPC) of human liposuction material by using specific antibodies coupled to magnetic beads. The CD133 antigen is a marker for early EPC, which was used for their initial isolation. CD34 a well known stem cell marker is lost in adipose stem cells (ASC) after one week in culture. Therefore this marker was taken to purify the CD133 enriched fraction. CD31 as a marker of late EPC was taken for their final enrichment. The EPC fraction achieved showed typical cobblestone morphology, FACS analysis revealed the expression of the endothelial cell markers CD31, CD34, VEGFR2 and VE-Cadherin. No expression was detected anymore for CD133, as well as for CD14 and VEGFR3 demonstrating the absence of leucocytes and lymphatic endothelial cells. The endothelial origin of the gained EPC was confirmed by tube forming structures in a matrigel assay and the uptake of acetylated LDL. A vessel forming experiment is performed to show functional integrity of isolated EPC. With support of FP7-Angioscaff.

51.P05
Labeling progenitor derived endothelial cells for studying cell-scaffold interactions

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Vascularization being a crucial process, the inclusion of PDECs (Progenitor Derived Endothelial Cells) to constructs is relevant in tissue engineering. To track cell presence in the matrix, fluorescent labeling by infection with lentiviral transduction was performed and its influence on PDEC viability, proliferation and differentiation was evaluated in vitro. The vector contained the tdTomato protein gene under the control of the Phosphoglycerate kinase promoter. For viral transduction, PDECs (from human cord blood) were mixed with 6.106 viral particles and cultured. The percentage of labeled cells was of 93–98%. Expression of tdTomato was observed under fluorescent microscope. There was no difference between PDECs labeled or not for cellular uptake of UEA-1 lectin and stainings for CD31, VE-cadherin and vWF (FACS). The surface marker CD45 was found at less than 0.5% for both cell types. PDEC proliferation (3 donors), with cells labeled or not was checked (10 000 seeded cells/cm^2) at days 1, 2, 3, 6 and 9 by neutral red and MTT, showing that it is not affected by labeling. Also, PDEC functionality was not modified in the capacity of labeled cells to form cord-like structures when cocultured with human osteoprogenitors.We conclude that PDECs tdTomato labeling does not alter the expression of specific endothelial markers, proliferation and capacity. We provide an improved qualification of PDECs for monitoring cell-scaffold interaction in tissue engineering.

51.P06
The role of blood-derived angiogenic cells (BDAC) with pericytic characteristics in early angiogenesis

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Pericytes are perivascular cells that are involved in blood vessel formation, maturation and stabilization. Although in the current view, pericytes are multipotent MSC-like cells residing in various tissues and responsible for the maturation and stabilization of blood vessels, pericytes involved in angiogenesis were reported to express hematopoietic markers. Here we propose the generation of blood-derived angiogenic cells (BDAC) with characteristics of pericytes. Comparison between BDAC and MSC-like pericytes derived from placenta (Pl-Prc) showed that both cell populations co-localized with tubular network formed by endothelial cells (EC) on matrigel. Both cell populations incorporated into EC spheroids, however when seeded into a collagen-I gel only BDAC migrated along the sprouts. Interestingly endothelial sprouting was enhanced in collagen I gels by BDAC, whereas it was inhibited by Pl-Prc. BDAC expressed many pro-angiogenic factors including VEGF, IL-8, MCP-1 and crucial proteases for endothelial sprouting MMP-9 and uPA. Interestingly inhibition if MMP-9 could significantly decrease sprouting. We propose the existence of at least two pericyte populations. Pericytes of hematopoietic origin are involved in early stages of angiogenesis like endothelial sprouting and resembled by BDAC, whereas MSC-like pericytes are rather involved in stabilization of blood vessels. BDAC represent promising candidates for therapeutic angiogenesis.

51.P07
Endothelium differentiation and regeneration from human adipose-derived stem cell by shear stress

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In the vascular system, endothelial cells (ECs) provide an interface between blood streams and vessel wall. The shear stress generating by blood flow might be a role to regulate endothelium differentiation and vascular development. Human adipose-derived stem cells (ASCs), isolated from adipose tissue, would be applied to regenerative medicine or cell-based therapy. Thus, we want to investigate that endothelium differentiation of ASCs combines both biochemical and mechanical stimulation. Human adipose-derived stem cells (hASCs) were isolated and cultured in DMEM, containing 10% FBS, and penicillin/streptomycin. We combined the endothelial growth factors and shear stress system to stimuli hASCs differentiation. RNA and protein level of EC markers were measured by RT-PCR and western blotting. DiI-LDL uptake and Matrigel assay were performed for EC functional analysis. Up-regulation of EC marker and function reveal the synergistic effect of endothelium differentiation on hASCs under biochemaical and mechanical stimulation.

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51.P08
Primary rat endothelial progenitor cells derived from bone marrow with high vascularisation potential for tissue engineering

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Introduction: Bone marrow-derived progenitor cells may contribute to formation of blood vessels. Endothelial progenitor cells (EPC) have demonstrated high potential to enhance therapeutic vascularisation. The aim of this study was to establish highly proangiogenic endothelial progenitor cells derived from rat bone marrow for vascularisation of bioartificial tissues and organs.
Material and methods: The mononuclear cell fraction was isolated from tibia and femur bones and subcultured. Molecular phenotype was characterised by FACS and PCR analysis. Functional capacity was investigated by tube formation on Matrigel and Fibrin. DiI-Ac-LDL uptake was used to confirm endothelial lineage up to 3 weeks after isolation.
Results: Progenitor cells expressed CD 146, CD 31 and VEGFR-2, markers of endothelial phenotype, in a distinct fashion. At 1 and 2 weeks after isolation, cells demonstrated tube formation on Matrigel and in a Fibrin matrix as well as DiI-Ac-LDL uptake, while control cells did not. Moreover, we were able to select EPC by DiI-Ac-LDL uptake followed by staining with FITC-UEA up to 3 weeks.
Conclusion: Primary EPC can be isolated with potent proangiogenic potential, as demonstrated by functional assays and expression of endothelial-specific surface markers. Combination of two independent fluorescent staining systems enables selection of EPC and long term tracking in vivo after transplantation, providing insights into their role in blood vessel formation in vivo.

51.P09
Pre-vascularising liver progenitor cell spheroids in vitro to increase survival in vivo

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Introduction: Cell death upon implantation in vivo is a major obstacle in tissue engineering and cell therapy. Implanting cells as 3D multi-cellular spheroids rather than single cells can improve cell survival. Furthermore, combining vascular cells within spheroids may improve in vivo cell survival by maximizing vascularization through inosculation. This project aims to combine liver progenitor cells (LPCs) with vascular cells to form capillaries within 3D multicellular spheroids to enhance survival post implantation.
Methods: Murine LPCs, liver sinusoidal endothelial cells (LSECs) and adipose derived mesenchymal stem cells (ASCs) were investigated for their spheroid forming ability using 20 000 cells/well in 20% methylcellulose. Proliferation and apoptosis were assessed by immunohistochemistry over 10 days. Various cell types were co-cultured to assess their ability to form a single spheroid and if capillaries could be formed within the spheroid.
Results: Each cell type formed a single spheroid/well with increasing apoptosis and decreasing proliferation over 10 days. In co-cultures, capillaries (CD 31 +ve) were seen in spheroids containing LPC/LSEC and LPC/LSEC/ASC.
Conclusion: Co-culturing LPCs with vascular cells shows capillary formation. Optimization experiments will increase capillary networks within LPC spheroids. In vivo implantation experiments will determine the success of inosculation in spheroid survival and LPC differentiation for liver replacement therapy.

51.P10
Effects on the regulation of VEGF by macrophages in triple-culture with outgrowth endothelial cells and osteoblasts

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Macrophages (MΦ) or monocytes (MNC) are known to play a significant role in the induction of new blood vessels. The co-culture system of endothelial cells and varying osteoblast cell-types represents a model to evaluate the different modulatory activity that MΦ may have on the other cells. The aim of this study was to compare the effect that MΦ/MNC (cell line THP-1) have on the release of VEGF in co-culture systems consisting of 1) human outgrowth endothelial cells (OEC) and human primary osteoblasts (pOB) and 2) OEC and the human osteosarcoma cell line MG-63. MG-63 are used as an osteoblast cell model in in vitro studies and has similar effects on endothelial cells as pOB in terms of promoting angiogenesis. Triple-cultures of pOB and OEC with MNC or MΦ (THP-1) resulted in an increase of the expression of VEGF. POB from at least three donors exhibited similar results. Triple-cultures of OEC and MG-63 with MNC or MΦ did not show any up regulation or increased release of VEGF. Triple-cultures with pOB, OEC and MΦ/MNC show a pattern of increasing release of VEGF mimicking what is observed in vivo under physiological conditions. The effect was not observed by using MG-63. These results show that in vitro models with primary cells more closely resemble the in vivo situation to stimulate MΦ/MNC to enhance the pro-angiogenic activity in the microenvironment. Further studies with other osteosarcoma cell lines are underway to determine if these cells show similar behaviour to MG-63.

51.P11
Cell interactions between human endothelial cells and mesenchymal stem cells in a three dimensional macroporous polysaccharide-based scaffold

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In bone tissue engineering, prevascularization of a scaffold by association with endothelial cells improves angiogenesis and bone formation. Crosstalk between endothelial cells and osteoprogenitors has been studied in 2 dimensions (2D) but remains unknown in 3D. Here, we used a macroporous matrix of polysaccharides that promote cell interactions. Cocultures of human Bone Marrow Stromal Cells (hBMSC) and human Endothelial Progenitor Cells (hEPCs) were performed. The aim was to investigate the existence of intercellular junctions in the 3D coculture and its effect on osteoblastic differentiation. Time lapse videomicroscopy performed during 24 h revealed that the porous polysaccharide-based scaffold generates formation of multicellular aggregates inside the pores of the matrix. The kinetic of cell aggregation depends on the mono- or coculture and the ratio of both cell types in coculture. Aggregates were constituted by a ring of hEPCs surrounding hBMSCs. Expression of Connexin43, VE-cadherin and bone specific markers cbfa1, alkaline phosphatase, type I collagen, osteocalcin, osteopontin, were studied by RT-Q-PCR and immunofluorescence for 12 days. Immunostaining evidenced VE-cadh in the ring of hEPCs and Cx43 punctated points between hBMSCs and hEPCs. mRNA levels of Cx43 and all the bone specific markers were increased with time of coculture. These data indicate that this 3D matrix enhances cell communication between heterotopic cells and drive hBMSCs towards osteogenic lineage.

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51.P12
Quantitative analysis of interactions between capillary networks and hepatocyte tissues in a microfluidic platform

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Increasing interest has focused on capturing the complexity of tissues and organs in vitro as models of human pathophysiological processes. In particular, a need exists for a model that can investigate the interactions in three dimensions between epithelial tissues and a microvascular network since vascularization is vital for reconstructing functional tissues in vitro. Here, we utilized a microfluidic device to develop a co-culture model of microvascular endothelial cells (MVECs) and hepatocytes. Hepatocytes and MVECs were cultured on each sidewall of a collagen gel scaffold between two microfluidic channels under static or flow conditions. Morphogenesis of three dimensional hepatocyte cultures was found to depend on the application of interstitial flow across hepatocyte clusters. In addition, MVECs formed three dimensional capillary networks that extended across an intervening gel to the hepatocyte tissues in hepatocyte-MVEC co-culture while they formed sheet-like structures in MVEC monoculture. Furthermore, an interface between the tip of the capillary networks and hepatocyte tissues was analyzed by confocal microscopy, and cell growth and migration in the capillary networks were quantitatively analyzed to investigate interactions between the capillary networks and hepatocyte tissues. The experimental approach described here is useful more generally for investigating microvascular networks within 3D engineered tissues with multiple cell types in vitro.

51.P13
Factors involved in capillary formation by endothelial cells in coculture with osteoblasts

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The survival and functioning of bone biomaterial requires a rapid and stable vascularization after implantation. One of the goals to speed the acceptance and recovery of an implant is to generate a pre-vascularized construct with the patient's own cells. In vitro co-cultures of human microvascular endothelial cells and primary human osteoblasts on bone biomaterials result in a tissue-like self-assembly of the cells, with endothelial cells forming microcapillary-like structures containing a lumen intertwined between cell layers of osteoblasts. These pre-formed capillaries have been shown to anastomose after implantation and rapidly integrate into the functioning host vasculature. However, the ability of endothelial cells to form microcapillaries differs depending on tissue source and donor. Which individual factors are involved in stimulating the randomly distributed endothelial cells in the co-culture to migrate and form microcapillaries? In order to be able to predict a reliable prevascularization with variable donors an understanding of the cell-cell communication and factors which lead to microcapillary structures is necessary. Therefore, we have begun examining different human osteoblast cell lines and osteoblasts isolated from different locations in the body to identify factors involved in this process. Moreover, we found morphological differences in endothelial cells from different source tissues, which also show distinct formations of microcapillary-like structures.

51.P14
Interactions between periosteal cells and blood vessels during bone autograft healing: implications for tissue engineering strategies

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Despite major drawbacks, autografts remain the golden standard to treat large bone defects. Cell-based bone tissue engineering is a promising alternative, but the lack of timely formed blood vessels often compromises the survival of implanted cells. Here, we examined the role of periosteal cells in blood vessel formation using a mouse segmental bone defect model. We verified the critical role of periosteal cells in autograft healing by showing that removal of the periosteum reduces the healing of autografts to the level of allografts. MicroCT-based visualization of the vasculature revealed a significantly lower number of blood vessels surrounding allografts compared to autografts, especially near the central graft region. Implantation of autografts from GFP-mice showed that the blood vessels in the callus were derived almost exclusively from the host. These data suggest that periosteal cells are critical for the attraction of blood vessels. Indeed, murine periosteum-derived cells (mPDC) cultured in hypoxia showed a faster and stronger increase of Vegf mRNA levels compared to bone marrow stromal cells. Also, conditioned medium of mPDC enhanced endothelial cell (EC) proliferation, while inhibition of mPDC-derived VEGF in cocultures induced manifest EC death. Our results show that periosteal cells are crucial for autograft healing not only by their strong osteogenic potential, but also their pro-angiogenic features and thus may provide an ideal cell source for bone regeneration.

51.P15
Coculture of Endothelial Progenitor Cells (EPC) and Coculture of Endothelial Progenitor Cells (EPC) and Mesenchymal Stem Cells (MSC) in polyurethane scaffolds (PU) promotes simultaneously implant neo-vascularization and bone neo-formation

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Vessel ingrowth from nearby tissues is insufficient in most large defect healing, current tissue engineering strategies focus on the neo-vascularization process. Our aim is to develop a pre-vascularized bone implant made of PU co-seeded with autologous MSC and EPC, and identify an optimized environment for both cell populations. MSC and EPC were isolated from human bone marrow (KEK_Bern126/03) using Ficoll and MACS respectively. PU scaffold were seeded with MSC alone, or with 25% or 50% EPC and in the presence of autologous Platelet Rich Plasma. Constructs were incubated (37°C-5%CO2) in endothelial cell culture medium (M200+LGS) or in IMDM+FCS+Platelet Lysate, alone or with Dexamethasone. After 1 week, samples were implanted subcutaneously in nude mice and sacrificed at 8 weeks. Other samples were kept in vitro until day 35. After 7 days, the presence of tubular structures positive for endothelial- (PECAM, vWF, LN) and pericyte-

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markers (CD146, NG2, αSMA) was observed only in MSC-EPC co-seeded samples and only in the presence of Dex. After 21 days, MSC-EPC samples were positive for ALP. In MSC samples, ALP only appeared after 35 days. After 8 weeks in vivo, matrix mineralization was clearly detected. We showed that EPC combined with MSC enhances and stabilises early vascular-like network formation in scaffolds, supports osteogenic differentiation of MSC and mineralization. The presence of PL and Dex in the medium has proven essential in these coculture systems.

51.P16
A distinct subpopulation of pig endothelial progenitor cells is the origin of vascular-like networks and supports pig endothelial cell proliferation in co-cultures with mesenchymal stem cells

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Vessel TE focuses on the coculturing of various cell types for the generation of endothelium lined muscular tubes. Endothelial cells (EC), requested for this purpose, normally appear as heterogenous population of cells when differentiated from EPC. Here, the role of giant Endothelial Progenitor Cells (gEPC) in cocultures with Mesenchymal Stem Cells (MSC) and vessel derived EC was analyzed. gEPC and differentiated EC stably expressing GFP or RFP were seeded onto monolayers of bone marrow derived MSC, all of porcine origin. After 14 days, cell type specific markers were investigated (EC, smooth muscle cells, pericytes) by immunhistochemistry. Appearing vascular-like networks were analyzed for density and structure length.MSC in contact with EC stained positive for sm-α-actin (ACTA2) and sm-myosin heavy chain 11 (MYH11). Proliferation of EPC was not observed, however, extensive vascular-like networks developed, which seemed to be formed mainly by the gEPC-subpopulation. gEPC, negative for ACTA2 and MYH11, exhibited a strong expression of CD31, more intense than normal EPC. As EPC, EC showed no proliferation on MSC, however, when tricultured with gEPC they proliferated and migrated until a confluent monolayer was reached. This study reveals (1) gEPC are the origin of vascular-like networks when cocultured with MSC, which in turn differentiate towards a smooth muscle cell phenotype. (2) gEPC have a proliferative effect on terminally differentiated EC when cocultured with MSC.

51.P17
Pericytes contribute to the stability of the vascular network of osteogenic tissue formed from cell sheet-based constructs

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Despite the increased use of cell sheets for tissue engineering, the application of this new technology for thick tissue reconstruction is being limited by old barriers such as the need to create a pre-vascular network to ensure proper nutrient and oxygen supply in vivo. In the present work, we created a cell sheet-based construct by co-culturing bone marrow-derived osteogenic and pericyte-like cells with human umbilical vein endothelial cells (HUVECs) with the purpose of enhancing the in vivo vascularization of newly formed osteogenic tissue. Human bone marrow mesenchymal stromal cells (hBMSCs) were isolated and cultured in medium supplemented with osteogenic factors or TGF-β1 to obtain either osteogenic or $CD146^+$ pericyte-like cells. Immunocytochemistry analysis of the co-cultures showed organized structures formed by $CD146^+$ hBMSCs and HUVECs over the osteogenic cell sheet, suggesting the existence of cross-talk between the co-cultured cell types. Nude mice were used to test the ability of those constructs to form functional and vascularized osteogenic tissue. Immunohistochemistry analysis of cell sheet-based constructs after 1 and 3 weeks of transplantation revealed the integration of HUVECs with neighboring host's vessels. Additionally, the analysis of the diameter of the blood vessels showed a higher mean diameter for the condition that combined pericyte-like cells and HUVECs, reinforcing the advantage of the proposed model regarding blood vessels maturation and stability.

51.P18
Assessing the ability of in vitro engineered vasculature in a collagen-GAG scaffold to enhance bone tissue regeneration in vivo using a rat calvarial defect model.

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In vitro engineering of microvasculature within tissue engineered (TE) constructs attempts to overcome graft failure due to avascular necrosis following implantation. We have recently developed a mechanism to engineer, in vitro, vasculature in a collagen-GAG (CG) scaffold using a delayed addition of human mesenchymal stem cells (MSCs) to pre-formed human umbilical vein endothelial cell (EC) networks. This study assessed the in vivo response and functionality of this co-cultured (CC) construct in a critically sized calvarial bone defect and compared it to vasculature formed using ECs alone and a cell-free CG scaffold (control). Animals were sacrificed at 4 and 8 weeks post-implantation. Quantitative analysis with microCT and histomorphometry demonstrated increased bone formation in the CC group compared to ECs alone at 4 weeks. Immunohistochemistry revealed the presence of M2 macrophages indicating a high level of remodelling and healing in the bone defects. In addition, positive staining for human CD 31 demonstrated the presence of human ECs 4 and 8 weeks post-implantation indicating that pre-engineered vessels had the ability to remain viable and become functional in vivo. These results reveal new information on vasculogenesis and the immune response of host animals toward a TE construct during tissue repair. In addition these data suggest that a CG scaffold containing pre-engineered vessels using a co-culture of ECs and MSCs may provide accelerated healing in bone defects.

51.P19
Enhancement of osteogenesis and angiogenesis by bone forming peptide 4 derived from BMP-7 prodomain

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Cells were cultured in medium for 6 days and treated with BFP 4 for 1 days and then subjected to various tests MTT assays, alizarin red S staining, alkaline phosphatase (ALP), cell migration, tube formation, osteogenic and angiogenic gene expressions, fluorescence microscopy, flow cytometric analysis and animal experiments. D1 cells were found to differentiate into osteoblasts in the presence of osteogenic differentiation medium. Osteogenic differentiation was enhanced when cells were treated with BFP 4, as determined by alizarin red S staining, ALP activity, calcium contents and osteogenic genes (ALP, Runx2, and osteocalcin) mRNA expression, and flow cytometric analysis for CD44 surface molecules. Angiogenic differentiation was enhanced when cells were treated with BFP4, as determined by cell migration and tube formation analysis. Furthermore, X-ray and morphology images showed

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that BFP 4 enhanced bone and blood vessel formation on animal experiments. These findings suggest that BFP 4 enhances osteogenic and angiogenic differentiation via osteogenic related protein expression and angiogenic related protein when administered to cells.
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51.P20
Periodontal regeneration: In vitro evaluation of prolyl hydroxylase inhibitors

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Prolyl hydroxylase (PHD) inhibitors hold a therapeutic potential to support periodontal regeneration. This assumption is based on the capacity of PHD inhibitors to stabilize hypoxia-inducible factor (HIF)-1, a central mediator of angiogenesis. The impact of PHD inhibitors on a broader cellular response, including protease activity and bone resorption, are however not fully understood. To demonstrate the responsiveness of the periodontium to PHD inhibitors, we determined HIF-1 and the production of vascular endothelial growth factor (VEGF) in periodontal fibroblasts. Following the same strategy, we determined the release kinetic of PHD inhibitor from bone substitutes. The impact of PHD inhibitors on plasminogen activation was evaluated by chromogenic assays, and the impact on the formation and activity of osteoclasts was assessed in murine bone marrow cultures. Our data show that non-toxic concentrations of PHD inhibitors increase HIF-1 and provoke the production of VEGF. PHD inhibitors released from bone substitutes within 48 h stimulate VEGF production. In addition, PHD inhibitors reduced plasminogen activation capacity of periodontal fibroblasts, and decreased the formation and activity of osteoclasts. Overall our results suggest that PHD inhibitors stimulate a pro-angiogenic response and have an anti-catabolic activity. These findings provide the basis for preclinical studies for PHD inhibitors loaded onto bone substitutes to support periodontal regeneration.

51.P21
Impact of human amnion-derived mesenchymal stromal cells and placental fibroblasts on the function of endothelial cells

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Introduction: Mesenchymal stromal cells derived from the human amnion (hAMSC) are multipotent cells which are immunologically well tolerated. This makes them promising candidates for cell-based therapies. In our study we investigated the paracrine effects of hAMSC and placental fibroblasts (PlFib) on the function of placental endothelial cells (PlEC).
Methods: Conditioned medium (Cdm) was prepared by incubating confluent hAMSC and PlFib with EGM-2 medium for 48 h. The impact of Cdm on the viability and network formation of EC was determined using LDH and Matrigel assays, respectively. The paracrine factors in Cdm were analysed by angiogenesis array kits and ELISA.
Results: Culture of PlEC in hAMSC-Cdm and PlFib-Cdm reduced the activity of LDH in the culture supernatant to 59 ± 26% and 59 ± 17% of control, respectively. As LDH is released by damaged cells, this points to a clearly enhanced endothelial viability. In the Matrigel assay, only PlFib-Cdm significantly increased the number of endothelial networks. Protein analysis of Cdm revealed a similar secretion of pro- and antiangiogenic factors such as angiogenin, IL-8, TIMP-1&2, while VEGF was only secreted by PlFib.
Conclusion: hAMSC and PlFib enhance endothelial cell viability via paracrine factors. Additionally, PlFib increase endothelial network formation by factors such as VEGF. Therefore, hAMSC might be valuable in therapeutic approaches to promote the survival of blood vessels without the risk of excessive angiogenesis.

51.P22
Unfolded Protein Response (UPR) as a possible target for angiogenesis regulation in tissue engineering

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Vascularization is important for most tissue engineering applications. Current strategies including the use of growth factors to control angiogenesis present several limitations. Therefore, targeting novel signaling pathways involved in the regulation of angiogenesis might improve vascularization in tissue engineering. We demonstrate here that the unfolded protein response (UPR) could be such a pathway. UPR is activated by accumulation of unfolded proteins following stress signals. After activating UPR, cells produce e.g. chaperones to counteract stress. However, it is also known that this pathway is involved in physiological reactions, such as inflammation and differentiation. We showed that BiP, an UPR regulatory protein, was induced in primary endothelial cells after stimulating with VEGF and bFGF. Anoxia, another angiogenic stimulus, also led to an increased BiP expression at mRNA and protein level. Importantly, down-regulation of BiP using siRNA inhibited the migration of endothelial cells in a modified scratch assay. Furthermore, the cells with down-regulated BiP exhibited reduced formation of capillary-like structures in an in vitro angiogenesis assay. Altogether, these data indicate an important role for BiP and the UPR pathway in angiogenesis regulation. We believe that the targeting of UPR could be used to control angiogenesis in different tissue engineering applications, in which stimulation or inhibition of vascularization is important.

51.P23
Hypoxia enhanced the induction of human amniotic mesenchymal cells into vascular endothelial lineage through up-regulation of the gene expression associated with angiogenesis

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Aim: Human amniotic mesenchymal side population cells (h-AMSPCs) have pluripotency and weak immunogenicity. These cells have promising features in regenerative medicine. The aim of the present study was to determine whether hypoxic condition is appropriate for the differentiation of the h-AMSPCs into vascular endothelial lineage.
Methods: The mesenchymal cells were isolated from the enzyme-treated amniotic membrane and were stained with Hoechst33342. The h-AMSPCs were negatively sorted by FACS. The h-AMSPCs were cultured in induction medium containing VEGF in normoxia (20%) or hypoxia (1%) for 1 or 2 weeks. The expressions of endothelial markers as KDR, Flt-1, vWF, VE-cadherin and VCAM were evaluated by real-time PCR and fluorescent immunostaining. Changes in the expression of angiogenesis-related genes were also investigated by DNA microarray.
Results: Gene expressions of KDR, Flt-1, VE-cadherin and vWF were most pronounced after 2 weeks cultivation. Protein expressions of KDR and VE-cadherin were more strongly observed after 2 weeks cultiva-

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tion under hypoxia. In the microarray data, gene expression of Angiopoietin, VEGFA and Thrombospondin, which were known to be up-regulated by HIF, increased under hypoxia.
Conclusion: The h-AMSPCs cultured under hypoxia differentiated into vascular endothelial lineage, probably due to the up-regulation of the gene expression associated with angiogenesis through HIF system.

51.P24
Cyclic mechanical strain guides capillary-like morphology in vitro

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Introduction: Stretching of tissue stimulates angiogenesis but increased motion at a fracture site hinders revascularisation. In vitro studies have indicated that mechanical stimuli promote angiogenic responses in endothelial cells, but can either inhibit or enhance responses when applied directly to angiogenesis assays. We anticipated that cyclic tension applied during endothelial network assembly would increase vascular structure formation up to a certain threshold.
Methods: Fibroblast/HUVEC co-cultures were subjected to cyclic equibiaxial strain (1 Hz; 6 h/day; 7 days) using the FlexerCell FX-4000T system and limiting rings for simultaneous application of multiple strain magnitudes (0–13%). Cells were labelled using anti-PECAM-1, and image analysis provided measures of endothelial network length and numbers of junctions.
Results: Cyclic stretching had no significant effect on the total length of endothelial networks ($P > 0.2$) but resulted in a strain-dependent decrease in branching and localised alignments of endothelial structures, which were in turn aligned with the supporting fibroblastic construct.
Conclusion: The organisation of endothelial networks under cyclic strain is dominated by structural adaptation to the supporting construct. It may be that, in fracture healing, the formation and integrity of the granulation tissue and callus is ultimately critical in revascularisation and its failure under severe strain conditions.

51.P25
Capillary-like structures adjacent to biofunctionalized microfibres generated by different supportive cell types

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Introduction: For an engineered tissue with more than 100–200 μm of thickness, blood vessels are necessary to supply tissue cells with nutrients. Thus, the goal of our studies is to locally control in vitro capillarisation by positioning of supportive and vessel-forming endothelial cells on biofunctionalized fibres within a 3D matrix. This support may then lead to capillary-like structure formation adjacent to the fibres.
Material and methods: Biofunctionalization of poly-DL-lactide acid (PDLLA) fibres is performed by aminofunctionalization and covalent binding of RGD peptides via hexamethylene diisocyanate (HDI). As supportive cells, human foreskin fibroblasts (HFF) were seeded on the fibres, followed by human umbilical vein endothelial cells (HUVECs). Fibrin moulding followed. After 9 days of co-culture, gels were fixed and immunostained (CD31). Quantification of capillary-like structures in the 3D fibrin matrix was carried out using two-photon microscopy and ImagePro® Analyzer software.
Results: When fibrin moulding was done after cell seeding, capillary-like structures formed on HFF as supportive cells adjacent to biofunctionalized microfibers.
Conclusions: Vascular network formation can be realised and controlled in the immediate neighbourhood of biofunctionalized fibres inside a 3D fibrin matrix. Supportive cells like HFFs are necessary to generate these networks next to the fibres. Two-photon microscopy helps to visualize and quantify the capillary-like structures.

51.P26
In vitro vascular-like network as a platform for functional tissue constructs

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The current limitation in developing 3D-tissue constructs is the lack of adequate vascularization. The vascular network should have features of mature and stable vessels. Preferably no animal components or unnatural scaffold material should be present. Since human adipose stromal cells (hASC) are known to secrete several angiogenic factors and promote vascular maturation, they are excellent building blocks for tissue constructs.We developed a natural-like, xeno-free in vitro vascular-like network. We induced hASCs to vascular-like network formation in co-culture with human umbilical vein endothelial cells (HUVEC). The aim was to find optimal culture conditions for the formation of vascular-like network with properties of mature tubules. The results show that hASCs induced tubule assembly, vessel maturation and also differentiated into endothelial cells themselves. hASCS and HUVECs self-assembled into a 3D network with a complete basement membrane and vessel supporting cells. Vascular endothelial growth factor and basic fibroblast growth factor were found to be essential for vascular-like network formation. In addition, serum-free/low-serum culture and addition of other growth factors improved network formation. This vascular-like network provides an excellent tool for developing natural-like, scaffold-free 3D-tissue constructs. Target cells from different origin can be added to the vascular-like network as the applicability of the system was tested in various conditions.

51.P27
Crosstalk between fibroblasts and endothelial cells promotes angiogenesis in vitro: putative role of alkaline phosphatase

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Interactions between the different cell-types, growth factors and extracellular matrix components involved in angiogenesis are crucial in the mechanisms of new vessel formation for tissue regeneration. The aim of the present study was to investigate if cocultured fibroblasts and endothelial cells (from macro- or microvasculature) could modulate growth factor production (VEGF, bFGF, TGF-β1 and IL-8), influence the formation of microcapillary-like structures by endothelial cells and

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affect fibroblast differentiation. Results obtained show that the two cells types interact with each other through exchange of growth factors (e.g., fibroblasts produce VEGF, which is known to influence angiogenesis). Fibroblasts promoted the formation and organization of capillary-like structures by endothelial cells, increased the amount of collagen in the cocultures and determined the expression of alkaline phosphatase. High alkaline phosphatase expression could be co-localized with capillary-like structures and the interaction between the two cells types induced fibroblast activation near microvessel-like structures.

51.P28
Generation of prevascularized muscle tissue constructs by coculture of skeletal muscle stem cells with endothelial cells

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Tissue engineered skeletal muscle constructs have been developed as alternatives to autografts to be used especially in regeneration of muscle tissue in congenital or accidental maxillofacial tissue defects. Skeletal muscle stem cells are able to form functional myotubes easily under in vitro conditions, especially under provision of biological cues and cyclic strain. With the aim of translating research to therapy, development of animal product-free protocols have gained significant importance. Human skeletal muscle stem cells (hSkMSCs) were isolated with enzymatic digestion from a small skeletal muscle biopsy obtained during orthopedic surgery, and characterised with flow cytometry and by differentiation into osteogenic and adipogenic lineages at the third passage. hSkMSC were seeded on electrospun parallel microfiber PHBV:PLDLLA:PGS mats to promote aligned myotube formation, which is necessary for unidirectional muscle contraction. Fetal bovine serum was replaced with human platelet lysate to make the protocol and the final product applicable to humans. Simultaneous construct vascularization protocol was developed by coculturing hSkMSCs with HUVEC or peripheral blood derived endothelial colony forming cells (ECFC) in a 1:1 ratio in media with different compositions. The endothelial cells formed both unidirectional and transverse capillary networks, and hSkMSCs were able to form aligned myotubes.

51.P29
Effect of neurotrophic factors on angiogenesis in reconstructed skin

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The neurotrophic factors (NF) are a class of secreted peptides. They are vital for the cutaneous innervation and the epidermis is known to be a major source of NF that remotely attract the nerve fibers. Interestingly, an angiogenic effect for the NF nerve growth factor (NGF) was described in other tissues. Beside their neuronal contribution, we hypothesized that neurotrophic factors influence the microvascular network in the skin. We characterized the endogenous expression and release of NF in human skin and we simplified the evaluation and comparison of their angiogenic properties by developing a unique endothelialized reconstructed connective tissue model (ERCT). Using the ERCT, we observed that the addition of NF to the culture medium induce a major increase of CLT formation and migration within the ERCT with 10 ng/ml NGF, 0.1 ng/ml BDNF, 15 ng/ml NT-3 and 50 ng/ml GDNF. Moreover, we assayed the phosphorylation events for some of the kinase effectors after stimulation with NF. Here we report the expression of TrkA, TrkB, p75, c-Ret and Gfrα-1, the cell receptors for these NF and we report for the first time an analysis of the intracellular kinase pathways activation following 15 min of stimulation with NF. We could also demonstrate that this effect was mediated through the TrkA and TrkB receptors for NGF, BDNF and NT-3. In conclusion, we generated an ERCT that allowed for the study of the role of neurotrophic factors on angiogenesis.

51.P30
Hypoxia mimicking small molecules induce an angiogenic trophic profile in human mesenchymal stromal cells

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The pro-angiogenic response of human mesenchymal stromal cells (hMSCs) is mediated by vascular endothelial growth factor (VEGF). We employed a high throughput assay on hMSCs containing a hypoxia responsive element from the VEGF promoter to identify novel small molecules which mimic hypoxia. The effect of several of these small molecules was cell type/species dependent, but we identified a molecule that induces high expression of HIF-1 target genes in hMSCs. Microarray data showed that the mechanism of action differs from desferoxamine (DFO - a known hypoxia mimic). At a protein level, we confirmed that the new molecule, in contrast with DFO, induces high levels of IL-8 expression suggesting a distinct mechanism from DFO-induced angiogenesis. Importantly, our new molecule alone was sufficient to induce vascular invasion of a Matrigel plug in vivo, making it a highly promising molecule for future revascularization strategies.

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52. Optical and MRI Imaging in vivo (in coop. EIBIR)

52.01
Keynote: Imaging challenges in tissue engineering

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The ability to engineer functional tissues could lead to new options for tissue reconstruction and replacement. Imaging technologies for 3D analysis of engineered tissues has been identified as a strategic priority for agencies involved in tissue engineering research. In this presentation, information will be provided on imaging challenges in tissue engineering and approaches that are being employed to address these issues described. A particular emphasis will be placed on techniques that can provide 3D imaging of engineered tissues without the use of exogenous contrast agents. Data will be presented using X-ray phase-contrast (PC) to image engineered tissues. These have shown promise for biomedical applications due to their ability to provide information on tissue structure through reliance on alternative X-ray properties (refraction, scatter) in addition to X-ray absorption. X-ray PC techniques can resolve both soft tissue and bone with excellent spatial resolution and deep tissue penetration. Hydrogels, polymer foams and tissue structure can be imaged using X-ray PC computed tomography without use of contrast agents. In addition, results will be presented showing how traditional brightfield, darkfield and phase contrast microscopy available in most research laboratories can be used for 3D imaging of engineered tissues in vitro for pre-implantation evaluation. Overall, this presentation will identify some of the recent advances in 3D imaging of engineered tissues.

52.02
Keynote: Molecular MRI of the joint

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Osteoarthritis (OA) changes in hyaline articular cartilage are characterized by important changes in the biochemical composition of cartilage. The macromolecular network of cartilage consists mainly of collagen and glycosaminoglycans (GAG). Special MR techniques attempt to selectively demonstrate the GAG components and/or the collagen fiber network of the extracellular matrix and are usually summarized as 'compositional imaging' of cartilage. These MR imaging techniques comprise: Delayed Gadolinium enhanced MRI of Cartilage (dGEMRIC) and sodium (23 Na) imaging which are based on similar principles, with positive sodium ions being attracted by the negatively fixed charged density of the GAG side chains. These electrostatic forces are responsible for a direct relationship between the local sodium concentration and fixed charged density with a strong correlation between fixed charged density and GAG content. T2 and T2* mappingT2 mapping has been used to describe the composition of hyaline articular cartilage in the knee joint on the basis of collagen structure and hydration. In addition to the transverse relaxation time (T2) of articular cartilage, T2* relaxation measures have recently been investigated for depiction of the collagen matrix. Chemical Exchange Saturation Transfer (gagCEST) CEST imaging have recently been presented as a technique with the potential to measure GAG content in cartilage.

52.03
Diffusion tensor imaging to assess collagen structure in cardiovascular bioengineered tissues

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Collagen is the principal load–bearing element of cardiovascular tissues. The 3D arrangement of these fibers is crucial to understand the mechanical behavior of the tissue. We evaluated a novel imaging methodology Diffusion Tensor Imaging (DTI) to assess the 3D collagen structure in bioengineered cardiovascular tissues, which is fast, non-invasive and non-destructive.Human venous vascular cells (p7) were seeded onto rapid degrading PGA (polyglycolic acid)/P4HB (poly-4-hydroxybutyrate) scaffold strips and cultured for 3 weeks. To validate DTI measurements, collagen in the tissue-engineered (TE) strips was stained with the fluorescently labeled CNA35-OG488 probe and visualized by confocal microscopy. An algorithm was developed in Mathematica in order to quantify the fiber orientations in the microscopy images. TE strips for DTI measurements were embedded in 4% type VII agarose. Fiber tractography was performed by an in-house-developed software program on the DTI images.DTI demonstrated an angled collagen alignment in the TE strips; likely in the direction of the internal stress. The DTI measurements were validated by the confocal microcopy measurements.This study demonstrated the feasibility of using DTI for evaluating the collagen orientation in bioengineered cardiovascular tissues as a fast and non-destructive method.

52.04
The use of fluorescent imaging to detect the neovascularization and differentiation of skeletal muscle in vivo.

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Introduction: A significant barrier for a progress in tissue engineering is the inability to monitor, in real time, the dynamic process of tissue regeneration. The first goal of this project is to develop a cell-labelling scheme that can be used for the development of a non-destructive imaging modality to monitor tissue regeneration in vivo. Another critical difficulty in cell therapy is the requirement for a functional vasculature to maintain viable cells. A second goal of this project is to develop a method to enhance tissue neovascularization.
Methods: Endothelial cells (HUVECs, labelled with DiI), pericytes (10T½ cells labelled with Qdot705) and GFP^+ myoblasts were the cell sources for these experiments. Cultures of fluorescently labelled cells, alone or together, were seeded on an acellular collagen scaffold and allowed to grow for 7 days prior to implantation into the subcutaneous space of nude mice for 8 weeks. Scaffolds were recovered for histological analyses.
Results: The presence of fluorescently labelled myoblasts and HUVECs were detected in scaffolds explanted after 8 weeks. Myoblasts were seen to have differentiated into mature myofibers and the presence of HUVECs on the scaffolds results in a significantly greater number of functional blood vessels integrated into the scaffold.
Conclusions: These experiments demonstrate that the use of fluorescently labelled cells is a viable method of tracing cell fate. Complementing the myoblasts with endothelial.

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52.P01
Preparation of molecular probe for photoacoustic tomography diagnosis

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Indocyanine green (ICG) is clinically used as a contrast agent. ICG is also useful for photoacoustic tomography diagnosis because it has an ability to absorb light in the near-infrared wavelength range. However, ICG is not always stable in the body, resulting in poor accumulation at the target site. This study is undertaken to design a novel delivery system which enables ICG to stabilize in the body and enhance the tumor accumulation. Gelatin was reacted with the N-hydroxysuccinimide ester group of 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE), followed by dialysis and lyophilization. Similarly, cholesterol was grafted into gelatin. ICG was mixed with DSPE- or cholesterol-introduced gelatin in the water to form the complexes. Following the intravenous injection into the Colon26 tumor-bearing mice, the body distribution of complexes was evaluated. The molar absorption coefficient of ICG complexed with DSPE- or cholesterol-grafted gelatin significantly increased compared with that of free ICG. The complex of ICG and DSPE-grafted gelatin showed stronger photoacoustic signal than that of cholesterol-grafted gelatin. The in vitro stability study revealed higher stability of ICG complex with the DSPE-grafted gelatin. The ICG complexed with the DSPE-grafted gelatin injected was accumulated in the tumor to a significantly high extent compared with free ICG. The DSPE-grafted gelatin is a promising delivery system to enhance the tumor accumulation of ICG.

52.P02
Spatially selective modification of polymeric scaffolds with markers for MR-Imaging

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Tissue regeneration is monitored most oftenly via tissue sections at certain time points of in vivo experiments. To overcome this drawback in animal studies a novel approach to observe tissue regeneration on polymeric scaffold structures is presented. The goal of this study is to modifiy scaffolds made from biocompatible polymers e.g. polylactid-acid (PLA) with MR-active markers like 19Fluorine. The regions of interest e.g. the suture of artificial vessels or newly designed Y-shape junctions can be selectively marked with highly fluorinated compounds to enhance visibility of these areas in MR-imaging. Our approach is based on photoactive compounds which can be immobilized onto polymer surfaces upon irradiation with light of a suited wavelength. Due to the high spatial resolution and adjustable parameters for photo immobilization laser irradiation is applied. The use of heterobifunctional linkers like 4-azido-2,3,5,6-tetrafluorobenzoic acid, succinimidyl ester (ATFB, SE, Invitrogen) and Sulfosuccinimidyl (perfluoroazidobenzamido) ethyl-1,3-dithiopropionate (Sulfo-SFAD, Pierce Scientific) facilitate a two step proceedure: First photo coupling of the compound onto the polymer surface, secondly wet chemical modification with a fluorinated compound like 3,5-Bis-(trifluoromethyl)-benzoylamine. Methods and preliminary results on achievable fluorine contents will be presented.

52.P03
Proton magnetic resonance imaging of resorbable hydrogels for tissue regeneration

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Magnetic resonance imaging (MRI) is a tomographic technique with high temporal and spatial resolution that can be applied for non-invasively track the degradation of biomaterials in vitro and in vivo. In this context, contrast enhancement agents such as gadolinium Gd(III),can help to differentiate between the biomaterial implant and the surrounding host tissue. The goal of this work was to synthesize labeled resorbable hydrogels based on PEGylated fibrinogen (PF) that can be used for characterizing and tracking the degradation of the materials in situ using MRI imaging. PF-GdDTPA was synthesized by conjugating diethylenetriaminepentaacetic acid-chelated Gadolinium (GdDTPA) to PF precursor and forming hydrogels by photopolymerization. The effect of different concentrations of PF-GdDTPA on proton relaxation times was determined. The R1 relaxivity of 1.3 mg/ml PF-GdDTPA was measured to be 2.2 (S-1), which was optimal for MRI imaging. Human dermal fibroblast cells were viable and proliferated normally in materials made from this concentration of PF-GdDTPA. In vitro constructs made from PF-GdDTPA hydrogel beads encapsulated in secondary unlabeled PF gels were used to demonstrate the contrast between the Gd-labeled and unlabeled regions. The labeled materials were implanted subcutaneously in rats and showed comparable contrast with the surrounding host tissue. We conclude that PFhydrogels with Gd (III) have the potential to enable noninvasive tracking of biomaterial resorption.

52.P04
Tracking of human muscle precursor cells by MRI and muscle regeneration in vivo

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Follow-up of cell survival and tissue formation is essential for improvement of cell therapies. Hence, tracking transplanted stem cells by a non-invasive manner is desirable. One method is Magnetic Resonance Imaging (MRI), where cells are labelled with Iron Oxide Nanoparticles (SPION). In this study we explored effects of increasing intracellular levels of SPIO and defined a safe concentration in which human MPCs can be easily detected by MRI, without loss of characterisations. MPCs were labelled with increasing concentration of SPION (100–1600 μg/mL). Then, labelling efficiency, cell viability, growth, molecular characteristics and differentiation were examined in vitro. SPIO labelled MPCs were injected subcutaneously into nude mice and followed for 4 weeks by MRI imaging. Muscle tissue formation was assessed macroscopically by organ bath and by immunohistochemistry. Labelling with more than 800 μg/mL of iron oxide reduced the viability of cells by 15–25%. In the long term, 800–1600 μg/mL SPIO decreased MPC differentiation ratio by 10%. However, 400 μg/mL SPIO has no effects on cell viability, growth, differentiation, and cells expressed muscle-specific markers and were detected by MRI for at least 4 weeks. Transplanted labelled cells formed a functional muscle tissue. Our data concludes that the optimized conditions of MPC labelling can be safely used in clinics to track sphincter muscle regeneration in patients under SUI treatment by cell therapy.

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52.P05
MRI relaxometry characterizes in vivo differentiation of muscle precursor cells

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We developed methods to assess the differentiation process of human muscle precursor cells (MPCs) in vivo by applying magnetic resonance imaging (MRI) relaxometry measurements. Cultured MPCs were mixed with collagen and injected subcutaneously into nude mice. Controls were injected with collagen. MRI was conducted on a 4.7 T scanner between days 3–28 post-injection. Measurements comprised T1, T2 using multi-echo-spin-echo and saturation recovery sequences and T2* quantification with a multi-echo-gradient-echo-sequences. Relaxation times were additionally measured in the paraspinal muscles. Animals were harvested at different time-points and the engineered muscle tissue was assessed by histology and immunohistochemistry. The engineered muscle tissue was always measurable with a decrease in volume over time. In control mice, collagen was resorbed in one week. Relaxometry measurements revealed a decrease of T1, T2 and T2* relaxation time during differentiation (initial measurement: T1 2172 ms +/− 141, T2 202 ms +/− 16, T2* 29 ms +/− 14, final measurement: T1 1240 ms +/− 17, T2 31.9 ms +/− 0.6, T2* 7.5 ms +/− 1.6), thereby approaching the physiological relaxation properties of muscular tissue. Cell differentiation and myofiber formation was confirmed by histology, immunohistochemistry and contractility confirmed by organ bath. This novel method might offer the possibility to non-invasively assess the effectiveness of cellular therapies for muscular disorders in near future.

52.P06
MRI and fluorescence for in vivo tracking of injectable microcarrier hydrogel implants

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Hydrogel microcarriers are a good platform for minimally invasive delivery of cells and bioactive factors. Microcarriers have a high surface area/volume ratio, which can provide several advantages for tissue regeneration, including more efficient interaction between implanted cells and surrounding tissues, increased nutrient availability and enhanced release of bioactive factors. In this study, we prepared hydrogel microcarriers from fluorescence/MRI probed materials by conjugating diethylene triamine pentaacetic acid-chelated Gadolinium (GdDTPA) and Cy5.5 dye to a resorbable PEG-Fibrinogen (PF) precursor. The hydrogel's in vivo subcutaneous resorption was documented by MRI and IVIS imaging for three types of constructs: preformed plugs, microcarriers and in situ polymerized material. We demonstrated the safety of PF-GdDTPA by in vitro viability and proliferation assays. MRI data revealed that all constructs were almost fully degraded in vivo within 3 weeks; however, the preformed plugs degraded significantly slower than the other two constructs with a similar volume. The release of covalently bound Cy5.5 into the surrounding tissues confirmed the implant resorption kinetics. Histological sections further substantiated the benefits of better integration of the microcarrier system. Finally, using bimodal imaging of co-localized MRI and fluorescence signals, we obtained a more complete picture of the in vivo implant resorption, which was correlated with histological data.

52.P07
Real-time imaging quantification of scaffold degradation using invisible near-infrared fluorescence

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Current scaffold degradation analysis methods require a lot of sample and animal and make inaccurate results due to their nonconsecutive single-time-point information. Moreover, in vivo does not correlate with in vitro results. Herein, we have developed a noninvasive quantitative method to investigate matrix degradation by employing invisible near-infrared (NIR) fluoroscence emitting dyes into collagen scaffold which comes from small intestinal submucosa. Biodegradability of ZW800-1 conjugated scaffolds was examined in vitro and in vivo using FLARE™ real-time intraoperative imaging system with an 800 nm emission filter set. To confirm accuracy and advancement of our system, existing invasive analyses including histology and gravimetric assay were carried out at the same time. Scaffold degradation showed confrontational curve against released dye over incubation time, which was quantitatively evaluated by fluorescence imaging. The results from optical fluorescence imaging were consistent with the data obtained from invasive method. This non-invasive method using NIR imaging fluorophore could enable degradation analysis of various biomaterials exactly, rapidly, easily and economically.

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53. Micro- and Nano-Tomographic Imaging in TERM

53.01
Keynote: In vivo monitoring of vascular development and regeneration

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In vivo monitoring of vascular development and regeneration through micro-computed tomography (micro-CT) requires the injection of contrast agents that provide good contrast, no extravasation and slow blood clearance. For this purpose, nanoparticle-based agents providing high soft tissue enhancement were developed for use in pre-clinical research. In the present study, a nanoparticulate contrast agent (Exi-Tron™ nano 12 000) was injected intravenously in C57BL/6 female mice. Following the injection, neck or lower hind limb tissues were monitored over 30 min with low resolution (70 μm) scans using in vivo micro-CT (Scanco vivaCT 40). The same protocol was applied for the following 5 days and 1 month after the first injection. The average X-ray absorption over time was calculated in blood, muscle and bone marrow. Nanoparticles provided a stable contrast within the first half-hour after injection. In the follow-up study, the daily injection was very well tolerated by all animals, as the nanoparticles were excreted from blood in less than 24 h. Dynamic measurements acquired 1 month after the first injection revealed that the dynamic response of all tissues went back to its first values. In conclusion, X-ray absorbing nanoparticles provided useful in characterizing different tissue behaviors with dynamic imaging facilitating in vivo monitoring of vascular development and regeneration.

53.02
Use of innovative techniques based on X-ray synchrotron radiation in advanced regenerative orthodontics

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Phase imaging is an innovative technique that offers an improved sensitivity compared to conventional attenuation-based techniques. The gain in sensitivity can be several orders of magnitude for soft materials, which makes it appealing for imaging of soft and/or thin tissues of biomedical interest. In particular, in-line phase tomography is the only phase tomography technique that has been recently successfully applied to the case of mostly weakly absorbing structures, in the presence of strongly absorbing ones. We used this technique to investigate the growth kinetics of regenerated bone in different dental implants retrieved from humans, where a large range of absorption contrast exists between bone and soft tissue or even between the Ti-based implant and surrounding tissues. Moreover, interesting results were obtained by X-Ray holotomography on a woven bone built in vitro by dental pulp stem cells and on a sample taken from the repaired mandible human bone. This innovative technique allowed not only the visualization and quantification of mineralized tissues, but showed that in both cases vessels are present. This is of paramount importance and demonstrates that in-line X-Ray phase tomography and holotomography appear to be important ways to investigate the cellular events involved in bone regeneration and represent promising tools for future clinical investigations of the cranio-facial tissues.

53.03
Imaging hydrogel-based engineered tissues with a benchtop X-ray imager

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Hydrogels have been explored in many tissue engineering applications. However, it is difficult to monitor the performance of these materials as they generate little contrast with conventional imaging technologies. X-ray imaging techniques based on phase-contrast (PC) have shown promise for biomedical application due to their ability to provide information about the X-ray refraction properties of samples. In this research, hydrogels were imaged using a newly developed benchtop X-ray in-line PC system. Porous PEG hydrogels and nonporous alginate beads were synthesized using standard methods. Following cell seeding and culturing in bioreactors, beads were imaged in vitro in cell culture media in both planar and CT mode. PEG hydrogels loaded with fibrin were implanted subcutaneously in rats, harvested and imaged. Alginate beads could be discerned from surrounding media. At later time-points, beads displayed higher contrast due to mineralization. The X-ray PC system was also capable of discriminating between underlying muscle tissue and invading fibrovascular tissue within the explanted PEG hydrogels without the use an exogeneous contrast agents. These samples suggest the significant potential use of a benchtop PC X-ray technique to visualize hydrogels in vitro and ex vivo for tissue engineering applications.

53.04
X-ray absorption and X-ray diffraction micro tomography of biodegradable polymer based composites

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Evaluation of degradation process of biodegradable materials is particularly important in tissue engineering. The aim of this study was to show how 3D imaging technique which combines X-ray absorption microtomography (uCT) and X-ray diffraction microtomography (XRD-CT) could be used to examine the in vitro degradation process of polycaprolactone–based composites. The measurements were performed at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) at the ID 22 beamline. Quantitative 3D morphological information with

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submicron resolution was obtained by uCT. The sample-to-detector distance was large enough so that edge-enhancement by refraction of X-rays was utilized (phase contrast imaging). The complementary information on phase distribution, changes in crystallinity and composition during degradation process was obtained by means of XRD-CT. Finally, it was possible to compare uCT and XRD-CT images of the same sample cross-section and demonstrate the capability of the technique for biomedical investigations.

53.05
Keynote: Multi-mode imaging of bone and fracture callus

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Bone is a dynamic tissue, continuously undergoing processes of remodeling, during which the local characteristics of organic tissue and mineral particles are changing, while bone forming osteoblasts get embedded in the bone matrix to become osteocytes. The situation is even more complex during bone healing where callus tissue is laid down, matures and eventually gets resorbed or remodeled into bone. To answer questions related to bone development, disease or regeneration, multi-modal analysis is needed to estimate structure, composition, cell activity and mechanical behavior within the same tissue. The presentation will review methodology being currently developed which combines position resolved x-ray diffraction, small-angle scattering and fluorescence spectroscopy with light microscopic imaging, polarized Raman imaging and nanoindentation mapping. This allows imaging hard and soft components of the bone and fracture callus and to estimate their properties in relation to the position of osteocytes in the tissue.

53.P01
Effect of micro-tomography radiation on cell viability and survival of live human mesenchymal stem cells

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Micro-tomography is commonly used for scaffold characterisation in tissue engineering. Cell visualisation is limited due to the absorption of X-rays and only fixed and stained samples can be imaged. New X-ray techniques, such as phase-contrast microCT, are undertaking cell imaging without markers or contrast agents but there is little information about the effect of this radiation on live cells. Human mesenchymal stem cells were seeded on tissue culture slide-flasks and cultured for 24 h prior to irradiation with a custom-made Nikon microCT. Irradiation regimes included different voltage (35–70 keV) and different power (4–25 W) for 20 min. Samples were maintained for 7 days after radiation and analysed for survival, DNA content, and gene and protein expression at 24 h and 7 days. Results 24 h post-irradiation revealed little difference in cell viability and density between exposed and non-exposed samples, regardless of energy or power levels. Samples analysed after 7 days showed lower cell viability and density that correlated to higher energy doses and exposure time. Further results of gene expression levels were not significantly different nor DNA levels after 7 days of irradiation at low dose. Mammalian cells can tolerate a limited exposure of ionised radiation. Cell survival decreases at high radiation dose caused either by increased voltage, current or exposure time.

53.P02
Quantitative analysis of vascular tree structure in decellularized liver for tissue engineering

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Introduction: Decellularized liver (DC-liver) has been expected to be a practical scaffold as a mold of fine vascular tree network for liver tissue engineering. However, the structure was discussed by only the appearance. Quantitative analysis of the structure is required for the development of practical tissue engineering technology.
Materials and methods: DC-liver of rat was prepared by using detergent. Vascular tree structures in natural liver / DC-liver were copied by resin. The preparation condition of resin sample was optimized to detect X-ray absorption of three-dimensional structure clearly. Three-dimensional numerical data of resin was obtained by 3D-CT.
Results and discussion: Molded resin with vascular tree network was successfully obtained. However, the X-ray absorption of resin was too weak to observe the fine network structure of resin. On the other hand, X-ray absorption efficiency was enhanced by coating the resin with contrast agent. Clearly image of a mold of vascular tree network was obtained. Internal 3D numerical information of vascular tree network in DC-liver could be obtained. This 3D numerical data provides useful information for the development of practical liver tissue engineering technology.
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53.P03
Analysis of vascularization in explanted chitosan based hydrogels using novel radiotracer

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As far as vascularization is the key challenge in tissue engineering, there is a need for objective tools able to monitore neoangiogenesis inside scaffolds. It is known that a molecular target involved in the angiogenic process is the $\alpha v\beta 3$ integrin. Radiolabelled peptides based on the Arg-Gly-Asp (RGD) sequence have been reported as radiopharmaceuticals with high affinity and selectivity for the $\alpha v\beta 3$ integrin and could therefore be useful as noninvasive molecular imaging techniques. In the present preliminary work, 99mTc-DTPA-bis (cRGDfK) was developed and evaluated in in vitro labelling of sections of bioresorbable chitosan based physical hydrogels that have been implanted subcutaneaously in rats for 1 to 60 days and shown to exhibit angiogenic behavior, on histological examination. The tracer structure presents a bifunctional chelator which is necessary for 99mTc labelling and 2 cyclic RGD peptides. Following incubation with 99mTc-DTPA-bis (cRGDfK) of high radiochemical purity on sections and a correct labelling yield, the sections were analyzed with quantitative high-speed and high-resolution autoradiographies (BetaImager and MicroImager, Biospace Lab, respectively) and areas of binding were identified and pro-

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cessed. Competition binding confirmed good affinity binding. Further studies are necessary but 99mTc-DTPA-bis(cRGDfK) represents a promising novel tracer for angiogenesis in non oncological applications. We thank ANR for financial support.

53.P04
Trace elements concentration in bone

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High concentrations of metals in the natural environment associated with industrial activity and increased migration of metals may cause an increase in the concentration of metals in living organisms, especially in the bone tissue, which reflects their total concentration in the body. Physiological basis for this assessment is based on the fact that the skeleton serves as a major reservoir for ingested heavy metals integrating them into bone matrix during calcification and where they remain until the bone is remodeled or resorbed. Investigations on the bone can be focused on three structural levels: mezostructural, micro, and nanostructural. In this study, we used X-ray fluorescence (XRF) to measure the residual amount of heavy elements in bone particles, obtained by a widely used method. The bones used to perform the experiments were collected from local hospitals, following certain surgical coxofemural prosthesis operations (according to agreed procedures on patient privacy and medical ethics). Studies carried out on samples taken from humans have shown that the variability in bone chemical composition is depending on subject living area, which is essential for understanding the contribution of these factors on bone mass and constitution. Comparing values obtained with concentrations of metals in bones reported by other authors allowed us to conclude that EDPXRF method can be used to assess tissue concentrations of natural elements.

53.P05
μCT evaluation of osteoarthritic changes in the tibia plateau of rats after destabilization of the medial meniscus

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Recently, a nondestructive method was developed to assess changes in quality and quantity of articular cartilage by micro-computed tomography (μCT) in combination with a negatively charged contrast agent. This method was used to study osteoarthritis (OA) development after destabilization of the medial meniscus (DMM) in a rat model. OA was induced in right knee joints of rats by DMM, contralateral knees served as intra-individual controls. At multiple time points after OA induction (2, 4, 6, and 16 weeks) proximal tibias were harvested and fixed in 70% ethanol. Prior to μCT scans specimens were immersed in contrast agent. Segmented images of tibia plateaus were assessed for mean attenuation, cartilage thickness and changes in morphology of the subchondral bone plate. Three dimensional (3D) reconstructions were used for macroscopic evaluation. Results were histologically confirmed in hard tissue sections from the same specimens. Analysis of 3D reconstructions and analysis of segmented images revealed progressing cartilage loss especially in the weight-bearing aspects of the medial tibial plateau. Cartilage damage was associated with increased X-ray attenuation in eroded areas and in focal cyst-like structures. The μCT results were confirmed by histology. μCT analysis is a valuable tool for quantitative assessment of changes in cartilage and subchondral bone plate in small animal models of OA.

53.P06
High resolution desktop tomography for evaluation of tissue ingrowth into polymer scaffolds

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Conventional methods for evaluation tissue engineered scaffold/cell constructs are based on 2D techniques such as histology, scanning electron and fluorescence microscopy. However, these methods are destructive and provide only discrete 2D information. In this work, the X-ray computed tomography method is applied to examine the in-vitro tissue ingrowth potential of biodegradable scaffolds. The capabilities of the method are demonstrated at varying resolutions using both absorption and phase contrast mode. Imaging of the scaffold/tissue composite at a scale of 5 mm and resolution of 5 μm allows visualization of the complex ingrowth of tissue into the biomaterial. Further imaging at 1 μm resolution allows to study of cell-tissue fine structure. These observations illustrate the benefits of tomography over traditional techniques for the characterization of tissue morphology and interconnectivity.

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54. Controlled Drug Release

54.01
Keynote: Controlled stem cell receptor activation using magnetic particle based approches

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One of the aims of controlled release technologies is to optimise localised targeting and regulation of key receptors on cells within tissues in the body. These technologies traditionally involve transport of growth factors and other critical agonists within nanoparticles or other biomaterial carriers to the tissue. Defining the release kinetics of the factors can control cell differentiation at the site. We have been developing a novel magnetic nanoparticle strategy for multifunctional localised control of stem cells in regenerative medicine applications which aims for temporal, spatial and traceable cell differentiation and tissue regeneration in tissues during clinical therapy. Magnetic particles have been used by multiple groups as tracking agents for MRI and targeting for hyperthermia and gene therapy applications. Our strategy involves a remote targeting approach to specific cell receptors for activation. By generating remote external oscillating magnetic fields, we can control receptor activation and promote cell differentiation along different lineages. Recent work includes optimising concentrations of particles to achieve multifunctionality, targeting, tracking and controlled differentiation. Multiple mechano-activatable receptors have been targeted including integrin, TREK ion channels, Wnt and growth factor pathways. Using a chick fetal femur organ culture model, we have demonstrated particle delivery and localised activation of specific receptors.

54.02
Controlled delivery of rhBMP-2 from OPF hydrogel composites for bone tissue engineering

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Considerable morbidity is associated with the repair of bone defects; however, the development of injectable materials could reduce the invasiveness of such procedures. It has been generally difficult to demonstrate extended release of growth factors from synthetic hydrogels alone. Therefore, we have designed hydrogel composites consisting of synthetic oligo(poly(ethylene glycol) fumarate) (OPF) and a naturally derived gelatin microparticle (GMP) drug delivery vehicle that also functions as a digestible porogen. The objective of this study was to evaluate the effect of different composite formulations on the release rate of rhBMP-2. We determined the degradation rate and mass swelling of composites up to 9 weeks and studied the effects of OPF: GMP ratio, GMP charge, and rhBMP-2 loading phase on the release rate of rhBMP-2. We detected rhBMP-2 concentration in the hydrogel supernatant over 4 weeks using 125I-radiolabeled rhBMP-2. Our results indicated significant effects of the loading phase of rhBMP-2 on its release kinetics; however, the OPF: GMP ratio and GMP charge had minor effects on release. Formulations with rhBMP-2 loaded into the GMP phase as opposed to the OPF phase demonstrated significantly reduced burst release with an increased release rate from days 3–17. Here, we demonstrated the development of a hydrogel composite that displays favorable degradation and rhBMP-2 release kinetics for in vivo bone repair.Funded by AFIRM (W81XWH-08-2-0032) and NSF (0940902).

54.03
FGF2 coacervate restores cardiac function

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Classic controlled delivery vehicles are particulates, gels and liposomes. We designed a new controlled release vehicle-a coacervate. The injectable coacervate is sub-micron sized oil droplets self-assembled from a biocompatible and biodegradable polycation, heparin, and fibroblast growth factor-2 (FGF2). FGF2 loaded into the coacervate at nearly 100% efficiency. In vitro assays demonstrated that the matrix protected FGF2 from proteolytic degradations. FGF2 released from the coacervate was more effective in the differentiation of endothelial cells and chemotaxis of pericytes than free FGF2. To test the utility of the FGF2 coacervate in cardiac regeneration, we used mouse infarct model where the left anterior descending coronary artery is ligated. One injection of the coacervate containing 500 ng FGF2 elicited comprehensive cardiac protection in vivo. The cardiac tissue is preserved better in the coacervate group than all the controls: saline, delivery vehicle, and free FGF2. Cardiac function as indicated by fractional area change (FAC) and end systolic and diastolic area is closer to normal in the coacervate group. FAC in the coacervate group is 50% and 88% higher than free FGF2 and saline respectively. We have previously demonstrated potent angiogenesis in subcutaneous tissues and excellent bioactivity of NGF using the coacervate. Taken together, these show the potential of polyvalent coacervate as a new controlled delivery platform.

54.04
Boosting osteogenic differentiation of MSCs by BMP-2 loaded silk fibroin nano particles

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The administration of BMP-2 in the tissue regeneration may be compromised by the blood from the cancellous bone surfaces after decortication washing away or diluting the therapeutic agents. Therefore to complement the loss of therapeutic agents, more growth factors unavoidable to be used. The BMP-2 loaded nanoparticles were designed to overcome the massive loss of therapeutic agents during bleeding, most importantly providing right pharmaceutical doses instead of the luxuriant doses applied currently. Silk fibroin particles had outstanding capabilities of delivering model drugs to cells as well as high cellular uptake efficiencies. Many studies have demonstrated that nanoparticles of sub-micron size have a number of advantages over microparticles as a drug delivery system, such as relatively higher intracellular uptake and available to a wider range of biological targets. Therefore, BMP-2 loaded silk fibroin nanoparticles were fabricated and used to promote osteogenic activity of bone marrow derived stem cells. Finally, better cytocompatibility was observed on stem cells cultured with BMP-2 loaded silk fibroin nanoparticles. Simultaneously, alkaline phosphatase activity of stem cells was also boosted, and cytotoxity of BMP-2 was minimized. Further alizarin red tests and calcium quantification examinations also proved that promising osteogenic differentiation outcomes of stem cells exposed to BMP-2 loaded silk fibroin nanoparticles compared with that of exposure to BMP-2 only.

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54.05
The importance of proper protein handling and detection for the design of a BMP-2 release system

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Although various promising strategies have been proposed for the delivery of BMP-2 for bone regeneration, there is currently no ideal system out on the market. Proper handling and detection of BMP-2 are two factors that are commonly overlooked, resulting in inaccurate characterization of BMP-2 delivery systems. In this work we employed radiolabeling with 125I in an attempt study the growth factor release from a hydrogel system both in vitro and in vivo. BMP-2 (InductOs, Pfizer) was radiolabeled using a modified chloramine-T method and adsorption to sample tubes was studied at different times and temperatures. In vitro release of BMP-2 was compared to the ALP expression. In vivo release was correlated to bone formation in an intramuscular ectopic model in male Sprague–Dawley rats. The results showed that Protein LoBind tubes exhibited the lowest BMP-2 adsorption. Both release studies resulted in a biphasic profile of biologically active BMP-2. Mineralization was observed in vivo after 8 days, with increasing mineral volume and mineral content until day 14. The study confirmed the superiority of radiolabeling over conventional methods such as ELISA, as well as the importance of cautions handling and reliable quantification techniques for successful design of BMP-2 delivery systems.

54.06
Delivery and efficacy of interfering RNA via a crosslinked collagen hydrogel in an excisional in vivo model and a human pre-clinical model to reduce fibrosis

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RNA interference has already been realised in clinical therapy wherein small interfering RNAs delivered systemically can inhibit mRNA and reduce protein expression 1. MicroRNAs (miRs) can silence gene expression endogenously and efforts exist at elucidating their roles in various states of physiology. Notably, miR-29B has emerged as a pivotal regulator of fibrosis and remodelling presenting an opportunity of intervention 2. Hence, the specific objectives of this project are to deliver siRNA mimicking miR-29B from a collagen type I hydrogel of miR complexes in vitro using a human skin model and in an in vivo rat model. Hydrogels formed using atelocollagen were neutralised, kept on ice and crosslinked using 4-arm-Poly (ethylene-glycol) succinimydl glutarate prior to gelation at 37°C. Within these hydrogels siRNA both in unprotected form and complexed with a hyperbranched polymer were mixed and applied to a wounded in vitro skin model previous described and on excisional wounds in vivo (analysed at 28 days). The combinatorial effect of scaffold and miR-29B reduced wound contraction in vivo (~15%). Current studies are verifying the silencing effect of this platform on the gene expression of fibrotic factors

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54.P01
Ionically crosslinked casein nanoparticles as a novel controlled delivery vehicle for flutamide

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A novel particulate delivery matrix based on casein (CAS) nanoparticles was developed for controlled release of flutamide (FLT). Nanoparticle formation was carried out by O/W emulsification stabilized by ionic crosslinking between positively charged CAS molecules and negatively charged sodium tripolyphospate (TPP). FLT-loaded nanoparticles were prepared by varying the TPP/CAS and FLT/CAS mass ratios. Fourier-Transform infrared spectroscopy demonstrated formation of nanoparticles via ionic interaction. TEM measurements showed that nanoparticles exhibited a spherical shape with a size around 100 nm. Both placebo and FLT-loaded nanoparticles were found to have a zeta potential of +16 mv indicating their stability to aggregation. The entrapment efficiencies of the particles ranged between 29% and 42%. In vitro release profile in PBS displayed that 50% FLT was released in the first 5 days. The pharmacokinetic parameters of FLT-loaded CAS nanoparticles were evaluated after i.v. administration to healthy rats. Compared to FLT, the FLT-loaded nanoparticles showed more sustained release, longer circulation time and well toleration during the in vivo studies with no apparent adverse effects. Results illustrated the feasibility of the technique for preparing FLT-loaded CAS nanoparticles which may be effective in prolonging release of anticancer drugs.

54.P02
Designing a novel system for strictly regulating the differentiation of transplanted neural stem/progenitor cells in the brain tissue

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Transplantation of neural stem/progenitor cells (NSPCs) is taken notice as a powerful method for treatment of Parkinson's disease. However, cell-based therapy for treatment of Parkinson's disease has big problems. One of problems is that the differentiation of grafted NSPCs cannot be strictly regulated in the brain. To overcome the problem, in this research, biodegradable microparticles (MPs) were designed to strictly regulate the differentiation of cell. MPs are constructed with PLGA as an outer phase, atelocollagen as an inner phase, and glial cell line-derived neurotrophic factor (GDNF) fused with collagen-binding peptide (CBP), that is GDNF-CBP chimeric protein. The main character of the designed MP is a novel function in which the release of neurotrophic factor encapsulated into MP is time-dependently regulated by using biodegradation of collagen with specific protease. GDNF-CBP was synthesized using Escherichia coli, and was stably-encapsulated using collagen-CBP binding. MPs were prepared using W-O-W emulsion technique. GDNF-CBPs encapsulated in MPs hardly released in PBS. In contrast, an immediate start of release of GDNF-CBP observed in collagenase solution. Bioactivity of GDNF-CBP released from MPs was analyzed with differentiation of NSPCs into neuronal cell, indicating that the released GDNF-CBP has a bioactivity. Consequently, it was shown that our designed MPs have ability for strictly regulating the differentiation of transplanted NSPCs.

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54.P03
Development of evaluation system of growth factors on their stability and biological activity

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Introduction: Growth factor is one of the key components to create a functional tissue. Recently, immobilized growth factor is expected for the development of practical tissue engineering technology. In this study, we synthesized heparin-collagen conjugate and developed an evaluation system of growth factors on their biological activity and the stability.
Materials and methods: Heparin-collagen conjugate was synthesized by using 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide and N-hydroxysuccinimide. Air-dried heparin-collagen film was used for not only the evaluation of growth factor-immobilizable efficiency but also in vitro cell culture. Primary rat hepatocytes and normal human umbilical vein endothelial cells (HUVEC) were used.
Results and discussion: HGF and VEGF were immobilized on this heparin-collagen film. The immobilized ratios of the growth factors were more than 90%. Primary rat hepatocytes and HUVEC could be cultured on this growth factor-immobilized heparin-collagen film even in growth factor-free culture medium. HUVEC proliferated depend on immobilized VEGF density. Based on the results, about 50% of immobilized VEGF was stably maintained their biological activity during 16 days even in culture condition. These results indicated that immobilized growth factors will be a promising material for the practical tissue engineering technology.
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54.P04
Injectable hydrogel containing affinity-driven self-assembled nanoparticles for therapeutic angiogenesis of hindlimb ischemia

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The proper spatio-temporal delivery of multiple therapeutics represents a major challenge in strategies aimed at inducing tissue regeneration and angiogenesis after major ischemic insults. To address this, we developed a delivery platform made of an injectable in-situ forming alginate hydrogel incorporating growth factors within self-assembled nanoparticles (NPs). The NPs are spontaneously formed as a result of electrostatic, affinity-binding interactions between heparin-binding proteins and the synthetic alginate-sulfate (AlgS). The NPs have an average diameter of 100–200 nm and negative surface charge (ζ potential = -20 mV), indicating that AlgS is present on the NP surface. When entrapped in NPs, the growth factors are protected from enzymatic degradation by trypsin, as shown by mass spectrometry (MALDI-TOF). In a model of hindlimb ischemia, the injection into muscle of NPs of the three angiogenic factors (VEGF, PDGF-BB and TGF-β1) in in situ forming hydrogel significantly enhanced blood perfusion (Laser Doppler imaging) and vessel density (α-SMA staining), as compared to animals treated with the factors dissolved in saline. The results strengthen our recent work, which revealed a long-term efficacy of this system in a model of acute myocardial infarction. The simple design and scalability of the delivery platform together with its efficacy in prolonging the activity of multiple growth factors makes it a valuable tool for clinical applications in regenerative medicine.

54.P05
Polycaprolactone and polycaprolactone/tricalcium phosphate scaffolds with the bactericidal properties

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Nowadays, scaffolds for tissue engineering (TE) should provide not only mechanical support for neo-tissue formation, but also actively participate in regeneration processes. In addition, to avoid infections or biofilm formation, also the controllable drugs release is desirable in TE. Therefore, aim of the study was to develop polymeric and composite scaffolds with bactericidal properties. Two types of cylindrical scaffolds (fi = 6 mm) were fabricated using rapid prototyping technique. The first one was made of PCL and 15% of gentamicin sulfate (GS). The second one was made of PCL, 5% of tricalcium phosphate (TCP) and 15% of GS. The scaffolds were characterized in the terms of structure and physicochemical properties using gel GPC, DSC, SEM and microtomography (μCT). The kinetics of drug release from the scaffolds was tested in vitro, and against three bacterial strains: Staphylococcus aureus, Pseudomonas aeruginosa and Staphylococcus epidermidis.The DSC and GPC results have shown no significant influence of TCP and antibiotic on the thermal properties and on the molecular weight of the polymer. The SEM observations and μCT scans have shown good distribution of TCP and GS in the polymeric matrix. The PCL scaffolds show faster drug release than the composite scaffolds. However, the both types of scaffolds provided similarly good bactericidal properties during microbiological tests. In all cases, during the two-weeks tests, the inhibited bacterial growth zones were observed.

54.P06
Polysaccharides impregnated vancomycin-PMMA implants for treatment of methicillin-resistant Staphylococcus aureus osteomyelitis

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The aim of this study was to enhance drug release from polymethyl methacrylate (PMMA) cement by incorporation of polysaccharides. The cement tablets comprising of PMMA, vancomycin, and polysaccharides (chitosan, alginate, pectin) were prepared using mould method. Surface morphology and porosity of these cement composites were studied by scanning electron microscopy (SEM). Vancomycin released from the cements was determined over 4 weeks. The drug was assayed using both UV-spectrophotometry at 280 nm, and microbiological method using agar diffusion technique against Micrococcus luteus. SEM micrographs showed highly porous structure of all polysaccharide-PMMA composites, whereas the pore was not found in the PMMA cement. Vancomycin release from the cement composites was remarkable increased as 6 to 7-folds above PMMA cement over 4 weeks. The highest drug amount was observed from the PMMA-alginate composite as 33.82 ± 0.06 mg, followed by pectin, and chitosan cements as 31.98 ± 0.35 mg, and 28.09 ± 0.33 mg, respectively, whereas 4.50 ± 0.03 mg was released from PMMA cement after 4 weeks. During 28 days the released drug was well above the MIC against Staphylococcus aureus ATCC 25923 (MIC = 0.5 mcg/ml), and methicillin-resistant Staphylococcus aureus (MRSA) DMST 20654 (MIC = 1 mcg/ml). These data indicate that polysaccharides impregnated in PMMA composites can be a potential candidate for enhanced antibiotic carrier for treatment of MRSA osteomyelitis.

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54.P07
Light triggered drug release

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In therapy of cancer the systemic application of drugs is the most common procedure (chemotherapy). Highly undesirable side effects of these drugs to the entire organism like bone marrow suppression, immunosuppression or alopecia (hair loss) is still a problem in this kind of treatment. By targeting and controlling dosage of anticancer drugs side effects can be minimized. Thus, product efficiency and safety, as well as patient convenience and compliance can be improved. In a novel approach the model drug/antimetabolite 5-Fluorouracil (5FU) is immobilized on/released from a microgel surface. By use of (2+2) Diels-Alder cycloaddition a dimer of 5FU is synthesized in a UV-photoreactor and subsequently bound to a beta-cyclodextrin cage. Photochemical cleavage of 5FU dimers occurs in a certain range of wavelength and allows a controlled - light triggered - release of 5FU. In this study the influence of different light sources, intensities and irradiation times on release kinetics is investigated. The drug release is quantified by high pressure liquid chromatography. Aim of the activities is the development of a drug loaded stent with targeted and controllable drug release by use of laser irradiation. Research on this novel drug release system is funded within the 'Boost Fund' program of 'Exploratory Research Space' at RWTH Aachen – ERS.

54.P08
Heparin mimetic peptide amphiphile nanofibers for angiogenesis

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Angiogenesis is a basic physiological process during both development and tissue regeneration. Formation of blood vessels is triggered by interaction with the glycosaminoglycans (GAGs) situated in extracellular matrix (ECM) and vascular endothelial growth factor (VEGF) secreted by endothelial cells. Since VEGF activity is modulated by heparin which is a component of ECM, mimicking the ECM is a promising approach in tissue engineering. Here, we demonstrate a novel peptide nanofiber system that mimics the heparin functionality to induce angiogenesis in pancreatic islets without addition of any exogenous growth factors in vitro. Viability of rat islets was examined by Alamar blue/DNA assay for 21 days. Sprouting was shown by lectin staining. In order to show the functionality of islets, glucose-stimulated insulin release assay was performed. Results indicated that heparin-mimetic PA increases the viability of islets compared to control group. These promising results stem from peptide nanofibers' bioactivity, which induce sprouting formation and mechanical support that keeps the islets intact. Also, glucose assay showed that cells are functional and produce insulin. In conclusion, we report that heparin-mimetic PA nanofibers designed by our group alleviates some of the handicaps of islet transplantation.

54.P09
Modeling of release kinetics of silver nanoparticles from novel alginate nanocomposites aimed for biomedical applications

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Alginate hydrogels with embedded silver nanoparticles (AgNPs) are very attractive for biomedical applications as antimicrobial materials due to the possibility of AgNPs controlled release. In order to predict the antimicrobial activity, it is necessary to know the release kinetics, which depends on the hydrogel size and shape as well as on the surrounding hydrodynamic conditions. In this study, we have produced alginate microbeads (1.9% w/v, ~600 μm in diameter) with incorporated, electrochemically synthesized AgNPs (1.8 mM) and studied the silver release kinetics in distilled water at 37°C in three different systems: static dishes, shaken flasks and packed bed bioreactors (0.27 ml/min perfusion rate). AgNPs release was monitored over 3 weeks by UV-Vis spectroscopy while total silver concentrations in water and in microbeads were determined by AAS. Release kinetics of AgNPs was modeled by internal diffusion within the microbeads followed by external mass transfer and silver oxidation, assumed to be a first order reaction. Internal diffusion was found to be rate limiting in all investigated systems with the AgNPs apparent diffusion coefficient in the hydrogel of ~10^(-11) cm^2/s, while the fluid flow increased the overall transfer rate for 65–85%.

54.P10
Genipin crosslinked gelatin hydrogel: Tolbutamide release and cytocompatibility

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Gelatin based drug carrier matrices have gained immense interest as a promising class of delivery system. The aim of this study was to develop model drug Tolbutamide -loaded-gelatin based gels. Gelatin gel due to its reversible gel-sol transition at ~35°C and potentially low gel strength is often chemically crosslinked to improve its thermal and mechanical stability. Here, gelatin matrices were crosslinked with genipin, a naturally occurring crosslinker for the release of tolbutamide. Tolbutamide (an anti-diabetic drug) was incorporated into the gelatin matrices to form drug loaded gel for the release study. Morphological analysis of crosslinked gels using confocal microscopy revealed network structure. The release of tolbutamide from the crosslinked gels indicated an initial increase upto 8 h. This was followed by a steady release state after 20 h (52%). Release kinetics was determined following Peppas model. The diffusional exponent (n) (0.84 ± 0.01) ($P < 0.05$) indicated anomalous release behaviour. Further, in vitro cellular compatibility and normal cell proliferation in AH-927 cell line was observed in live dead assay and fluorescence microscopy studies using propidium iodide staining. The studies supported that the matrices display excellent compatibility without compromising the cellular integrity and thus, can be utilized as safe carrier matrices for drug transport.

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54.P11
Click-hydrogel therapy to prevent re-synostosis in pediatric mouse model

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Craniosynostosis is the pathologic fusion of cranial sutures and requires calvarial remodeling. In many cases, rapid re-closure of the skull occurs and is associated with a high incidence of complications. The objective was to develop a novel injectable click hydrogel for controlled delivery of the BMP antagonist Gremlin to delay bone growth. Our click hydrogel cross-links spontaneously in less than 1 min via Cu-free click-chemistry between poly(ethylene-glycol) and our newly dibenzocyclooctyne cross-linker. Release kinetics were performed in vitro and in vivo by incorporating a fluorescently labeled protein into hydrogels. A posterior frontal defect was made in infantile (21-days post-natal) C57Bl/6 mice (5 mice/group/time). Defects containing one of the following treatments and evaluated by μCT and histology 5 and 14 days later: empty defect, hydrogel+carrier, hydrogel+300 ng rhGremlin, and, hydrogel+500 ng rhGremlin. Increasing the concentration of the cross-linker resulted in a more controlled and linear release. Quantification of fluorescent images of the defect showed that more protein was retained when delivered with the hydrogel. There was a dose-dependent decrease in defect width and bone volume in the defect with hydrogel containing Gremlin. The hydrogel was able to provide controlled delivery of rhGremlin to delay the post-operative re-synostosis and has potential to greatly reduce the complications associated with the surgical management of craniosynostosis.

54.P12
Antioxidative and physicochemical characteristics of hydroxyapatite-calcium sulfate bone cements impregnated phenolic compounds crosslinked fish gelatin-chitosan

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Hydroxyapatite and calcium sulfate are widely used as bone cements due to their biocompatibility and nontoxicity. However, poor physical characteristics of the cements such as high friability, and fast disintegration in body fluids limit their applications as drug carriers. This study was focused on improvement of physicochemical properties of bone cements by incorporation of chitosan and marine collagen. Triphala, Thai herbal medicine comprising of polyphenolic compounds, was used as crosslinking agent of gelatin and chitosan. The cement tablets were prepared using mould method and investigated for their physicochemical properties. With increasing of triphala concentrations of the cement composites, increased hardness and decreased friability were observed. SEM micrographs showed alteration of surface morphology of the triphala incorporated cement composites. These tablets were stable in PBS at pH 7.4 over 1 month. DPPH free radical scavenging assay revealed remarkable antioxidant activity of the cement composites. These data suggest that triphala incorporated in chitosan-fish gelatin bone cements may be suitable as skeletal drug delivery systems and bone tissue engineering.

54.P13
A biodegradable, delayed burst-release device for the delivery of vaccine 'booster shots'

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A single-administration vaccine delivery device (SAVDD) has been conceptualised to achieve spontaneous burst release of a vaccine with a tailorable lagtime, using a biodegradable polymer capsule. The SAVDD is implanted subcutaneously and designed to provide a burst-release of vaccine at a specific time up to a year after implantation, by varying the composition and geometry of the polymer capsule. Biodegradable PCL-PEG co-polymer capsules were prepared by melt electrospinning. Polymer degradation was evaluated in phosphate buffered saline at 37°C using dye marker technology, followed by implantation in an in vitro porcine skin tissue culture model. Preliminary degradation studies have shown that it is possible to tailor the rate of polymer degradation enabling burst-release of the vaccine with a tuneable lagtime. Vaccine stability has been assessed using Chlamydia Muridarum MOMP-MBP. Protein degradation assays (nanodrop, BCA, SDS Page) were conducted from -20 to 37°C with varying protein concentrations. The development of this SAVDD aims to provide a generic technology platform that can be applied to any repeat administration vaccine. This technology platform can improve both the logistics and efficacy of immunisation programs around the world, particularly where access to medical care is limited, such as in remote communities, developing countries, and for the immunization of wildlife.

54.P14
Investigation of copper-alginate microbeads as antimicrobial materials for potential biomedical applications

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Alginate hydrogels cross-linked with copper ions are promising biomaterials for biomedical applications due to their biocompatibility, bioinductivity and antimicrobial properties. While highly-hydrophilic alginate matrices can serve as cell carriers or wound dressing, Cu ions provide antimicrobial activity and as essential micronutrients and cofactors for many enzymes, promote angiogenesis and healing processes. In this study, we have optimized production of Cu-alginate microbeads ($\sim$ 500 μm) by electrostatic extrusion of 1.9%w/v Na-alginate solution into 0.135 M $CuSO_4$ gelling solution. Release kinetics of Cu ions in saline solution (0.9% w/v NaCl) as well as in distilled water were investigated in conjunction with studies of antimicrobial activity of the microbeads in suspensions of E. coli and S. aureus. Experiments were performed in shaken flasks at 37°C for 24 h (4 g of microbeads in 10 ml of solution). It was found that Cu ions quickly induced alginate gelation resulting in the Cu concentration of 0.157 mM/g in the hydrogel. However, when Cu-alginate microbeads were placed in water or saline solution, Cu ions were released over 5 h period to the final concentration in solution of $\sim$20 mM. This release trend was found to be very efficient inducing quick bactericidal effects in both cultures even after 1 h of incubation. These results suggest that copper release rate and antimicrobial activity of Cu-alginate could be optimized for each particular application.

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54.P15
Evaluation of release properties of protein loaded microspheres

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The study of in vitro release profiles of proteins delivery systems gives in advance information on the in vivo drug release behavior and success of the system. The purpose of this study was to assess the effects of different types of release enviroments (phosphate buffer saline, cell culture medium with serum and synovial fluid) on the in vitro release of a model protein from poly (ϵ-caprolactone) (PCL) microspheres. Microspheres were prepared by modifying the double emulsion-solvent evaporation method. Encapsulation efficiency of protein loaded microsphereres was found 54.03 ± 1.32%. SEM examinations showed that microspheres had spherical shape and rough surface without pores. The mean particle size of protein loaded PCL microspheres was 5.79 ± 0.12 μm. Release profile of the protein from microspheres was studied in PBS for 14 days at 37°C and the amounts of total and biologically active protein released from microspheres were determined by BCA and ELISA, respectively. Amount of total protein released from PCL microspheres was found higher than that of amount of biologically active protein released from PCL microspheres. Additionally, release profile of model protein from microspheres will also be carried in cell culture medium containing 5% FBS and in human synovial fluids for 14 days. The released amounts of total and biologically active protein from PCL microspheres in PBS, synovial fluids and cell culture medium will be compared at the end of this study.

54.P16
Poly-ϵ-caprolactone/poloxamine matrices for drug delivery and bone regeneration applications

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The design of implantable devices that can locally provide both high levels of antimicrobial drugs and osteoconductive properties is an interesting way to improve current therapeutic strategies for osteomyelitis management. We have previously observed that certain poloxamine varieties (X-shaped PEO-PPO copolymers) can induce differentiation of mesenchymal stem cells to osteoblasts (Rey-Rico et al. Eur Cells Mater. 2011; 21:317–340). Physical blends of poly-ϵ-caprolactone (PCL) and different varieties of poloxamine (Tetronic® 908, 1107, 1301 and 1307), also incorporating ciprofloxacin, were prepared by hot melting, through a solvent-free and eco-friendly methodology. Blending of PCL with poloxamine led to matrices with mechanical properties similar to those of fresh cortical bone. Implantable devices were able to sustain the release of ciprofloxacin for more than six months being its release rate accelerated when poloxamine was present in the matrices. All implants of PCL: poloxamine showed good cytocompatibility with osteoblasts. Matrices prepared with Tetronic® 908 enabled the differentiation of mesenchymal stem cells to osteoblasts. Overall, PCL poloxamine implantable matrices, with tunable size and form, are potentially useful not only for the local treatment of bone infections requiring antibiotic release over a long period of time, but also as scaffolds for bone regeneration.

54.P17
Characteristics and release profiles of PEG-PCL-PEG microspheres containing immunoglobulin G

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Polyester-polyether type block copolymers have attracted much attention in the area of drug delivery systems with their capability in providing biomaterials having a broad range of amphiphilic characteristics. The aim of the present work was to prepare and characterize immunoglobulin G (IgG) loaded poly(ethylene glycol)- poly(ϵ-caprolactone)-poly(ethylene glycol) (PEG-PCL-PEG) microspheres as potential drug carriers. PEG-PCL-PEG triblock copolymer was synthesized by ring-opening polymerization of ϵ-caprolactone initiated by MPEG and characterized. IgG loaded PEG-PCL-PEG microspheres were prepared by using the double emulsion-solvent evaporation method. Encapsulation efficiency of microspheres was 74.32 ± 3.45%. According to the scanning electron microscopy images, microspheres had spherical shapes and rough surface without pores. Release profiles of γ-irradiated and non-irradiated microspheres were studied in PBS (0.01 M, pH 7.4) for 7 days and the amounts of total and biologically active IgG released were quantitated with BCA and ELISA methods, respectively at different time points. The IgG release profiles of γ-irradiated and non-irradiated PEG-PCL-PEG microspheres showed similar trends. No significant difference was observed between the amounts of IgG released from γ-irradiated and non-irradiated PEG-PCL-PEG microspheres ($P > 0.05$). This study showed that PEG-PCL-PEG microspheres are promising systems for the controlled release of proteins.

54.P18
Chondrocyte extracellular matrix modification by indomethacin controlled release from alginate gels

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are currently used in the treatment of different types of arthritis such as osteoarthritis (OA) reducing its sympthoms. There are some clues showing that Indomethacin may be reducing several inflammation mechanisms. However, there is a need for further information about how Indomethacin modifies the chrondocyte extracellular matrix (ECM). Naturally derived alginate gels were used as a scaffold in order to provide an ideal environment for chondrocyte culture as well as a drug delivery system. Matrilins are known ECM proteins which play a key role in cytoskeletal dispositions and tissue remodelling. Indomethacin was loaded (0.1 μM–160 μM) into alginate gels and released over a maximum period of 15 days. Human chondrocytes isolated from arthrosic knees were cultured in the scaffolds for 5, 10 and 15 days. The expression of Glycosaminoglycans(GAGs) and Matrilins (1, 3) was studied by means of qPCR. Inflammatory cytokines were also measured (IL-6,TNF-alpha) along with MMP-13 expression. Live/Dead rate was calculated by Confocal Microscopy. As a result Indomethacin was successfully released showing a low cytotoxic rate and exerting a effect in GAGs metabolism which seemed to be modulated by a combined Matrilin expression.

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54.P19
Modulating drug release kinetics of PLGA microspheres by fabricating PLGA-alginate core-shell drug delivery devices with microfluidic method

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Controlled drug delivery devices offer numerous advantages compared with conventional drugs. Among all the devices, PLGA microspheres are of particular interests for biocompatibility, biodegradability and easy administration. However, control of the drug release kinetics is still a major challenge due to the complex release mechanisms [1]. Recently, near zero order release were observed from PLGA microspheres / alginate beads composite devices [2]. However, such structure eliminated the easy administration property. In this study, PLGA-alginate core-shell devices were developed by microfluidic method to modulate the drug release kinetics while keeping the microspheric geometry. PLGA-alginate core-shell devices were fabricated by O/W/O emulsion systems utilizing capillary microfluidic devices [3]. The resultants were characterized by SEM, microscopy, in vitro release test and MTT assay. The core-shell structure was confirmed by SEM and microscopy. The drug release kinetics of PLGA microspheres were significantly modulated by the shell layer. Initial burst was suppressed and a near zero order release was achieved when the shell layer was saturated with released drug. PLGA-alginate core-shell devices were biocompatible and may be promising tools for controlled drug delivery.

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54.P20
Antibiotic-eluting calcium phosphate foams for bone regeneration

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Calcium Phosphate Cements (CPCs) are used as synthetic bone grafts and have been shown to have high potential not only for bone regeneration, but also as local drug delivery systems due to different properties, among which their nano- and microporosity. However, this intrinsic porosity of CPCs does not always allow the drug to be fully released to the surrounding media. Calcium Phosphate Foams present an additional macroporosity in comparison with CPCs, fulfilling the requirements of pore size and interconnectivity that are relevant for bone regeneration. Moreover, the open macroporosity is expected to strongly modify the drug release kinetics. It is the aim of this work to develop antibiotic-eluting self-setting calcium phosphate foams. To this end, Doxycycline hyclate is added in an alpha-tricalcium phosphate slurry, which after foaming is able to set, producing a solid hydroxyapatite foam. The effect of the antibiotic addition on the setting reaction and structural parameters of the foam is studied, and the antibiotic release profile is determined for different antibiotic concentrations. Results show that calcium phosphate foams are able to release higher drug percentages (26.01% ± 8.83 at 100 h) than their unfoamed counterparts (5.10% ± 1.49), and the antibiotic release is sustained for longer periods of time. Moreover, increasing the amount of doxycycline in CPFs leads to important structural effects in the foam architecture, such as an increase in pore interconnectivity.

54.P21
Insulin entrapment and release from zinc-containing silica microparticles

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The major problem associated with oral delivery of insulin is the low bioavailability in the stomach due to the susceptibility to enzymatic attack. Microencapsulation of an active material is considered for protection and controlled release. The encapsulation of proteins in zinc silica matrix offers several advantages. Inorganic silica matrix isolates and constrains the movement of the biomolecules preventing their aggregation and denaturation, while the zinc oxide improves the stability of the system especially at low pH values. The purpose of this study was to encapsulate insulin into zinc silica microparticles and microspheres using sol-gel and spray-drying methods, respectively, and to characterise these particles with respect to average size, specific surface area, porosity and morphology. The insulin secondary structure was assessed using FTIR spectroscopy and indicates a high structural integrity of insulin in sol-gel derived particles. For release assays, the as prepared samples were immersed in solutions with different pH values and kept at 37°C for different periods of time. The amounts of withdrawn solutions were analyzed by UV-VIS spectroscopy in order to identify the insulin level for a prolonged period.

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54.P22
Core-shell PVA/PCL nanofibres with liposomes as a novel drug delivery system

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The incorporation of growth factors (GF) into liposomes in the core/shell nanofibres may improve the stability of GF and positively influence the tissue regeneration. In the study, the core/shell polyvinyl alcohol/poly-ϵ-caprolactone (PVA/PCL) nanofibres with liposomes-incorporated bFGF, IGF-I, and TGF beta 1 (Lip-PVA/PCL) in the core, and control nanofibers with GF blend (PVA/PCL) were prepared and tested their effect on the viability and proliferation of mesenchymal stem cells (MSCs) using MTS assay, and BrdU and PicoGreen assays, respectively. Cells on the scaffolds were visualized using confocal microscopy. The 22 day-release profile of GF was evaluated using ELISA assays. MSCs on Lip-PVA/PCL scaffolds showed significantly higher both proliferation and viability compared to PVA/PCL. The release profile of GF in Lip-PVA/PCL was lower and sustained release of GF was observed compared to PVA/PCL. The core-shell PVA/PCL nanofibers with liposomes can be used as the novel drug delivery system. Supported by the research plans AV0Z50390703 and AV0Z50390512, MSMT research programs NPV II 2B06130 and 1M0510, grant no. MSM0021620849, and project ERA-NET CARSILA,

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54.P23
BMP-2 release from low-temperature processed calcium phosphate foams

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Although it has been shown that the specific release of BMP-2 can modulate the osteogenic capacity of calcium phosphate (CaP) bone grafts, the interaction BMP/material in the release behavior is a crucial aspect which remains largely unknown. Thus, this work aims to determine the in vitro release profile and the biological activity of BMP-2 loaded CaP foams with different compositions and to assess their osteoinductive capacity by subcutaneous implantation in athymic nude mice. The foams were obtained at 37°C by foaming an alpha-tricalcium phosphate (α-TCP) slurry and the progression of the hydrolysis reaction from α-TCP to hydroxyapatite (HA) was adjusted to obtain specific proportions of the two phases. 1ug of rhBMP-2 was then adsorbed on the foams containing 50, 80 and 100% HA respectively. In vitro results showed that the BMP-2 released from the foams and its activity was strongly dependent on the composition of the foam, being more sustained in the samples containing 80% HA, and with a higher activity as determined by osteocalcin expression in human mesenchymal stem cell cultures. The 8 week-ectopic implantation of the foams corroborated this trend as shown by the significant increase in bone neoformation, bone marrow and blood vessels formation. In conclusion, the composition of the CaP substrate strongly influenced the protein release pattern, which in turn resulted in a different in vivo performance of the BMP-2 loaded scaffolds in terms of osteoinduction potential.

54.P24
Intrinsically magnetic Fe-substituted hydroxyapatite for medical application

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Nowadays the use of magnetic stimulation or guidance in regenerative medicine is coming up as one of the most attractive concept to design magnetisable scaffolds. Considering the importance of having magnetic scaffold based on no-toxic magnetic nanoparticles (MNPs), the present work is focused on the development of an innovative biocompatible and bioresorbable superparamagnetic phase by doping hydroxyapatite (HA) with Fe ions. XRD and Rietveld analysis confirm that the new phase is an HA with a disordered structure where $Fe2^+$ and $Fe3^+$ occupied the two independent calcium sites with a specific coordination so to generate intrinsic superparamagnetism. Magnetic Fe-HA can be used as a conceptually new type of biomaterial for hard tissue regeneration or as a valid bioactive substitute for the not biodegradable magnetite MNPs. The possibility to functionalize the Fe-HA MNPs with growth factors or drugs results to be an innovative delivery system that can be used both for regenerative medicine and for cancer therapy, due to the possibility to move and to fine control, by an external magnetic field, the release of the bound molecules in the target bone site. Moreover, thanks to the hyperthermia effect induced by the Fe-HA magnetic properties (4.2 emu/g at 2T), these new Fe-HA MNPs can be used in cancer therapy: they exhibit an increase in temperature of about 40°C in 60 s under the exposition of magnetic field. Studies in vitro and in vivo have assessed the biocompatibility of Fe-HA.

54.P25
Evaluation of phytoncide-eluting stent coated with biodegradable PLGA

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Drug-eluting stent (DES), metallic stents coated with drug-loaded non-degradable or degradable polymers, has become a standard treatment in percutaneous coronary intervention (PCI). Among drugs for DES, it has been known that sirolimus with immunosuppressive activity and paclitaxel with potent anti-proliferative effect are the most representative drugs. This is why they can prevent in-stent restenosis that induces the proliferation of smooth muscle cells. However, it was recently reported that there were potential risks that inhibit endothelialization and delay vessel healing. Phytoncide, a substance released from trees and plants, has many attractive effects such as human NK cell activity and an anti-oxidant. In particular, monoterpenoids in phytoncide were known as compounds with anti-oxidative and anti-microbial activities. Hence, we investigated in vitro bioactivity and release behavior of phytoncide as an alternative drug of sirolimus and paclitaxel. Cell viability and anti-proliferative effects of phytoncide and sirolimus on smooth muscle cell (SMC) and endothelial cell (EC) were characterized by CCK-8 assay. In vitro migration assay was performed to evaluate the inhibitory effect on cellular migration. For drug release in vitro, phytoncide incorporated in poly(lactic-co-glycolic acid) (PLGA) was coated on stainless steel by our electrospray method. Obtained results suggest that phytoncide could be promising to replace sirolimus or paclitaxel as a drug for DES.

54.P26
Hollow calcium carbonate/calcium silicate microspheres for drug delivery

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Bioceramic microspheres for bone regeneration applications should have good bioactivity, biodegradability, and controlled drug-release ability. Calcium silicate (CS) exhibits good bioactivity and controlled drug-release ability. The main disadvantages of CS are its low degradability and difficult to fabricate into spherical form. In the present study, hollow calcium carbonate/calcium silicate (CC/CS) hybrid microspheres with desirable properties were prepared using the CO2 bubble template method. The morphology and composition were examined by scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Additionally, methylene blue (MB) was used as model for evaluating the feasibility of CC/CS microspheres on drug-delivery system. The cell viability of MG63 was used to examine the biocompatibility. Results showed that monodisperse hollow microspheres with size of about 10 μm could be obtained by controlling the concentration of Si precursor. XRD and FTIR demonstrated that the hollow spherical structure consisted of vaterite. Moreover, the loading capacity of MB was remarkably enhanced from 35 to 105 μg per mg of particles while Si was incorporated into CC in the microspheres. The viability of MG63 cultured with CC/CS microspheres revealed good biocompatibility, comparable to the tissue culture plate control. It is concluded that the hollow CC/CS microspheres might have the potential for bone repair and drug-delivery system.

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54.P27
Monodisperse hydrophilic multi-block copolymer microspheres for controlled intrarenal drug delivery

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The increasing prevalence and treatment costs of chronic kidney disease call for novel therapeutic strategies that prevent disease progression at an early stage. We propose polymer microspheres (MSP) with physical and chemical properties tailored to controlled intrarenal drug delivery as a tool to modulate processes responsible for chronic kidney disease, such as inflammation and fibrosis. In this study we generated monodisperse, hydrophilic-phase-separated-multi-block copolymer-based MSP and investigated their intrarenal biocompatibility and thus adequacy for future therapeutic application. Monodisperse MSP (30 μm) were injected under the capsule of healthy F344 rat kidneys. Kidneys were retrieved 3,7,14 and 28 days after MSP implantation. General histology was evaluated by PAS staining. Renal interstitial presence of macrophages and myofibroblasts as a result of MSP implantation was studied by IHC. MSP were detected in the subcapsular space at all time points and showed little degradation in time. MSP implantation did not affect tubular integrity at any time point. Macrophages and myofibroblasts were virtually absent in the renal interstitium. We report for the first time the intrarenal biocompatibility of MSP designed for intrarenal drug delivery. Based on their slow degradation rate and the lack of adverse effects of their subcapsular implantation on the renal tissue, we propose monodisperse MSP to be a promising tool for sustained, controlled intrarenal drug delivery.

54.P28
Multi-factor release systems for regenerative applications in tissue engineering

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As regenerative implants are used for the treatment of cartilage and bone defects, a major challenge lies in the development of suitable mechanisms of growth factor delivery as naturally occurring concentrations are needed to enable regenerative properties of incorporating implants. Biomimetic materials for implants become more popular due to their improved compatibility, and they also offer an ideal platform for growth factor release systems mimicking the ECM as a reservoir for growth factors. By modulating the binding affinities, different release kinetics can be achieved and finally used to create a modular setup of chronological assessable multi-factor release systems whose composition then could be adapted to various application-specific manners. To investigate the influence of modulated heparine affinities on the release kinetics, GDF-5 was equipped with different artificial heparine binding domains. Although both, the DNA recombination and the protein dimerization could be confirmed successfully, in vitro ALP assays revealed a loss of their biological activity and SPR interaction studies with the cellular receptors BMPR-IA and BMPR-IB showed a significant decrease in their binding affinities. Assuming protein misfoldings as a possible explanation, the purification and renaturation steps performed need to be analyzed in detail and subsequently adopted appropriately to generate biologically active muteins whose binding kinetics then can be examined and further refined.

54.P29
A bone graft composite of demineralized bone matrix and alendronate-loaded hydroxyapatite microsphere ameliorates osteoporosis

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Osteoporosis is an abnormal reduction in bone mass and bone deterioration leading to increased fracture risks. Despite variety of therapeutic approaches were developed, many adverse effects were found and needed to be solved. We have developed a new bone graft composite (HAP-ALD-DBM) that is hydroxyapatite-based microsphere loaded with alendronate, demineralized bone matrix (DBM), and carboxyl methyl cellulose (CMC) combinations for prevention of osteoporosis, when it is used bone repairs and regenerative processes. The HAP-ALD-DBM and HAP-ALD (the same microsphere without DBM) were evaluated in vitro chemical characteristics and in vivo biocompatibility and safety, in particular for ameliorating osteoporotic processes by the grafts. The experimental results showed the bone graft composite is highly safe and biocompatible with animals tested. Furthermore, new bone deposits occurred in the area of bone graft implanted sites after 13 weeks. On the basis of serological tests, calcium contents, bone density revealed differences among treatment groups. However, HAP-ALD-DBM bone graft composite showed clear evidences of bone regenerative effects on bone repairs. And it showed the greatest increase of bone mass and density in radiological and histological findings at graft sites.

54.P30
Development of novel statin releasing biodegradable nano-scaled fiber for bone tissue engineering

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Introducton: Tissue engineering is an effective approach for the treatment of bone defects. Statins have been widely and safety used for the treatment of hyperlipidemia for more than two decades. Some studies have reported pleiotropic effects of statins on bone formation. Meanwhile, electrospun biodegradable fibers have a similar structure with the natural extracellular matrix (ECM), and have shown applicability to carriers for drug delivery system (DDS) and scaffolds for bone tissue engineering. We developed simvastatin-releasing, biodegradable fiber scaffold (SRBFS) by electrospun procedure. The objective of this study was to investigate the feasibility of SRBFS for the treatment of bone defects in comparison with β-tricalcium phosphate (β-TCP) which is clinically used frequently as a bone repair material.
Materials and methods: Non-healing, critical-sized (4 mm) calvarial defects were created in the left parietal bone of 8 weeks-old male mice. Defects were treated with SRBFS, biodegradable fiber scaffold without simvastatin, and β-TCP particles. Histological, biochemical analysis and micro-CT scan analysis were performed for 6 and 18 weeks after implantation.
Results: The new bone formation in the SRBFS was significantly higher than in the other two groups both 6 and 18 weeks after implantation.
Conclusion: These results suggested that our strategies using statin releasing biodegradable fiber scaffold may be favorable for the treatment of bone defects.

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54.P31
Enhanced orthotopic bone regeneration promoted by intracellular delivery of dexamethasone

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Cell engineering approaches are a promising tool to promote the regeneration of functional bone, particularly regarding the use of osteoprogenitor cells. The main goal of this work was to direct rat bone marrow mesenchymal stem cells (rBMSCs) towards the osteoblastic phenotype, through the use of dexamethasone-loaded amphiphilic nanocarrier intracellular delivery systems (dex-micelles), in order to induce new orthotopic bone formation in vivo. Dexamethasone is a glucocorticoid known to stimulate osteogenic differentiation of MSCs in animal models and humans. The dynamic nature of the nanoparticulate carriers allowed a pH-responsive release of the drug, promoting a faster delivery under acidic conditions as the ones observed in the endossome compartment. Internalization studies with rBMSCs showed an effective internalization of the dex-micelles up to 24 h of incubation and the consequent intracellular release of dexamethasone up to 7 days led to increased levels of osteogenic-related markers. The implantation of gelatin hydrogels seeded with rBMSCs pre-cultured with dex-micelles promoted a highly significant enhancement of de novo bone formation in a rat ulna critical size defect, in a dose-dependent manner. One month post-implantation, new tissue with clear hierarchical organization was found in the groups loaded with the nanocarriers. These results highlight the potential of the proposed intracellular delivery system as a powerful approach to promote bone regeneration.

54.P32
In vivo bioluminescent imaging of enhanced skeletal muscle regeneration by enzyme modulated oxygen generating system

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This study addressed the hypothesis that skeletal muscle regeneration can be enhanced by controlled oxygen producing system which is modulated by an enzyme, catalase. Furthermore, this regeneration process in vivo was tracked by firefly luciferase expressing cells using bioluminescence (IVIS) imaging (BLI). A severe animal model (BALB/c Nude Mouse) of muscle injury was used by combining myotoxin induced direct skeletal muscle damage with induction of ischemia to the hind limb. Enzyme modulated oxygen microsphere system loaded with firefly luciferase expressing cells were injected in sample group ($n = 5$). Animals were observed by BLI for 6 weeks. Finally, animals were sacrificed and evaluated by histology and molecular analysis. Localized and sustained release of oxygen enhanced cell engraftment, limited fibrosis, induced new vessel formation and accelerated formation of muscle tissue traced by molecular and imaging analysis. The enzyme modulated oxygen producing microspheres can effectively promote skeletal muscle regeneration in vivo by enhanced local angiogenesis and reduced necrosis.

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55. Bioreactors for Translational Research

55.01
Keynote: Bioreactors for development of healthy and diseased myocardial tissue models

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The ability to generate cardiomyocytes from either embryonic stem cells or induced pluripotent stem cells (PSC) provides an unprecedented opportunity to establish human in vitro models of cardiovascular disease as well as to develop replacement cardiac tissue for possible implantation. Although advances in stem cell biology enable us to reliably obtain cardiomyocytes from stem cells, cardiomyocytes derived from human PSC are representative of early human development and are not equivalent to adult cells. Our goal was to develop in vitro models that enable induction of maturation and hypertrophy in human iPSC derived cardiomyocytes. The cells were cultivated in cylindrical microwells microfabricated in PDMS with centrally positioned suture. Gel compaction enabled generation of a highly aligned tissue termed biowire (100 mm in diameter and 1 cm long). The tissues were stimulated using biphasic suprathreshold pulses, at the frequencies up to 6 Hz. Quantitative PCR indicated a significant downregulation of atrial natriuretic factor (ANF), a marker of fetal development and alpha-myosin heavy chain, expressed in higher quantities in fetal human cardiomyocytes. Stimulated samples exhibited improved calcium handling compared to non-stimulated samples (e.g. responsiveness to caffeine). After addition of a hypertrophy agonist, isoproterenol, for one week the up-regulation of atrial natriuretic factor and brain natriuretic peptide was induced.

55.02
Options for long-term monitoring strategies in bone tissue engineering

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Monitoring strategies play a pivotal role when experimental results are to be fed into computational simulations, as each sample can be assessed individually and over time. Only when taking the changing environmental status into account, influencing factors of tissue development can be identified and their effect quantified. Long-term cultures provide the possibility to assess influencing factors not only in parallel but also sequentially. We designed spinner flask bioreactors for non-destructive monitoring of mineralized ECM development of individual samples with μCT. MSC were cultured on silk scaffolds and their mineralized ECM development was compared to static culture. The setup enabled long-term tissue culture and showed continuous deposition of mineralized ECM over 2 years. Dynamic culture resulted in significantly increased ECM volume and a more homogenous distribution of the tissue, especially at early timepoints. μCT monitoring of spinner flask bioreactors provides a tool to assess long-term effects of mechanical stimulation in bone tissue engineering. This technology may be expanded to investigate other parameters such as drug effects or co-cultures with bone resorbing cells in order to truly understand 3D cell behavior in vitro. This valuable data will serve as input for computational simulations and enable translation of in vitro knowledge to the in vivo situation.

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55.03
Real-time and in-situ control of environmental parameters in a modular bioreactor

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Many researchers now recognize the importance of the external environment in which cells are cultured for cell function and differentiation. Most of the systems able to apply physiological-like stimuli also need a classical incubator or a specifically designed system to control the environmental parameters at some distance from the cells. Here, a standalone platform for cell, tissue and organ culture is described. The SUITE (Supervising Unit for In-vitro Testing) system can control local environmental variables like pH, temperature and hydrostatic pressure over long periods, to provide the optimal environment for cells outside the classical incubator and also to apply mechanical and chemical stimuli to simulate the physiological milieu. The SUITE platform is used with Multi-Compartmental modular Bioreactors (MCmB) to perform dynamic cultures of hepatocytes as in-vitro liver model. Preliminary tests demonstrated the capability of the system to maintain the target parameters for more than 72 h generating different hydrostatic pressures (20–30–40–50 mmHg). Then, two bioreactors were connected in series and cultured for 24 h in the SUITE platform with hydrostatic pressures of 20–30–40 mmHg. Static and dynamic controls were placed in the classical humidified incubator at 37°C, 5% CO_2. The results show that cell function is enhanced in SUITE at up to 30 mmHg of hydrostatic pressure, as confirmed by viability, metabolic function and morphological analysis.

55.04
All-in-one rapid-prototyped bioreactor/implant for semi-automated generation of tailor-made critical size bone tissue substitutes

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Additive Manufacturing (AM) offers the possibility to design and build products with high geometrical complexity and in smaller batch numbers. In this work we applied AM to design and build a disposable bioreactor in combination with a scaffold. This work applies rapid prototyping to design and fabricate scaffolds pre-enclosed into a disposable and ready-to-use perfusion chamber. Computerized tomography was used to scan a 3 cm sheep tibia section and generate a porous computer model replica. A fully enclosed perfusion chamber was designed around the replica. As a proof of principle the perfusion chamber and porous implant within were prototyped in one single piece by fused deposition modeling of ABS. The device's watertightness was confirmed by micro-CT and high flow perfusion studies. Micro-CT also confirmed that although prototyped in one piece, one inside the other, the implant and surrounding chamber were not merged together at any point. Opening the chamber we observed that perfused fluids had totally and homogenously reached the highly interconnected porous network of the scaffold. This device is currently being fabricated from PLA for performing in vitro perfusion cell cultures in its interior. This work demonstrated the possibility to produce easy- and ready-to-

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use fluid-efficient culture chambers pre-enclosing tailor-made and patient-specific scaffolds.

55.05
Capacitive stimulation enhanced osteogenic differentiation of primary human mesenchymal stem cells

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Electricity is a novel modality in Tissue Engineering that has the ability to enhance proliferation and differentiation of various musculoskeletal cell lines in vitro and in vivo. In our work we are developing Electrical Stimulation into an effective modality for the creation of bone implants. A capacitive bioreactor, developed by our group, was used to deliver electrical stimuli with 3 V/mm field strength to primary Mesenchymal Stem Cells cultured in osteogenic medium. A computerised model was created in the commercial software MATLAB, based on the Method of Moments that allows the calculation of the of the electrical field strength inside the bioreactor. Stimulation was delivered with 500 Hz frequency, and 1 and 1000 μs pulse width for 3 h per day. Cell numbers were assessed with Picogreen assay, metabolism with Alamar Blue, differentiation by Alkaline Phosphatase assay and the qRT-PCR of the bone related markers BMP-2 and ALPL at day 4 and 7. Proliferation was significantly lowered by 1 μs pulses both at day 4 ($P < 0.005$) and 7 ($P < 0.005$), but this coincided with a significant enhancement of metabolic ($P < 0.005$ and $P < 0.05$) and alkaline phosphatase activity ($P < 0.005$ and $P < 0.005$). At day 7 expression of the gene ALPL was increased 3-fold ($P = 0.04$) due to 1 μs pulses. The presence of BMP-2 was not detected in controls, but was detected in 1 μs treated samples. This shows the great potential that capacitive electrical stimuli have for tissue engineering.

55.06
Engineering of human bone tissue from pluripotent stem cells in perfusion bioreactors

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Bone tissue engineering aims to develop functional grafts for reconstructive surgery, by methods designed to mimic processes during native bone formation. Human embryonic stem cells (ESC) and induced pluripotent stem cells (IPS) represent promising cell sources for these goals, as they can provide unlimited numbers of tissue progenitors, and give rise to any cell type in the body. We developed a stepwise protocol to engineer fully viable, compact bone-like tissue from ESC. ESC-mesenchymal progenitors were derived from pluripotent colonies, expanded and cultured on osteoconductive scaffolds in bioreactors with interstitial flow of medium for 5 weeks. The resulting tissue constructs contained significantly higher cell numbers, alkaline phosphatase activity, osteopontin release into the culture medium and deposition of mineralized bone matrix (staining positively for osteopontin, bone sialoprotein, osteocalcin and osteoid) compared to statically cultured constructs. Notably, ESC-engineered bone maintained its phenotype stability, underwent further maturation and supported vasculature ingrowth during 8 weeks in vivo, without a single incidence of tumor formation. In contrast, undifferentiated ESC implanted on osteoconductive scaffolds consistently formed teratomas over the same time period. We propose that engineering of bone-like tissue from pluripotent stem cells can advance fundamental studies of osteogenesis, as well as translation into regenerative medicine applications.

55.P01
Development of the automatic cell processing machine for the clinical application of the cartilage regeneration

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In order to make regenerative medicine practicable, we have been developing automatic cell processing machines. Cell culture for the regenerative medicine has been performed by cell culture experts in cell processing facilities, not only been making great cost but also will have latent contamination risks and human errors. As these machines are able to be settled in lower grade air clean room, the initial and running cost are deduced. Because the clean robot arms automatically deal with cell culture procedure with preset programs, it is not necessary to worry about the cross-contamination nor to take A patient for B by mistake. At this time, the machines are aiming at the clinical application of cartilage regeneration. In its regeneration, bone marrow MSCs from the patient are cultured to obtain the necessary cell numbers and cellular quality. The compatibility between human-handling and robot-arm handling cellular quality has been verified in Osaka University Hospital utilizing our machine. There is one benefit that the robot has high repeatability though they cannot realize the differences among individuals. Then, we have let responsible culture person enable to make decisions of the timing and conditions of primary culture and sub-culture so flexible. When we verified the compatibility, cell number, viability and cell surface markers were confirmed. From now on, the clinical studies are going utilizing our machine. This research is supported by Japanese government, NEDO.

55.P02
Development of automated 3-dimensional tissue fabrication system (Tissue Factory)-2: Confirmation of an aseptic process

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Aseptic processing is the most demanding of pharmaceutical manufacturing processes. We are developing a new concept cell automated production system (Tissue Factory) by adopting modular of small isolators which have uniformed docking ports. Every module can be decontaminated individually by hydrogen peroxide steam, and has an automated process manufacturing function, for example cell isolation, cell primary culture, cell expansion, cell sheet forming, and cell sheet layering. Several proper modules can be attached on the system and interlocked to manage various production lines when necessary. We have innovated the flexible modular platform (FMP) concept, and tried to construct aseptic processing procedures for the system. In this study, we prepared several modules for cell sheet forming and layering (cell seeding/medium changing module, cell sheet layering module, CO2 incubator module, sample loading module, and transfer module), and the aseptic processing reproducibility of one combinatorial process was investigated (Operational Qualification). The maintenance of optimal germ

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free condition was carried out repeatedly. This research is granted by the Japan Society for the Promotion of Science (JSPS) through the "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)".

55.P03
Development of automated 3-dimensional tissue fabrication system (Tissue Factory) 1: automation system toward clinical practice in regenerative medicine

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We have succeeded in a novel treatment in regenerative medicine using "cell sheet", and the treatment have been applied to clinical practices of the cornea, heart, esophagus, periodontal ligament, and cartilage. The approach to industrialization is one of the critical issues to broadly distribute the treatment, and we are developing an automated 3-dimensional tissue fabrication system (Tissue Factory) for mass production of living materials, where we had newly designed to be the flexible modular platform (FMP) for saving the common apparatuses which can be shared in multi-manufacturing lines. The exterior of modules has adopted an isolator technology for excluding from some major contamination risks. Tissue factory has been developed separately as cell isolation & primary culturing system, cell expansion culturing system, and cell sheet assembly system. In this study, we had designed a prototype of cell sheet assembly (cell sheet forming and layering) system, and the prototype faced several challenges of automated production control system for FMP. This research is granted by the Japan Society for the Promotion of Science (JSPS) through the "Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST Program)".

55.P04
Development of automated 3-dimensional tissue fabrication system (Tissue Factory)-5: cell sheet assembly process

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We develop an automated 3-dimensional tissue fabrication system 'Tissue Factory'. The central challenge is to lead to more efficient cell-processing facility by introducing the novel system rather than conventional cell-processing facility with fully equipped clean rooms. For minimizing the manufacturing space and maximizing the flexibility for multi-purpose manufacturing facility, we applied a flexible modular platform to our system. In this study, we underwent the cell sheet assembly process, where a multilayered cell sheet was fabricated by stacking single layer cell sheets. Three processing modules (seeding, incubation and sheet assembly) and two supportive modules (transferring and gateway) were connected as a system. Human skeletal muscle myoblasts were seeded in temperature-responsive culture dishes and cultured overnight utilizing the seeding module and the incubation module. On the next day, the single layer cell sheets in the dishes were stacked into a multilayered cell sheet utilizing the sheet assembly module. The whole process was conducted automatically except for the preparation of materials. After the adjustment of the process parameters, multilayered cell sheets were successfully fabricated. These multilayered myoblast sheets kept viable in the following static culture for 3 days at least with the live cell ratio over 95%. This study demonstrated the feasibility of automatic cell sheet assembly process using our system.

55.P05
Development of automated 3-dimensional tissue fabrication system (Tissue Factory) 3–1: automated cell isolation system from tissue for regenerative medicine

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Currently, cell therapy is considered to be one of the most effective treatments for damaged tissue. The cell isolation from tissue is the most important processes. However, most part of the process depends on manual operations, which requires highly sophisticated techniques to stably acquire high quality cells. In this study, we introduce a newly developed cell isolation system which enables isolation of target cells from tissue specimen. As the model process, the isolation of skeletal myoblasts from skeletal muscle tissue was performed the serial procedures of mechanical mincing, enzyme digestion to prepare the cell suspension under aseptic condition. The culture system has five functional units: pipetting unit for cell suspension, liquid pump unit for medium supplying, centrifugation unit for cell washing and cell isolating. The cell isolating unit consists of three parts: a stirrer device, a chamber, and a rotate controller. Tissue specimens were put in the chamber, and pre-warmed enzyme solution was added. Then a stirrer device was rotated intermittently at 37°. After this process was completed, skeletal myoblasts were acquired and suspended in a culture medium. The number of isolated cells per weight of tissue specimen was examined by comparing the machine operation and the conventional manual one. The successful performance was accomplished in the isolation of skeletal myoblasts from porcine muscle tissue, contributing to labor-saving and operational stability.

55.P06
Innovative low-cost microgravity bioreactor for hydrogel-based myocardial regeneration

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Hydrogel-based injectable scaffolds offer a promising strategy for the regeneration of injured cardiac tissues. An innovative low-cost perfusion bioreactor was designed for testing and culturing cell-seeded hydrogel microspheres in microgravity condition (MC). Optimal design and culture conditions were identified by a multiscale computational fluid dynamic (CFD) approach providing a comprehensive investigation of the functioning of the device. The bioreactor geometry allows the generation of stationary vortices within the culture chamber, which maintain in mixing suspension the microspheres and assure an adequate oxygen transport, guaranteeing MC without using rotating components. Within the culture chamber are located a filter for preventing accidental outputs of microspheres, and a check valve for guarantying the unidirectionality of the flow. For assisting the bioreactor design and for identifying the proper operating conditions, multiphase CFD simulations were performed, by adopting a finite volume method. According to the CFD results, experimental tests demonstrated the suitability of the bioreactor: the combination of operating conditions and properly

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shaped chamber walls give rise to flow separation, with the ensuing formation of stationary vortices. The balance between hydrodynamic and gravitational forces guarantees MC, avoiding both specimen sedimentation and shear stress values critical for cells. Work carried out in the scope of BIOSCENT European Project.

55.P07
Versatile use of biomimetic bioreactors for functional evaluation of nanocomposite alginate based hydrogels

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We present here utilization of perfusion bioreactors and a bioreactor with dynamic compression, which mimic physiological conditions in vascularized tissues (20–50 μm/s fluid velocities) and mechanically loaded tissues such as articular cartilage (0.1–1 Hz compression frequency, 5–10% strain), respectively, for functional evaluation of novel alginate based hydrogels with incorporated silver nanoparticles (AgNPs) of different compositions (blends with PVA and PVP) and in different forms (discs, beads, microbeads, microfibers). These hydrogels are attractive as antimicrobial wound dressings and soft tissue implants as well as scaffold components in tissue engineering. We have combined conventional methods for determination of hydrogel antimicrobial activity in suspensions of E. coli and S. aureus and cytotoxicity in monolayer cultures of bovine calf chondrocytes, with bioreactor studies of chondrocytes immobilized in alginate and of articular cartilage discs in direct contact with nanocomposite hydrogels. Main functional parameters (AgNPs release kinetics, mechanical properties and cytotoxicity) were significantly affected by the surrounding environment. We have developed predictive models for AgNPs release and mechanical behavior for the case of nanocomposite alginate microbeads when applied topically (static environment) or implanted in vascularized and/or dynamically loaded tissues.

55.P08
Development of automated 3-dimensional tissue fabrication system (Tissue Factory) 4(2); Survival rate analysis of floating human myoblasts to develop a subculture equipment for a mass cell production

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Several clinical applications of cell sheet technology have been made, and there are great hopes to be popular therapies. Especially, a heart disease has been treated with laminated tissues of myoblast cell sheets in Japan. For cell expansion of myoblasts, the closed vessel culture system is the promising tool which can perform the automated culture operations under asepic condition accompanied by several liquid handling for cell and medium transfer during the passage. The passage operation of cell seeding and harvesting allows the adherent cells to be suspension, which is considered to reduce the viability. In the present study, the influence of the floating time was examined. The human skeletal Muscle Myoblast (HSMM) cells were maintained in SkGM-2 (Lonza). The number and ratio of the survival floating myoblasts was estimated after 0 (control), 1, 2, 3, 4 h under 37°C and gentle shaking. To estimate the growth activity, the cells were seeded in 8-well plates. The number of the attached cells was measured and the ratio of myoblasts in the population was evaluated by immunostaining. The ratio of the survival floating myoblasts within 2 h was more than 80% and no difference of growth activity, considering that 2 h suspension of cells allow the automated operations. This study is performed under the support of the 'Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST)', Japan.

55.P09
pH-triggered automatic medium exchange: a step forward toward GMP-compliant tissue engineering manufacturing processes

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Medium exchange is one of the most frequent steps during cell cultures. Facing the need of reducing contamination risks and preserving the homeostasis of the culture milieu, we developed an automatic medium exchange system for feedback-based control of pH within a user-defined range of values. A sensing system for on-line monitoring of pH was developed. Appropriate hydraulic circuits and a closed-loop control unit have been implemented to manage medium conditioning according to the sensing apparatus. The operating parameters, such as pH thresholds and pumps flow rates, are arbitrarily set by the operator in an easy, intuitive and versatile control panel: real-time graphs of pH values are shown and data logged. A Petri dish with modified plug was used as culture chamber: reliability and sterility tests, and cell cultures trials were designed to validate the device. Mechanical and electrical reliability of the apparatus during operation were verified. Medium pH could be efficiently maintained within user-defined ranges. The sterility of the whole system was also positively verified. In conclusion, the developed device is a versatile and reliable tool, proving how automation of cell cultures (limiting labor-intensive operator handling) could improve safety and standardization of tissue engineering processes in order to achieve GMP graft production. Moreover, control of environmental parameters such as pH to drive cell behavior is an invaluable tool in tissue engineering.

55.P10
Development of automated 3-dimensional tissue fabrication system (T-Factory) 3–2: Real-time Multi Sensing System

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We developed an automated 3-dimensional tissue fabrication system (T- Factory) with flexible modular platform (FMP) realizing on-demand connection and disconnection modular-type devices. To manage the automation system for primary cell culture which includes the variance of the profile, 'monitoring' is one of the critical functions. In this study, we proposed a real-time multi sensing system in a customized culture vessel, which can measure temperature, pH, CO2, motion and position in the modular device. The captured data were transmitted to the central computer by a telemetry system. This monitoring system realizes the estimation of the culture status and the automation stability, helping the culture traceability. In addition, we installed a novel noninvasive method for pH monitoring, which is based on spectrophotometry. This proposed system is expected to contribute to advances in automation systems for regenerative medicine.

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55.P11
Multimodality, multispectral and 3D light microscopy of engineered tissues without dyes

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Light microscopic imaging of live cells and tissue typically requires fluorescent or absorbing dyes. For future monitoring of engineered tissue, dyes may be toxic or alter cell behavior. Future applications will be in monitoring clinically functional tissue for transplantation, where dyes may be prohibited altogether. Methodologies for producing high-contrast and 3D views of tissue constructs without dyes are being developed. These methodologies include multispectral collection with several wavelengths and multimodality imaging, including phase-contrast, darkfield and forms of transmitted light brightfield modalities. Early approaches to software algorithms include new 3D deconvolution algorithms, principal component analysis for generating high-contrast images of desired structures and 3D visualization algorithms using the VTK software library. Proof-of-principle tests will be presented. Samples that were used include 2D and 3D constructs relevant to the study of tissue engineering, including vascular aggregates, smooth muscle aggregates and cultures with multiple cell types, such as stem cells and adipocytes. Three dimensional deconvolutions of these modalities and samples will be presented. Feasibility tests that show promise to spectrally discriminate cell types and cell components, such as lipid droplets versus other components, will be shown. These preliminary tests show promise for future development of this technology.

55.P12
Metabolic change of cultured cell sheets depending on oxygen supply

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Introduction: Our group has been trying to fabricate thick, cell-dense cardiac tissues by layering cell sheets in vitro. In the perfusion cultured cell sheets, anaerobic metabolism was predominantly observed. To confirm this observation, here, we try to quantitate the metabolic change of the cell sheets depending on oxygen supply, under static culture conditions.
Methods: A cell sheet was cultured on collagen gel using two systems, namely systems I and II. In system I, the sheet was immersed in the culture medium constantly. In system II, the medium was placed under the gel and was permeated through the gel. Thus the sheet contacted with air for a brief period after the medium exchange. The medium was exchanged and collected every other day.
Results/Conclusion: Net lactate production by a cardiac cell sheet in system II was significantly smaller than that in system I. As a result, the molar ratio of lactate produced to glucose consumed was 1.4, suggesting that aerobic cell metabolism had occurred partially. Interestingly, when a fibroblast sheet was used, the reduction in the lactate production did not occur. Considering the fact that the cardiac cells consume oxygen more rapidly than the fibroblasts, the observed metabolic change reflects the shortage of oxygen in culturing cardiac cells. Therefore, developing a system supplying enough oxygen to the rapid consuming cells, like cardiac cells, is crucial for tissue culture in future studies. Granted by the JSPS (FIRST Program).

55.P13
Streamlined bioreactor-based production of engineered grafts

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The majority of all tissue engineering approaches require extensive cell expansion in monolayer culture (2D) prior to cell seeding & culture in 3D scaffolds. However, expansion in 2D is typically associated with cell dedifferentiation or a progressive & dramatic loss of stem/progenitor cell function. Moreover, due to the large number of manual & labor-intensive manipulations that must be performed by lab technicians, conventional 2D expansion procedures possess inherent risks of contamination, high intra- & inter-operator variability, limited scale-up potential, & high manufacturing costs in the long-term. As an alternative, we describe a bioreactor-based approach to streamline the production of engineered grafts within a single closed system, which eliminates the need for 2D cell expansion. Cells, freshly isolated from a tissue biopsy, can be seeded & expanded directly in the pores of a 3D scaffold within a perfusion bioreactor, & perfusion cultured until the graft is sufficiently mature for implantation. This streamlined approach will be illustrated in the context of engineering of human cartilage grafts, osteogenic grafts, & vascularized bone grafts. The streamlined process could serve as the basis of manufacturing systems requiring the minimal number of processes & unit operations, thereby facilitating simplified & compact bioreactor designs with limited automation requirements, & with the likely result of lower operating costs & increased compliance to safety guidelines.

55.P14
No guidance documents or standards are available to assist the development of bioreactors for tissue engineering applications

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Due to the fact that there are extremely varying conditions in the human body, a variety of bioreactor types for all fields of application have been developed over the last few years. These bioreactors are essential for routine manufacturing of tissues for clinical application and in studying the effect of different parameters on cell behavior. Today, there are no specifications supporting the design of new bioreactors under standardized conditions. Therefore, it is important to establish a working group of scientific and industrial experts that can identify shared problems, identify risk factors and define fundamental common requirements. The technical committee CEN/TC 316 'Medical devices utilizing tissues' of the European Standardization Organization (CEN) is organizing this year a workshop in Berlin, Germany towards standardization of Bioreactors. The presentation will provide insight into the current activities for standardization on the European and international level.

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55.P15
Development of a cell culture system providing an oxygen gradient condition

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This study describes a cell-culture system allowing culture cells to be exposed to an oxygen gradient condition, which was created with the diffusion of PDMS from a micro channel at the bottom of a bleeding ground for cell culture. Oxygen density and gradient in the ground were controlled by altering culture-chamber design, and the volumetric flow rate of gas in the micro channel. The oxygen gradient system was demonstrated to be a powerful tool for drug screening and biological studies for regeneration medicine. Hypoxia culture condition of tissue and stem cells have recently attracted attentions for forming tissue structure, imitating human body, and analyzing the differentiation of stem cells. Cells in tissue in vivo are always exposed to an oxygen gradient from vessels. However, in vitro investigations analyzing cell functions have been performed with a uniform hypoxia or controlled oxygen conditions using devices such as a hypoxia incubator. The system composed of an oxygen gradient PDMS-chamber made with a micro-fabrication technology, and a hypoxia desiccator realized an oxygen density gradient from 0 to 159 mmHg in the surface of the chamber. In addition, cells in the hypoxia region of chamber were stained with pimonidasole, a hypoxia marker, which was confirmed by an immunofluorescent technique. The proposed oxygen gradient system was able to be readily applicable to efficient and precise studies investigating of the tissue function or differentiation of stem cell.

55.P16
Mesenchymal stem cells cultured in a bidirectional flow system resulted in superior cell proliferation and osteogenetic induction

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Mesenchymal stem cells (MSCs) are capable of differentiating into osteoblasts, chondrocytes or myoblasts depending upon culture condition. However, MSCs present very low concentration in bone marrow. A bidirectional flow system with was developed for culture, expand and induce MSCs into osteogenesis. A comparison between static and spinner-flask culture with this flow system was examined. Total DNA was measured to evaluate cell growth. Cultured media with osteogenetic supplement were sampled and the concentrations of glucose, lactate and calcium (Ca) were quantified. Bone markers such as alkaline phosphatase (ALP), osteocalcin (OC) and intracellular Ca content were examined. After 4 weeks culture, highest cell numbers was acquired from the bidirectional flow system and the glucose consumption in it was obviously higher than the other two groups. After 3 weeks, MSCs cultured in spinner-flask and this flow system with high ALP concentration expression. The content of OC in static cultured MSCs was dramatically higher after 4 weeks culture. At the same time, Ca concentration in medium was decreased and high Ca concentration in cell was detected due to intracellular deposition. In conclusion, MSCs cultured in static state had no proliferation but mineralization. Otherwise, cells in this flow system could be expanded and induced into osteogenetic lineage without mineralization. The bidirectional flow system ultimately is applied in bone regeneration for personal MSCs cultivation.

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56. Automatization of Bioreactor Technologies for 3D Tissue Cultures

56.01
Keynote: Bioreactor technology and automatization in the production of human 3D tissues

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Bioreactors can be designed to maintain physiological parameters at desired levels, enhance mass transport rates, and expose cultured tissues to specific stimuli. The requirement of functional tissue engineering includes (1) cellular components with appropriate functions, (2) a scaffold usable as structural template, (3) a bioreactor providing the necessary biochemical and physiological regulatory signals guiding tissue development. To ensure homogeneous conditions within the bioreactors methods of topology and shape optimization are applied. The results are compared with analytic models, from which a general parametric description of the design is obtained and tested. During the talk a state of the art review of specific reactors and the automatization of singles production steps and whole processes will be provided. A major topic will be the discussion of concepts which are important in the development of reactors and technologies that can be use for production of clinical scale tissue. At the end of the talk a general overview of a fully automated process to produce skin equivalents will be given. However, without a comprehensive understanding of each of these components, bioreactor design and tissue growth to manufacture product will remain at a relatively rudimentary and limited level. Increased fundamental understanding of the issues can have a dramatic impact on the ability to generate tissue-engineered product safely, economically, and in the numbers that are required.

56.02
Bioreactor systems for expansion and differentiation of human mesenchymal stem cells

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A main challenge in cell therapies and other tissue regeneration approaches is to produce a therapeutically significant cell number. For expansion of mesenchymal stem cells (MSC) the cultivation on 2D plastic surfaces is still the conventional procedure, even though the culture conditions differ significantly from the 3D environment in vivo. Additionally, static amplification of MSC is a labour-intensive procedure. We therefore used bioreactors (rotating bed or wave bioreactors) in order to maximize ex vivo expansion of MSC and for guided differentiation towards the osteogenic lineage. The integration of sensors for online monitoring of various parameters (e.g. pH, pO_2, pCO_2) ensured cultivation under well controlled and reproducible conditions. MSC derived from umbilical cord and human adipose tissue were used for the study. Cell expansion in a rotating bed bioreactor provided a high number of MSC. The use of micro carrier cultures in wave bioreactor was also shown to be a suitable and effective method to expand adherent cells under stirred dynamic or flow conditions. After expansion cells were differentiated towards the osteogenic lineage. Cells lack the ability to grow in 3D direction and build functional tissue in vitro. Thus, it is necessary to seed and culture cells on 3D matrices to obtain functional implants. After expansion cells were seeded on ceramic 3D matrices. Osteogenic differentiation was induced under dynamic conditions.

56.03
Assessment of the performance of a novel hydrostatic force bioreactor- changes of pH and oxygen tension during cell culture

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pH and oxygen tension are crucial parameters during bioprocesses, which are known to influence the cell cycle, cell growth, cell differentiation and the production of extracellular matrix. Despite their importance, limited information seems to be available about pH and oxygen changes in three-dimensional cell culture systems. The aim of this study is to investigate the performance of a hydrostatic force bioreactor by measuring the changes of pH and dissolved oxygen in bicarbonate-buffered and HEPES-buffered cell culture media. Moreover, it examines the effect of hydrostatic force on the viability of chick fetal femur derived stem cells cultured in 3D collagen gels. The time, pressure and frequency of the applied hydrostatic force were altered in order to determine their effect on pH and dissolved O_2. pH changes of media samples were determined via a colorimetric approach. The concentration of dissolved oxygen was measured using an optical micro oxygen sensor. Increasing hydrostatic force, duration and frequency resulted in an initial increase of pH and oxygen concentration. Oxygen concentration and pH then equilibrated and during extended loading periods no further increase was observed. Initial results suggest further that an increase in hydrostatic force had a stronger influence on pH and dissolved O_2 than frequency of modulation.

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56.04
Increased flow velocity positively influences osteogenic differentiation of human stem cells in a perfusion bioreactor

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Introduction: Bioreactors are widely used to mimic the in vivo environment and to monitor and control in vitro culture conditions. Perfusion bioreactors have been shown to enhance the mass transfer to the interior of a 3D scaffold and exert shear forces on cultured cells. The velocity of perfusion flow mainly triggers the extent of shear stress acting on the cells. We investigated the effect of two different flow velocities on human mesenchymal stem cells (hMSCs) cultured in a perfusion bioreactor.

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Methods: hMSCs were cultured on silk fibroin scaffolds of 8 mm in diameter and 1–2 mm in height. Scaffolds were cultured under dynamic (0.2 and 12 ml/min) and static conditions in osteogenic medium for 7 weeks. The development of mineralized extracellular matrix (ECM) was monitored with micro-computed tomography.
Results: Cells cultured under low flow conditions did not produce mineralized ECM but showed increased levels of DNA and non-mineralized ECM. High flow conditions resulted in mineralized ECM production with similar DNA levels compared to the static group.
Conclusions: Flow velocity had a distinct influence on cultured hMSCs. Low flow velocity triggered cell proliferation whereas high flow velocity induced osteogenic cell differentiation. Therefore, a high flow velocity is preferable to induce osteogenic differentiation of hMSCs.
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56.P01
Fabrication and 3D culture of customized artificial bone by oscillatory flow

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As the bone defects of the patients were usually various and complicated in shape, it would be favorable to produce a custom-made engineered bone graft according to the anatomical information of a target defect from a patient. We used a segment of rabbit femur as bone defect model for reconstruction in this research, and fabricated a custom-made ceramic scaffold according to the CT data by sterolithography successfully. Furthermore, the customized scaffold was cultured with rabbit MSCs by a novel oscillatory perfusion system. The results showed that the ceramic scaffolds not only have customized contour the same to the CT data, but also have interconnected customized channels designed for 3D perfusion culture. After 3D culture by oscillatory flow, the cells grew homogeniously throughout the scaffold, but the cells within the scaffolds culture in a static condition died after the prolonged in vitro culture. The DNA content and ALP activity was also significantly higher in perfusion group than static group ($n = 3$, $P < 0.05$). Therefore, in this study, the customized artificial bone with 3D cellular osteogenic activity was fabricated successfully and proved to be promising for clinical application.

56.P02
Recapitulating endochondral ossification in custom perfusion bioreactor for bone grafts engineering

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The purpose of this study was to develop an in-vitro biomimetic bioreactor capable of mimicking physiological niches essential for endochondral ossification by embryonic stem cells (ESC). Ultimately, deriving bone grafts with physiological properties to promote better integration and regenerative capacity. An ESC engineered construct was cultured in a bioreactor that allows media modifications based on the stages of differentiation. Nutrients and oxygen were delivered across an agarose bedding to allow for the generation of gradients within the bioreactor. Differential pO_2 distribution ranging from 84 mmHg to 0 mmHg was observed. After 52 days, a rigid ivory-toned construct was derived. It was stiffer (144.1 Pa) than its cell-free counterpart (21.5 Pa). Calcium deposition was validated with positive alizarin red straining. Real-time PCR and immunofluorescence staining had shown inhabitation by chondrocytes and osteoclasts, with positional variability observed. The oxygenated section was composed of pre-chondrocytes and osteoclasts, while chondrocytes was found in the hypoxic area. Although calcium deposition was detected, osteoblast was absent. A mineralized construct comprised of chondrocytes and osteoclasts was derived. In the future, mechanical loading will be applied on the construct to drive further maturation, prior to transplantation into critical size skeletal defect model. Once validated, this technology may provide an alternative approach for producing custom implants.

56.P03
Cyclic hydrostatic pressure enhances osteogenesis in cell-seeded scaffolds

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Cyclic hydrostatic pressure (CHP) has been proposed as an important stimulus by which osteochondral and progenitor cells sense and respond to mechanical loading in vivo. In our previous work we have demonstrated that CHP applied in a custom designed bioreactor stimulates enhanced osteogenesis in the chick foetal femur in vitro, particularly an increase in the bone density. In this current investigation we have employed the same bioreactor strategy to mechanically stimulate hydrogel and solid scaffolds seeded with embryonic or adult stem/progenitor cells. We found that CHP results in an increase in the construct's density, supporting our previous conclusions that cyclic pressure stimulates enhanced bone formation (via matrix mineralisation). The mechanisms underlying this effect are under investigation to further evaluate this bioreactor technology as a useful means to mechanically precondition 3D scaffolds for applications in osteochondral tissue engineering.
Methods & Results: Bone-forming cells were isolated from foetal chick femurs and seeded into hydrogel scaffolds (Matrigel in this example). A regime of one hour stimulation per day at 1 Hz, cycling between 0 and 280 kPa was applied for 7 days. End point analysis was by μCT, histology and qPCR. Cell-seeded hydrogels which experienced CHP in the bioreactor were both denser than unstimulated controls and contained a larger mineralising phase (41.7% of the total gel volume, compared to 35.3% for unstimulated gels).

56.P04
Dynamic culture and mild hypoxia synergistically enhance osteogenic differentiation of human periosteum progenitor cells on 3D scaffolds

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We aimed at exploring the interaction between two important in-vitro environmental factors: hypoxia and dynamic culture and developing an optimized 3D culture condition for scaffold in skeletal tissue engineering. 100 000 human periosteum derived progenitor cells (hPDCs) were firstly seeded into titanium scaffold made by selective laser melting (SLM) methods. Seeded scaffolds were divided into four different culture conditions: (i) 20% O_2^+Static (ii) 20% O_2^+Dynamic; (iii) 5% O_2^+Static; (iv) 5% O_2^+Dynamic for an additional 3 weeks. Upon harvesting, samples morphology were characterized with live/dead staining and assayed for cellularity, metabolic activity, ALP, and calcium deposition. A statistical DOE approach was adapted to analyze the interactions between variables and then identify the best combination of factors that would yield maximum in vitro mineralization. The results show that dynamic culturing of hPDCs in 5% O_2 resulted in

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enhanced osteogenic differentiation compared to static control. Higher cellularity, a more homogeneous distribution of hPDCs throughout the scaffolds and higher ALP protein level and calcium deposition were found in 5% O_2 under dynamic culture. The multi ANOVA analysis also confirmed that there is a significant interaction between these two investigated factors ($P < 0.001$).We have demonstrated here that dynamic culture imparts biophysical stimuli and improved nutrient supply to cells resulting in higher osteogenic differentiation.

56.P05
Dynamic seeding of large cancellous bone scaffolds with mesenchymal stromal cells: spatial cell distribution using perfusion, rotation and static seeding techniques

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Due to the high socioeconomic relevance of musculoskeletal disorders innovative concepts for bone regeneration are essential. In this context, a major prerequisite for successful dynamic cultivation of large-scaled three-dimensional porous scaffolds is the homogenous cellular distributions (Rauh et al., Tissue Eng Part B, 2011). The aim of the study was to compare the spatial distribution of single-cell-derived immortalizied hTERT-expressing human mesenchymal stromal cells (MSCs) on high-volume porous scaffolds using perfusion-based, rotational, and static seeding techniques. Bovine cancellous bone (BCB) and tricalciumphosphate (TCP) cylinders (Ø20 mm × 25 mm) were seeded with 6.3×10^6 MSCs for 2 h in triplicates using a rotational seeding device, a perfusion bioreactor system and static steel multi-well chamber. With a cutting device the samples were cut along the central axis. All devices were custom-built. Cell distribution was examined using confocal laser microscopy and cell quantification measuring DNA and LDH content. The rotational seeding technique resulted in improved distribution of viable MSCs compared to static and perfusion seeding. Seeding efficiencies were up to 31% higher on BCB compared to TCP. Rotational seeding improved seeding efficiency up to 26% compared to static and perfusion-based techniques. We conclude that rotational cell seeding should be considered for bone tissue engineering strategies using large-scaled porous structural scaffolds.

56.P06
Development of automated 3-dimensional tissue fabrication system (Tissue Factory) 4(1): development of large scale expansion culture system

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Cell sheet engineering is paid to attention as a promising technology that spreads the regenerative medicine. We are developing a large scale expansion culture system for distributing a large amount of cells in order to popularize this high level medical treatment. We adopt the closed vessel cell culture method for the system, which has a merit in preventing contamination because of less cell exposure time to outer environment. Additionally, this system is expected to have cameras to monitor cell condition. Under those specifications, the increase of cell culture area per volume of the system should be considered. We investigate the area per volume between two cell culture vessel layouts: wide area plane culture and tube inner wall culture and confirmed that both methods do not have a remarkable difference. Therefore wide area plane culture method is selected in the point of easiness of observation using cameras. Moreover, the level regulation mechanisms which maintain horizontalness of culture vessels using acceleration sensor feedback are installed because the influence of the inclination of culture vessels will be large in the wide area plane culture method. This development is performed under the support of the 'Funding Program for World-Leading Innovative R&D on Science and Technology (FIRST program)'.

56.P07
Flow perfusion co-culture of articular chondrocytes and mesenchymal stem cells

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Articular chondrocytes (ACs) and bone marrow-derived mesenchymal stem cells (MSCs) are two common cell sources for articular cartilage engineering. However, their use is not without challenges. Isolation of adequate numbers of ACs leads to donor site morbidity and in vitro expansion results in a loss of phenotype. Conversely, large numbers of MSCs can be isolated, but their chondrogenesis is limited. Co-cultures of ACs and MSCs are gaining popularity as an alternative to monocultures. The objective of this work was to evaluate AC and MSC co-cultures in flow perfusion conditions. Bovine ACs and a 1:1 ratio of bovine ACs and rabbit MSCs were seeded on poly(ϵ-caprolactone) microfiber scaffolds and divided into static and perfusion cultures for 14 days in chondrocyte growth medium. DNA and glycosaminoglycan (GAG) content ($n = 8$) were quantified, and histological sections were analyzed ($n = 4$). Both AC and co-culture groups had significantly higher levels of DNA and GAG in perfusion than in static conditions. Furthermore, the need for ACs was successfully diminished in static cultures as there was no statistical difference in GAG or GAG/DNA levels between the cell populations. In perfusion, however, the GAG/DNA was significantly greater in ACs (4.1 ± 0.5 $\mu g/\mu g$) than in co-cultures (3.2 ± 0.3 $\mu g/\mu g$). While both cell populations were enhanced by perfusion, more work is needed in order to fully utilize perfusion conditions in the co-cultures. (This work was funded by the NIH R01-AR57083.)

56.P08
Development of hASC on 3D microcarrier system

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Finite amount of cells that can be extracted from an individual remains an impediment and the stem cells may face the problems of losing their original phenotypes after a series of passaging. Bioengineered-microcarriers are composed of alginate core embedded with paramagnetic particles and coated with covalently bound adhesion molecules, such as, collagen, gelatin and laminin. These specially designed microcarriers have the advantages of high surface to volume ratio, efficient oxygen and medium nutrient diffusion which are beneficial for cell proliferation and maintenance of cell phenotype. In this study, hASC (human adipose-derived stem cell) was selected as our model for comparison of the cell conditions in 2D and 3Dculture. Cell adhesion assays were performed first for the selection of the appropriate ECM coating. BioLevitator, a bench-top incubator which can increase the mass transfer between cells and microcarriers by tuning the rotating condition to

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thoroughly mixing was used in the 3D culture system. Cells morphology and proliferate status were observed by DAPI and Phalloidin staining. Cells from either 2D or 3D culture were replated for the examination of their differential ability toward osteogenic and adipogenic lineage. Increases of cell viabilities and growth rate by culturing cells in environment similar to physiological environment were found. There is a trend that the differentiation ability of hASCs on 3D culture system was higher than 2D.

56.P09
Electrospun biaxially distensible bilayer membranes to treat cleft palate

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Cleft palate occurs in over 1 in 1000 live births worldwide. Current treatments are multi-staged, spanning childhood. Current implants don't grow with the child, nor allow for the development of a hard palate. This study aims to develop an electrospun scaffold that can be used for cleft repair, encourage growth of a hard palate, and biaxially distend with the child. The aim is to produce a bilayer membrane to allow growth of bone on one side, and oral mucosa on the other, allowing slow growing bone tissue to develop. The scaffold must also be capable of biaxial distension to accommodate the growth of the palate. Bilayer membranes of nanofibrous polyhydroxybutyrate-co-valerate and microfibrous polyL-lactide were produced by sequential electrospinning onto a rotating drum. Embryonic mesenchymal progenitor cells and dermal fibroblasts were labelled with fluorescent dyes and seeded onto either side of the membrane. Cells attached and proliferated but did not cross over even after 7 days of co-culture. A distensible bilayer membrane was created by electrospinning onto a biaxially distensible surface (a latex balloon) and the effects of biaxial distension on cells were investigated through the development of a dynamic balloon bioreactor. In summary we describe a bilayer nanofibre/microfibre membrane capable of segregating labelled cells for at least 7 days. This study now needs to be extended to characterise the response of cells on the scaffold during biaxial loading.

56.P10
Shear force stimulation of adipose-tissue derived stem cells in a novel bioreactor

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Introduction: Adipose-tissue derived stem cells (ASCs) have been identified as a possible source for cartilage tissue engineering. Although differentiation with growth factors is possible, the mechanical properties of these constructs are rather poor. Therefore, our concept aim to enhance differentiation by applying a combination of shear and pressure forces on a scaffold seeded with ASCs.
Material and methods: The bioreactor system consists out of two piezo motors and one cantilever with a stamp on it. The first piezo motor is connected to the cantilever and presses the stamp onto a fibrin scaffold with the embedded cells inside. The second motor is a linear element that is responsible for the bidirectional movement of the lower part, where the scaffold is fixed.
Results: Constructs were stimulated applying a force of 300 mN over 3 weeks, 3 days per week, without destroying scaffold or cells. The first findings indicate that cell proliferation is reduced, compared to unstimulated cells. After stimulation, chondrogenic differentiation was demonstrated with alcian blue histochemistry.
Conclusion: ASCs can be stimulated in this system with various parameters. Therefore, different parameters will be tested on passage 2 ASCs of rats with variation in duration from 10 to 60 min, force from 100 to 600 mN, frequency from 2 to 5 days per week. Financial support from FFG (#818412) and City of Vienna is gratefully acknowledged.

56.P11
Computational and experimental approach to understand the dynamic cell seeding

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Proper cell density and spatial distribution in a 3D scaffold are essential to morphogenetic development of an engineered tissue. The aim of this study was to combine computational and experimental techniques to study cell seeding under dynamic conditions. Rapid prototyped polycaprolactone scaffold with diameter of 5 mm and height of 1.5 mm (Biotek, USA) was used in a custom-made microfluidic chamber, thus enabling live visualization of the seeding process. The scaffold morphologies were reconstructed from micro CT images and the fluid volume was created similar to the microfluidic chamber. Computationally cell motion was represented as particles in a fluid medium using a multiphase Lagrangian formulation implemented in Ansys Fluent. Spherical particles (10 μm) were dragged by the fluid flow and cell adhesion was simulated. Experimentally, polystyrene fluorescent microspheres (10 μm) were used, and the fluid flow was controlled with a syringe pump. Identical inlet fluid flow condition was applied corresponding with a constant inlet velocity of 0.05 mm/s. Live image of the seeding process in the microfluidic chamber enables to record particle trajectory and velocity and possible zone of cell adhesion. In both cases when the particles are near the scaffold, there is an increase of particle velocities. The correspondence in terms of particles adhered are shown. The combination of experimental and computational analysis provides a strong platform to optimize cell seeding.

56.P12
In vitro and in silico study of cell growth in porous scaffold under dynamic flow

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The use of bioreactors for cultivating bone-forming cells on a three-dimensional porous scaffold material resolves mass transport limitations and provides physical stimuli, increasing the overall proliferation and differentiation of cells. Despite the recent and significant development of bioreactors for tissue engineering, the underlying mechanisms leading to improved bone substitutes remain mostly unknown. Previous studies have shown that numerical simulations can be a powerful tool to predict tissue development in complex environments. However, current models often present a poor representation of local physics and comparisons with experiments generally do not lead to a quantitative agreement. In order to experimentally reproduce the fluid flow through a porous scaffold, three-dimensional, micro-architectured micro-fluidic chambers have been designed. Osteoblast cells have been cultivated in micro-systems with and without flow, and cell proliferation dynamics have been monitored with image analyzing. Simultaneously, a numerical model has been developed in order to predict cell growth under fluid flow. Cell population dynamic is simulated using a three-dimensional cellular automaton, while the fluid

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flow is described using the Lattice-Boltzmann method (LBM). Experiments and numerical results show the influence of fluid induced shear stress on cell proliferation.

56.P13
Characterisation of a uniaxial tensile strain bioreactor

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Uniaxial strain bioreactors are used to investigate the mechanobiology of cells in vitro, and for mechanical conditioning of cell-scaffold constructs. Bioreactors create a controlled environment that more closely resembles in vivo conditions than conventional cell culture techniques. A custom-designed bioreactor imparting cyclic uniaxial tensile strain to cells cultured on a hyperelastic membrane was characterised. DIC and laser displacement data showed near-uniform and uniaxial strain across the well verifying finite element modelling. Micro-crystalline domains are evident with high resolution atomic force microscopy that after plasma-etching are obscured by formation of an amorphous oxide layer. Wettability studies show hydrophilicity increases as a function of both plasma power and etching duration. X-ray photoelectron spectroscopy revealed that plasma-etching of the Chronoflex culture membrane increased surface oxygen while nitrogen and silicon remained unchanged. Adsorption of serum proteins to the modified surface increased nitrogen content and improved attachment of osteoblast-like cells. Poor cell attachment was found with non-etched membranes; clustered cells were evident, and scanning electron microscopy showed that these clusters were bound to the membrane via single cells. Osteoblastic cell morphology and spreading on plasma-etched membranes was similar to that of TCP. Changes in cell morphology and metabolism were observed in cells exposed to cyclic strain.

56.P14
Dynamic culture in a rotating bioreactor gives rise to enhanced cell distribution, viability and morphology

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In vitro culture conditions play a crucial role in cell distribution, viability, proliferation and differentiation, driving subsequent tissue development. Significantly better results were reported when scaffolds were seeded and cultured under dynamic conditions in bioreactors, compared to static controls. The aim of the present study was to investigate the effects of dynamic conditioning on cell distribution, viability and morphology onto long tubular porous scaffolds using our double-chamber rotating bioreactor, which allows proper cell seeding, mass transport and hydrodynamic stimuli within a construct of clinically relevant dimensions (L = up to 10 cm, d = 2.5÷10 mm). Primary porcine aortic smooth muscle cells, primary bovine chondrocytes and human mesenchymal stem cells were separately seeded on (1) non-woven 100% benzilic ester of hyaluronic acid and (2) non-woven 20/80 PGA/PLLA scaffolds. Dynamic culture under two different conditions, single phase (medium) and two-phase (medium/air) rotation, were investigated and compared with static controls. Histological and immunohistochemical analysis were performed, as well as scanning electron microscopy, at different timepoints (1 day, 7 days, 21 days, 34 days). Higher cell survival and much better colonization throughout the scaffold thickness with respect to static controls were obtained. Evaluation of ECM synthesis is currently on-going on late timepoint sections stained with Masson's trichrome and subsequent morphometric analysis.

56.P15
An innovative acoustic permeability measurement system for tissue engineering scaffolds

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Permeability is a key parameter to evaluate the transport performance of the scaffolds. Existing permeability systems show limitations related to (i) long measurement time (e.g. gravity driven systems) and (ii) necessity of controlling sensitivity of three transducers, i.e. pressure flow systems. To overcome these shortcomings, we present a new rapid permeability evaluation technique based on acoustic pressure measurements by using only a transducer, i.e. a low-frequency microphone. The permeability system consists of a condenser microphone and a cavity closed with a scaffold holder and a piston. Technically, an alternating airflow waveform is generated in the cavity by the motion of the piston and the microphone, located in the cavity, is used to measure the sinusoidal pressure component. The permeability is determined by the ratio between the root mean square (RMS) value of the pressure measured into the closed cavity and the RMS volumetric airflow rate. Preliminary tests were performed on a low-porosity rigid calcium carbonate commercial scaffold used for the regeneration of bone tissue (porosity = 50%, pore diameter = 150 μm). The experimental results provided a permeability value k = (2.85 ± 0.10)e-11 m^2, with a confidence level of 95%, in accordance with published permeability values. In the next future, this system will be used to measure permeability of scaffolds, with different porosity, over a wide order of magnitude (from $1e^{-18}$ m^2 to $1e^{-7}$ m^2).

56.P16
Replicating the 3D cardiomyocyte environment in the squeeze pressure bioreactor

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Cardiac cells are normally subject to mechanical stresses during hemodynamic loading/unloading in the heart, modulating their physiological development and functions. Furthermore, a 3D structure can increase differentiation of cardiac cells. Here, the SQPR (SQueeze PRessure) bioreactor is used to apply a cyclic hydrodynamic non-contact pressure on a H9c2 murine cardiac cell line in combination with scaffolds, to recreate a functional cardiac environment. The system is composed of a control unit and a chamber with a piston which moves up and down, creating a local overpressure on the base of bioreactor, where the cell construct lies. A CFD model identified pressure peak values around 1 kPa. The system was set to a 1 Hz cyclic loading frequency for 24 h. 2D (i.e. collagen sandwich) and 3D hydrogel (i.e. gelatin porous) scaffolds were fabricated and compressive mechanical tests were performed to evaluate the mechanical properties of both constructs. Typical values range within 1 to 10 kPa. Cellular experiments were performed seeding the 2D scaffolds with 6×10^4 cells/cm^2 and the 3D constructs with 1×10^6 cells/cm^3. The results showed that stimulated cells have good viability compared to controls, indicating that the SQPR does not damage the cell culture. Moreover, morphological and histological analyses show that after stimulation cells adopt a more cardiotypic phenotype with an organized cytoskeletal structure and elongated F-actin fibers.

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56.P17
Electro-mechanical sensorized bioreactor for cardiac tissue engineering

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In cardiac tissue engineering, a number of studies demonstrated the role of physical stimuli in improving functional and mechanical properties of the engineered cardiac constructs. We designed and developed an electro-mechanical sensorized bioreactor for delivering controlled stretching and electrical stimulations for growth and differentiation of stem cells cultured on scaffolds.The bioreactor is composed of: a sterile culture chamber for housing cell-seeded scaffolds and the culture medium (working volume = 70 ml); a mechanical stimulation system, with dedicated grasping system, to provide cyclic stretching loading (strain up to 20%, cycling frequency up to 2 Hz); an electrical stimulation system to provide electrical monophasic square pulses (1–6 V/cm, 0.25–10 ms, 1–5 Hz); a sensing system for measuring the load applied to the constructs; a recirculation system for the automated medium change; a control system for data acquisition and mechanical stimulation. Operating tests demonstrated the suitability of the bioreactor in delivering mechanical and electrical stimuli to mimic the physiological ones, providing information on forces acting on the constructs. This bioreactor is a multipurpose adaptable system for dynamic culture of cell-seeded scaffolds, in order to obtain functional cardiac constructs. Study carried out within the scope of BIOSCENT European Project (ID 214539).

56.P18
A multi-stimuli environment for cardiac tissue engineering

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The identification of the ideal cell source to generate cardiac tissue able to integrate into the host myocardium and with the contractile system is crucial for cardiac engineering. Amongst different cell sources so far proposed, human adult Cardiac Progenitor Cells (hCPCs) show the ability to proliferate and differentiate toward cardiac lineages when grown in appropriate microenvironmental conditions. It is widely accepted that conventional 2D cultures may provide a physiological environment for growing cells. For this reason the need to have an engineered microenvironment, matching physiological requirements, is crucial. A 3D context with spatial and time varying distribution of regulatory factors using mechanically matched scaffolds and bioreactors could represent an in vitro cell culture model being able to more closely reflects the in vivo conditions. In the present study, the possibility of using biocompatible and biodegradable scaffolds of collagen based or derivatives hydrogels in combination with Linneg/Sca-1pos hCPCs gathered from human heart biopsies was investigated. Bio-constructs were placed in the low shear, high flow MCmB (MultiCompartment modular Bioreactor) and the combined effects of dynamic culture conditions and 3D scaffolds on cell morphology and differentiation were studied in order to investigate the possibility of fabricating stem cell-derived cardiac patches to replace infarcted tissue.

56.P19
Development of a skin culture model using a bioreactor with a combination of shear and compressive mechanical stimulation

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A skin culture model has been developed to evaluate the degradation and burst release kinetics of a subcutaneously implanted vaccine delivery device. The tissue culture model aims to preserve the physiological and immunological properties of the native tissue in order to provide an environment which represents the implantation site of the vaccine delivery device, without the need to conduct live animal studies. Porcine skin explants were cultured with the epidermal side at the air-media interface, in a combined compressive/shear bioreactor system to simulate the dynamic environment of native tissue and encourage perfusion of oxygen and medium through the tissue. A prototype of the vaccine delivery device was then implanted in the hypodermal region of the explant to evaluate the degradation and release kinetics of the active compound, using a fluorescently labelled marker, ovalbumin. Physiological and immunological changes to the dermal tissue were assessed using a haematoxylin and eosin stain, in conjunction with immunohistochemistry for key biological markers such as collagen, keratin and Langerhans cells. The shear/compressive bioreactor has demonstrated potential to increase the viability of the porcine skin explants, thus creating a better model for live native skin. This model will reduce the need for animal experiments while assessing the degradation and burst release kinetics of the vaccine delivery device during the optimization of the technology.

56.P20
Establishment of 3D culture system for primary hepatocytes 'Regulation of cell membrane polarity for tissue engineering'

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It is important for liver to be constructed 3 dimensional (3D) structure for maintaining sufficient hepatic function. In addition, the cell membrane proteins of hepatocyte are maintained the polarization of vessel and bile canaliculi side in vivo. However, the polarization of cell membrane proteins cannot be maintained in 2D culture system. Therefore, the artificial culture system is necessary to solve this problem. In this study, we report the artificial technique that can regulate the polarization of cell membrane proteins of hepatocyte. We used PVLA[poly(N-p-vinylbenzyl-4-O-ß-D-galactopyranosyl-D-gluconamide)] as artificial extracellular matrix. PVLA is the specific artificial polymer that is adhered to hepatocytes through interaction between the asialoglycoprotein receptor (ASGPR) of hepatocytes and the galactose moieties of PVLA polymer. We confirmed that the polarization of ASGPR can be regulated by using PVLA coated dish. In addition, we confirmed that the spheroid of hepatocyte is formed by the cell migration. And the spheroid formation maintains the liver specific function and regulation of cell membrane polarization. Next, we fabricated complex gel structures by using 3D bioprinter, since it is important for primary hepatocyte to be maintained 3D environment. We selected the alginate gel to form the gel structure rapidly and be used as the scaffold to seed hepatocyte. These findings may open a new horizon by developing the artificial organ for TE.

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56.P21
Towards engineering vascularized adipose tissue in a custom-made bioreactor system

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In adipose tissue engineering for reconstructive and plastic surgery, adequate vascularization of large constructs still represents a crucial challenge. Therefore, objectives of this study were (1) to establish co-culture conditions for adipose-derived stem cells (ASC) and microvascular endothelial cells (MVEC) on a decellularized porcine jejunum segment, and (2) to perform first steps towards the use of this cell carrier in the generation of vascularized adipose tissue in a custom-made flow-through bioreactor system. The decellularized jejunum segments were seeded with human ASC and human MVEC in coculture (400 000 cells/cm^2 each, seeded on opposite sides of the matrix) and a coculture medium based on PGM (Lonza) and VascuLife (CellSystems) was established. After adipogenic induction in well plates, Oil red O staining for lipids indicated strong adipogenesis of ASC, which was in distinct contrast to uninduced controls. Lipid content increased over time during the 14 days of culture (histology, triglyceride quantification). A fraction of the ASC was shown to migrate towards MVEC, which were stained positive for CD31. When cultured in the bioreactor system, either under static (no flow) or flow (3.8 ml/min) conditions, successful adipogenesis of ASC was also demonstrated. In ongoing work, the decellularized jejunum is intended to be utilized as the basis for engineering adipose tissue in a bioreactor system specifically fostering the development of vascular structures.

56.P22
Application of a laser-based sensor for real-time oxygen monitoring in three-dimensional tissue cultures

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Clinical-size tissue engineered constructs pose a challenge to cell nutrition and survival. Oxygen concentration within 3D scaffolds determines cell viability and metabolism. Current technologies for oxygen monitoring include fibre optical sensors for direct insertion or indirect assessment by differential oxygen sensors. A real-time two-frequency phase-modulation technique to determine phosphorescent lifetime of oxygen-sensitive probes encapsulated in microbeads was applied towards mammalian static and dynamic cell culture systems. The oxygen sensor system consisted of an optical device with phosphorescent microbeads and a laser light source, a minaturized electronical device with implemented lock-in-amplifyer and frequency generator and software for data collection and analysis. Cylindrical collagen sponge-like scaffolds with microbeads were seeded with adipose-derived mesenchymal stem cells and cultured statically and dynamically in a laminar flow bioreactor up to 3 weeks. Signal detection was reliable at a depth of 10 mm inside the collagen scaffold. MSC demonstrated a gradual decrease in oxygen concentration in the scaffold centre from 21% down to 10%. The multifrequency phase-modulation technique allows measurement of phase shift of luminescent signals at two different frequencies and therefore can overcome autofluorescent signals from the tissue itself. The technique is applicable for continuous oxygen monitoring in static and dynamic culture of 3D tissue.

56.P23
Differentiation of murine embryonic stem cells (mESCs) into type II pneumocytes in a 3D sparged bioreactor

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Current practise on lung disease involves lung transplantation, which is limited by organ availability and immune rejection. Cellular therapies using ESCs offer an attractive possibility as long as challenges such as controlled and efficient differentiation are addressed. ESCs are traditionally cultured using conventional culture, which is labour-intensive, difficult to scale-up and cannot recapitulate the proper microenvironment that controls differentiation. Herein, mESCs were encapsulated in alginate hydrogels and were cultured in static cultures and a sparged bioreactor whereby gas (5%CO_2 and 20%O_2) is injected into the bioreactor. The A549 conditioned medium was used to induce differentiation toward the type II pneumocyte. The differentiated cells were assessed for lung cell markers: SPA, SPC, AQP5, CC10 and FoxA2. Relative expression of SPC markers reached the maximum level, ~10-fold increase, at day 14 and day 20 for 3D static cultures and the sparged bioreactor, respectively. After day 20, SPC expression was decreased in the static cultures in contrast to the sparged bioreactor. At day 30, both systems expressed endodermal makers, FoxA2, observing a significantly higher level in the sparged bioreactor. In conclusion, the gassing in the sparged bioreactor not only enhanced the differentiation of mESCs into type II pneumocytes but also mimicked the in vivo environment in the lung which allows the cells to maintain the lung genotype and morphology in long term cultures.

56.P24
Microarray analysis of human MSC in 3D collagen culture: 10% uniaxial cyclic tensile strain upregulates genes associated with musculoskeletal and cardiovascular development

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Mesenchymal stem cells (MSC) have gained great interest for cell based therapy and tissue engineering applications. As progenitor cells in bone, MSC function in an active mechanical environment. Previously, we have shown that cyclic tensile strain of magnitude 10% accelerates and increases osteogenesis of human MSC and ASC. The aim of this study was to use microarray to investigate potential mechanisms of hMSC response to mechanical load (10% cyclic tensile strain) in 3D collagen culture in the absence of soluble osteogenic induction factors. 79 genes were identified in response to 10% uniaxial cyclic tensile strain. Top five physiological development and functions associated with dataset included tumor morphology, tissue development, tissue morphology, development in musculoskeletal system, and development in cardiovascular system. Musculoskeletal system development and function showed the upregulation of genes associated with proliferation of muscle cells; clu, plau, plaur, trib1, and vegf a and differentiation of bone cell lines; bhlhe40, igf1r, irs1. Cardiovascular system

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development and function showed the upregulation of genes associated with vascularization; igf1r, irs1, plau, vegfA and migration of endothelial cells; hlx, plaur, tnfsf12a, vegfA. The top ten mapped pathways included embryonic stem cell pluripotency, and the roles of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis with the upregulation bmp1, wnt5b, pik3cd, and tcf4.

56.P25
Engineering cartilage: human adipose stem cells detect and respond to physiologic levels of hydrostatic pressure

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Human articular cartilage functions under a wide range of mechanical loads in synovial joints, where hydrostatic pressure (HP) is the prevalent actuating force. We hypothesized that formation of engineered cartilage can be augmented by applying such physiologic stimuli to adipose stem cells, cultured in hydrogels, using custom-designed hydrostatic pressure bioreactors. To test this hypothesis, we investigated the effects of distinct hydrostatic pressure magnitudes on cartilage formation in vitro by human adipose stem cells (hASCs) encapsulated in gellan gum (GG) hydrogels. To this end, we applied a pulsatile regimen to hASC-GG constructs (0.5 Hz) and varied the amplitude of loading, by generating both low (0.4 MPa) and physiologic (5 MPa) HP levels. hASC (10 million cells/mL) were encapsulated in GG hydrogels (1.5%) and cultured in chondrogenic medium under three regimens for 4 weeks: (1) 0.4 MPa Pulsatile HP; (2) 5 MPa Pulsatile HP; and (3) Static. Greater chondrogenic differentiation and matrix deposition were obtained for physiologic loading (5 MPa), as evidenced by gene expression of aggrecan, collagen type II and sox-9, metachromatic staining of cartilage ECM and immunolocalization of collagens. We thus propose that hASC detect and respond to physical forces, thus resembling joint loading, by enhancing cartilage tissue development in amplitude-dependant manner.

56.P26
The influence of scaffold mechanical manipulations on embryonic stem cells differentiation

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Controlling embryonic stem cells (ESC) proliferation and differentiation to form complex, viable three-dimensional (3D) tissues is challenging due to their pluripotency and their potential therapeutic implications. We previously showed that polymer scaffolds which serve as mechanical and biological supports for cell growth and functionality can promote proliferation, differentiation and organization of ESC into 3D structures. The ability to create such 3D constructs can enhance our understanding of embryogenesis mechanism. During this process cells sense a range of mechanical forces which play a critical role in the gastrulation phase by transforming the embryo from a simple sphere of cells to a multi-layered organism. In this study we used mechanical manipulation to mechanically influence the differentiation of mouse ESC within 3D structures and to better understand the involvement of forces in embryogenesis. Using advanced bioreactor we apply dynamic or static forces on the cells within 3D environment and examine their differentiation. Our results show that external signal mediated to the cells through the scaffold can influence their differentiation with different response for each of the germ layers. Additionally, different type of mechanical manipulations induced different response of the cells.

56.P27
Mechanical stimulation of mesenchymal stem cells in a 3D glass scaffold using oscillating and unidirectional flow

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Bioreactor conditioning seems to accelerate cell differentiation and matrix production for bone tissue engineering but it is unclear what the optimal mechanical stimulus is. The aim of this study was to investigate the effects of oscillating and unidirectional flow (3, 5 and 10 ml/min) on a human mesenchymal stem cell (MSC) cell line h-ESMP (Cellartis) within porous glass scaffolds. Glass scaffolds (NSG GROUP, 10 mm diameter and 3 mm height) were seeded with 2.5×10^5 cells and cultured in standard MSC media with dexamethasone (DEX) (100 nm). Cell-seeded scaffolds were subjected to fluid flow for 1 h on days 4, 7 and 10 and samples were tested for cell metabolic activity by MTS activity and Alkaline Phosphatase (ALP) activity by the p-nitrophenol phosphate reaction at day 7 and collagen and calcium production at day 14 of culture. Preliminary results indicate that both flow regimens maintained cell viability in the scaffolds. More specifically ALP, calcium and collagen production were higher in those scaffolds subjected to oscillating flow than those of unidirectional flow. Scaffolds that had been subjected to an oscillating flow of 3 ml/min were shown to produce the highest level of ALP and those that were subjected to 5 ml/min produced the highest level of calcium and collagen. These results indicate that short bouts of oscillatory flow may stimulate osteogenic differentiation of MSCs in bioreactor conditioning of tissue engineered bone.

56.P28
Effects of hydrodynamics on undifferentiated mouse ES cells proliferation in a suspension bioreactor

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Introduction: ES cells and iPS cells have much attention as a cell source for a regenerative medicine technology. To realize the therapeutic potential of these stem cells, a mass cultivation system for undifferentiated cells must be established. We focus on stirred-tank as a culture vessel for ES cell culture. In this study, we evaluate the effect of shear condition on ES cells proliferation in suspension bioreactor.
Materials and methods: A single-cell suspension was added to a 100 ml spinner vessel containing 50 ml of culture medium supplemented with 1000 U/ml LIF. The culture was stirred continuously for 3 days at various agitation rates. Then, cells were harvested and were dissociated to obtain single-cell suspension. The cells were reseeded back in the spinner vessel. Every 3 days, ES cells were passaged in a same procedure.
Results and conclusion: ES cells formed multicellular aggregates spontaneously. It was found that high cell density culture could be achieved in the suspension bioreactors as long as the maximum shear stress was maintained within the range of 0.4 and 0.6 Pa. The resulting bioprocess achieved an 11-fold expansion in 3 days. The high expansion ratio was maintained at least five serial passaging. ES cells within the aggregates maintained in an undifferentiated state throughout the culture period. These results indicated that this suspension culture process provides an alternative to the conventional culture process on feeder cells.

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56.P29
Hydrogel microsphere cell carriers for cell expansion in suspension culture: implications for stem cell therapeutics

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The development of scalable methodologies for handling human stem cells (hSC) necessitates an approach premised on the use of three dimensional (3D) suspension cultures that are readily adaptable to large-scale bioreactors. In this project, encapsulating fibrinogen based hydrogel biomaterials were used for developing a 3D bioprocessing methodology for stem cells through a straightforward cell inoculation into microspheres, in situ self-renewal in the microsphere culture system and mild cell recovery into a solution phase. For creating the microsphere carriers, we employed a temperature-responsive semi-synthetic hydrogel material produced by the conjugation of poloxamers to fibrinogen. Mesenchymal cells were encapsulated and cultivated in the hydrogel microspheres using suspension bioreactors for up to 14 days. The viability, cell cycle and proliferation were characterized and indicated comparable expansion results to 2D tissue cultures on petri dishes. Cell recovery from the hydrogels was accomplished enzymatically with high yields and minimal disruption to cell viability. We conclude that a biomaterial-based method can be successfully applied for suspension expansion of cells with controlled cell proliferation through cell-cell and cell-material interactions. Moreover, hydrogel microsphere cell carriers such as the ones described herein may offer a tangible solution to the growing demand for commercial-scale stem cell bioprocessing practices.

56.P30
Development of bioreactor for culturing 3D hydrogel structures containing living cells

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It is essential to keep sufficient delivery of nutrients and oxygen to the periphery in engineering large sized tissues. For this purpose, the bioreactors for perfusion culture play an important role. Various 3D hydrogel structures containing living cells can be designed and fabricated using 3D bio-printer we have ever developed. 3D structures with multiple perfusion canals can be designed and fabricated. Then, the custom-made bioreactors were developed to culture such 3D bio-constructs. In order to achieve a fluent flow, flow-canals with uniform multiple micro channels of 180 μm in diameter were installed in the culture chamber. The bioreactor circuit was composed of a peristaltic pump, air trap, a gas exchanger and the designed culture chamber. Then, we investigated the flow in the chamber by flow visualization using fluorescent ink and the samples of non-woven mesh. As a result of the flow visualization, gentle and uniform flow was observed in the chamber, which is thought due to micro-channel. The trace of the flow was decided by the thickness of the fluorescent inks remained in the non-woven mesh. Compared to the chamber without micro-channels, the distribution of stain was significantly uniform. In conclusion, the bioreactor system in which fabricated 3D tissues can be cultured with uniform and gentle flow was developed. Using this bioreactor, fragile 3D hydrogel structures can be cultured towards culturing large sized thick artificial tissues.

56.P31
Site-directed cell differentiation on a single multicellular spheroid using multilayer microfluidic system

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Multicellular spheroids which are formed following rearrangement and compaction of cell aggregates, have been widely use as a 3-dimentional culture model to supplement traditional monolayer cultures. Because of their tissue-like characteristics, spheroids culture is suitable for various applications, including tumor metastasis and invasion research, drug screening, and serving as building units for tissue engineering. This abstract presents a microfluidic system which can induce cell differentiation at a specific site on a single spheroid. A thin PDMS membrane contained multiple orifices (200 μm) was bonded between two PDMS micro-channels to create a multilayer microfluidic chip. The designed geometry could trap a spheroid, partitioning the spheroid into two parts which allows different parts of a spheroid exposing to different stimuli and later differentiating into different cell types. Our preliminary results showed that spheroids could be trapped on the membrane under continuous flow. To mimic the different stimuli, two fluorescent dyes, CMFDA (green) and CMTMR (red), were injected into different channels. After treatments, a chimeric spheroid was shown one part with red and the other part with green fluorescence. In conclusion, cell differentiation cues can be delivered by fluid flow to a selected part of the spheroid. This technique may contribute to tissue engineering, drug screening, and biological studies.

56.P32
Perfusion seeding of porous scaffolds: a bioreactor-based procedure suitable for the establishment of uniform 3D-structured co-cultures

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Interstitial perfusion has been proved successful for the development of 3D tissues, whereby it is crucial to provide a high initial number of viable cells with a homogeneous distribution throughout scaffolds. The present study aims at characterizing and enhancing a perfusion seeding technique based on a previously validated Oscillating Perfusion Bioreactor (OPB). Design of Experiments: statistical methods were used to assess the effects of process parameters, considering flow speed, cell seeding density and seeding time as process variables and cells and commercial scaffolds as inputs. OPB enabled to reach up to 70% seeding efficiency associated to significantly higher cell viability as compared to static controls (170% relative to static, $P < 0.0001$) for densities up to 4×104 cells/mm^3. Scaffold cross-sections showed higher uniformity for perfusion seeded compared to statically seeded constructs. In order to assess the possibility to guide the structural organization of the construct obtained with our procedure, we performed preliminary experiments with a two steps sequential seeding method of MSC and HUVEC. With our procedure we generated constructs with HUVEC networks embedded on uniformly MSCs-lined scaffolds, as opposed to statically or one-step seeded mixed populations constructs. In conclusion results demonstrate that our bioreactor-based seeding procedure can represent a step forward in the achievement of structural control in organized 3D multicellular systems.

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56.P33
Stem cell expansion in a fluidised bed bioreactor for accelerated osseointegration of bone substitute material

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The aim of this work is to enable the culturing of human stem cells within a porous bioceramic scaffold in a novel fluidised bed bioreactor. As such, the populated scaffold would be superior to the current synthetic bioceramic bone graft materials commercially available. The presence of a viable cell population will promote more rapid osseointegration thus accelerating the healing process. Particles of hydroxyapatite / tricalcium phosphate (4-6mm in diameter) have been fluidised successfully. The results combined with data from terminal velocity experiments and fundamental theory of fluidisation, have been used to develop a CFD model of the bioreactor in FLUENT. This model utilises a combination of the Eulerian multiphase model and the dense discrete phase model to calculate the velocity, pressure drop and shear stress within the bioreactor allowing control of its operation. Tests to establish the shear stress in relation to attachment and detachment of a cell line, using a purpose-built flow chamber, will enable the final design of the fluidised bed to be optimised in this FLUENT model. This analysis will inform the next stage of the project where suitable conditions will be derived to seed and culture a cell line within the fluidised bed. This model has the scope to be adapted to encompass the differing requirements of a range of cell lines in conjunction with bone graft material of different geometries, compositions and properties.

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57. TE for In Vitro Tests

57.01
Keynote: Incorporating microfabricated models of gastrointestinal tract into 'Body-on-a-Chip' devices

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A 'Body-on-a-Chip' device is a physical representation of a PBPK model where tissue engineered constructs of selected organs replace the corresponding differential equations and is used to predict human response to drug candidates. Such devices are microfabricated to facilitate low cost drug tests. The GI tract controls entry of orally delivered drugs into the systemic circulation. Here we described two approaches to construction of a realistic GI tract model consisting of epithelial, goblet, and M cells. This model produces mucus and can transport particles across the cell layer. While the cells form microvilli on a flat membrane, macrovilli require a supporting structure. We have done this using a novel fabrication technique to make collagen pillars. These pillars have physiologically realistic height to diameter ratio. As an alternative we have constructed a S4-8 membrane (controlled pore size from 0.5 to 3.0 μ) that drops over silicon pillars. The pillars are then etched away leaving a porous structure. Cells have been grown on both of these structures. We will compare these two approaches, demonstrate the ability to construct multi cell type systems with full functionality and test a GI tract module integrated with systemic circulation and other organ modules such as liver.

57.02
Digital microfabrication of tissue arrays for pharmaceutical investigations

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Presently, monolayer cell cultures are frequently used to investigate potential pharmaceutical agents. The issues at hand are that these models give very little in terms of feedback on the effects of the microenvironment on chemotherapeutic and the heterogeneity of the targeted biological investigation. Micro-Electro-Mechanical Systems (MEMS) technologies have been very attractive and demonstrate the potential for many applications in the field of tissue engineering, regenerative medicine, and life sciences. These fields bring together the multidisciplinary field of engineering and integrated sciences to develop biological microfluidic models that aides the exploration of pharmaceuticals and its effects on tissues and organs. The Digital Micro-mirror Microfabrication (DMM) system has the capabilities to fabricate many advantageous tissue models. Amongst them, the microfluidic tissue array demonstrates promising tissue engineering, regenerative medicine, and life sciences in vitro tissue applications. Unlike many conventional microfabrication approaches, this digital microfabrication process eliminates the need for mask by incorporating a dynamic mask-less fabrication technique. Tissue arrays developed by this digital microfabrication process create a cell friendly environment that enhances cell attachment and proliferation within the modeled microarchitecture.

57.03
Engineering a functional in vitro model of the spinal stretch reflex arc

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In vitro systems that will be developed for drug discovery and toxicity testing need to move beyond the single cell systems to functional tissue. However, creating single organ systems in many ways doesn't address the issue of system level function or neuronal control of those tissue systems. We are developing two test-beds, one based upon cognitive function and the other motor control circuits, specifically the reflex arc. This specialized circuit translates information from the motor cortex to a motoneuron, which then activates muscle fibers, in turn a specialized part of the muscle, called the spindle, relays information based upon this movement to a sensory neuron back to the original motoneuron. We have created each segment of this system utilizing rat cells and have now repeated multiple segments utilizing segments derived from human stem cells. These cellular systems have been integrated with BioMEMs devices, including microcantilever systems that allow for non-invasive stimulation and monitoring of the devices. Glial cells have also been integrated with certain segments for understanding the myelination process. This talk will focus on progress to date and building these systems as well as their eventual utilization in the drug discovery process. These systems can be used to address applications, such as spinal cord injury, myotrophic lateral sclerosis, neuropathic pain, demyelinationg diseases as well as being useful in studies of fundamental neuroscience.

57.04
Thymus engineering: 3D in vitro model to culture functional adult thymic epithelial cells

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The lack of available models for in vitro culture of functional adult thymic epithelial cells (TEC) is a limiting factor for the engineering of a thymic organoid and the understanding of the development of a competent immune system. Therefore, we aimed to establish a 3D model for ex vivo culture of functional TEC. We first demonstrated that a small number of primary adult murine TEC seeded onto a scaffold could support T-cell maturation in vivo, but to a limited extent. To increase the potency of the graft, we aimed to expand TEC in vitro. However, TEC expanded under conventional conditions (in 2D on plastic) lost their specific phenotype and functionality. In contrast, TEC expanded for 12 days in 3D in a fibrin hydrogel formed EpCAM+ cell aggregates and showed phenotypic properties similar to freshly extracted TEC in terms of size and marker expression (e.g. MHC II). Moreover, 3D-expanded TEC maintained their functionality in vitro as assessed by their capacity to support and activate $CD4^+/CD8^+$ double positive thymocyte precursors. Ultimately, TEC functionality was confirmed in vivo by ectopically implanting fibrin constructs in athymic mice and detecting mature T-cell of thymic origin in the peripheral nodes after 6 weeks. We have developed an in vitro 3D model that maintains the functionality of expanded TEC, paving the way to a better understanding of TEC/thymocyte cross talk and to unprecedented approaches for the treatment of thymic-derived pathologies.

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57.05
3D bioengineered model of the tumor-stroma microenvironment of late stage ovarian cancer

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Introduction: The tumor-associated stroma promotes cancer progression, yet the precise interactions between cancer cells and their stromal compartments are poorly defined. We discovered mediators of metastasis by employing a bioengineered 3D approach that mimics the tumor-stroma microenvironment of late stage ovarian cancer.
Methods: We established an integrated 3D co-culture model of ovarian cancer and stromal cells using imaging and proliferation analyses. In patients, ovarian cancer cells form spheroids attaching to the stromal layer. Spheroids were grown in biomimetic polyethylene glycol-based hydrogels and layered onto electrospun-fabricated polycaprolactone meshes allowing adhesion of stromal cells. A whole human genome microarray was conducted to identify genes differentially regulated upon 3D co-culture.
Results: Spheroid growth was enhanced and more genes were differentially expressed upon 3D co-cultures. Regulation of biosynthesis and transcription were altered in cancer cells, while inflammatory and migratory responses changed in stromal cells after 3D co-culture.
Conclusions: We unraveled pathways that may be crucial in the etiology of ovarian cancer metastasis.

57.P01
Engineered silk fibroin matrices for 3D in vitro tumor modeling

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Tissue engineering has provided a major impact on regenerative medicine and may have an equally significant impact on personalized cancer medicine. Breast cancer metastases are usually not a curable condition. However, proper treatment can prolong life, delay the progression of cancer, relieve cancer-related symptoms, and improve quality of life. 3D in vitro model systems that are able to mimic the in vivo tumor microenvironment are now essential in cancer research. 3D silk fibroin protein matrices from non-mulberry silkworm Antheraea mylitta were investigated as potential biomaterial for this purpose. A model of in vitro breast cancer was developed and studied for the efficacy of anticancer drugs paclitaxel, celecoxib and ZD6474. Human metastatic breast adenocarcinoma cells were co-cultured with human osteoblast-like and mesenchymal stem cells. The results indicate that a mylitta fibroin scaffold can provide an easily manipulated microenvironment system for tumor modeling. The engineered tumor construct shows different zones of cell proliferation like an avascular tumor. Higher drug concentrations and synergistic combinations were required to achieve reduction in cell viability and invasive potential in 3D cultures. Metastatic breast cancer cells were found to suppress osteoblast proliferation, alter their morphology and functioning, induce bone loss and increase resistance to drugs. 3D in vitro tumor models may be a better system to evaluate cancer treatment strategies.

57.P02
Development of an in vitro platform for the study of drug-induced cardiotoxicity

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Introduction: Drug induced cardiotoxicity accounted for 19% of drugs withdrawn from the US market in the last 40 years. Early detection of compounds that are cardiotoxic during the in vitro stage would reduce the incidence of drug induced cardiotoxicity. We propose that recreating the anisotropic cardiomyocyte culture will increase the sensitivity of the platform for drug screening and provides a better environment to study CYP metabolism in the heart.
Methods: HL-1, H9C2 and primary cardiomyocytes were cultured on gratings of two different dimensions: One with pitch of 1micron and depth of 200 nm and the other with pitch of 2 μ and depth of 300 nm. Alamar blue, Immunostaining, RT-PCR and CYP gene induction studies by Beta Napthoflavone (BNF) were carried out.
Results: Cells were viable on the gratings. The F-actin and sarcomeric actinin and CX43 staining revealed a more aligned sarcomeric structure for the cells on the gratings which translated to increased force of contraction. There was significant CYP 1A1 induction upon treating the cardiomyocyte culture with BNF, a known CYP 1A1 inducer.
Discussion and conclusion: These preliminary results concur with the existing report that anisotropic culture restores phenotype and function of the cardiomyocytes. It shows its usefulness for CYP induction in cardiomyocytes. Further experiments are aimed at understanding how culture configuration affects cardiomyocyte sensitivity to drugs and its effect on CYP metabolism of drugs.

57.P03
Extracellular matrix deposition and scaffold biodegradation in an in vitro three-dimensional model of bone: x-ray computed microtomography study

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The development of an in vitro model of bone and the optimization of tools for the comprehension of the biological processes occurring during bone repair remains a major goal in the field of bone tissue engineering. Recently, taking advantage of a model based on the three-dimensional co-culture of osteoblasts and osteoclast precursors in SkeliteTM scaffolds we showed an enhancement of osteoblast differentiation in the 3D mineralized environment that, in turn promoted earlier osteoclast differentiation. The same study demonstrated that the increased osteoblast differentiation in the 3D model led to a deposition of extracellular matrix faithfully reflecting the bone morphology. Here we evaluated the same in vitro model by both X-ray computed microtomography and histological analysis, thus fully evaluating bone deposition and degradation processes. Either osteoblasts or a combination of osteoblasts and osteoclasts were seeded on SkeliteTM scaffolds. Scaffold biodegradation and an increased bone deposition, together with a more organized extracellular matrix were observed in the co-cultures, highlighting the role of osteoclasts in the guidance and regulation of bone deposition. These results confirm the potential and the relevance of co-culturing osteoblasts and osteoclasts in order to resemble the native tissue. Finally, the approach here presented could be useful in

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the future to validate and develop new in vitro culture systems in the field of bone tissue engineering.

57.P04
3D in vitro model of tumour microenvironment

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It is thought that altered communication in the tumour microenvironment (TME) results in epithelial-to-mesenchymal transition (EMT) which drives cancer development and progression. To elucidate the functional role of TME in cancer progression a three-dimensional (3D) in vitro model was created.HCT116 epithelial colon carcinoma cell line was cultured in alginate-chitosan-hyaluronan (Alg-Cs-HA) capsules in mono- or co-culture with neuroblastoma IMR-32 fibroblasts for 20 days. Capsule internal structure, cancer cell morphology, viability, metabolic activity, gene expression profiles and protein expression were analysed. Capsules exhibited an irregular, porous internal structure. Distinctive morphology of epithelial cancer cells within Alg-Cs-HA capsules was observed. Encapsulated HCT116 cells initially exhibited increased metabolic activity, followed by a decrease and plateau formation. Large changes in gene expression levels were observed in a number of EMT-related genes. Of those the expression of vimentin, Slug, Zeb1, MMP-2 and -9 was upregulated whereas E-cadherin, cMet, TGFβ1 and integrin α5 was decreased with time and upon co-culture with fibroblasts. Epithelial cell aggregates showed altered adherens junction morphology and strong MMP2 protein expression. Current results indicate that Alg-Cs-HA capsule may provide relevant 3D in vitro model for studies of interactions within TME and potentially a model for therapeutic target validation for initial drug screening.

57.P05
Towards convenient in-vitro culturing of basement membrane containing tissues: development of a new gradient perfusion bioreactor

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Membranes play an important role in the vital functions of many organs. To recreate and study these membranes in vitro, an organotypical environment is key. A new dual chamber perfusion bioreactor was developed, which holds space for a single membrane (Ø 9 mm). The possibility to supply a continuous flow of separate media to each side of the membrane, combined with optical and (small magnification) microscopic accessibility, enables the use of this bioreactor for a variety of applications in both membrane tissue engineering and the assessment of membrane functionality. A bioartificial kidney epithelial membrane, composed of human kidney-2 (HK-2) epithelial cells cultured in confluent density on an electrospun microfibrous polymer mesh, was used as a model system to demonstrate the functionality of the new bioreactor. The HK-2 cells were shown to remain viable even after a prolonged perfusion culture period (20 days) inside the bioreactor. Furthermore, preservation of the renal epithelial phenotype was indicated by RT-PCR by the expression of characteristic renal transporter genes. A gradient of media over the tissue engineered membrane enabled leakage experiments inside the bioreactor by which the monolayer integrity of the HK-2 epithelial cells on the membranes was assessed. Further possible applications using this bioreactor, which will be explored in future experiments, include transport studies and co-cultures.

57.P06
Towards a 3D cellular model for predicting in vitro the outcome of medical therapies: A case in photodynamic therapy (PDT)

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The need to obtain cell culture systems that recreate the in vivo cellular environment has challenge researchers to develop synthetic scaffolds inspired on extracellular matrix. These culture systems provide to cells a truly three-dimensional (3D) space for growing, essential for receiving the appropriate chemical, physical and mechanical cues. We have developed a 3D model, consisting of a hydrogel (the self-assembling peptide RAD16I) and human normal dermal fibroblasts (hNDF), to predict the outcome of photodynamic therapy (PDT), compared with monolayer cultures (2D). PDT is a therapeutic modality for treating various ophthalmic, dermatologic, cardiovascular and oncologic diseases, which involves a photosensitizer (PS), light and molecular oxygen to generate singlet oxygen, a phototoxic agent. Interestingly, we found that cells in 3D respond differently to PDT than in 2D. We observed that cells in RAD16I spontaneously up-regulated a subset of hypoxic markers, together with a radial uptake of the PS. These results demonstrated that cells in 3D are exposed to non-uniform distribution of oxygen and drug, which produces a heterogeneous population of cells that differ in their response to PDT, creating a cellular niche more resistant to the therapy. Instead, 2D cultures are characterized by uniformly rich nutrition and oxygenation. These data suggest that 3D models represent a powerful bridge between 2D systems and tissues for predicting in vitro the effect of medical therapies.

57.P07
Towards an in vitro model of macrophage-mediated degradation of polymer scaffolds

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Resorbable polymers are used in the human body as drug carriers and as scaffolds for tissue regeneration. Resorption of the polymers is based on intrinsic properties and the inflammatory process regulated by macrophages and fibroblasts. Macrophage-mediated degradation involves enzymes and/or reactive oxygen (ROS) species. The aim is to establish an in vitro model of polymer scaffold degradation in the presence of macrophages and fibroblasts that can be used to predict the outcome of the in vivo inflammatory niche. Macrophages (J774) and fibroblasts were cultured on poly(trimethylene carbonate) (pTMC) network films in the presence of inhibitors for degradation pathways in heat-inactivated medium. Degradation was quantified through polymer mass loss. Macrophage activity was measured through cytokine release. Cell coverage was calculated from confocal images. ROS were detected using CM-H2DCFDA.Cholesterol esterase is the main contributor to macrophage-mediated degradation as assessed by the degradation inhibition by diethylumbelliferylphosphate. ROS(indirectly inhibited by apocynin through NADPH oxidase and nitric oxide synthase) also contribute to the degradation of PTMC networks, as clear ROS activity patterns can be associated with sites of polymer surface erosion. The interaction between fibroblasts and macrophages creates an additional level of

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complexity towards a representative in vitro model to investigate the mechanism of in vivo degradation of biodegradable polymers.

57.P08
Microfluidic gradient systems to generate defined cell microenvironments and study cellular fate processes

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Cell microenvironments are the main driving force in cellular fate processes and phenotype expression in vivo. In order to mimic specific stem cell niches, and study cellular responses under those conditions in detail, we need the ability to create and control cell microenvironments in vitro. This includes the capability to modify surface properties and liquid composition in cell culture. In particular, the ability to generate spatial and temporal controlled liquid gradients, using microfluidic systems, is of high relevance to study concentration dependent cell responses. In this study, a diffusion based gradient generator was characterized both by computational fluid dynamic simulations, as well as experimentally. The microfluidic network was combined with a microstructured growth substrate to study myoblast differentiation and alignment in response to chemical and topographical stimulation. The same microfluidic network was also used to investigate different aspects of endothelial cell migration along chemotractant gradients and stem cell differentiation. The developed platform allows monitoring phenotype expression of cells in highly controlled gradient environments of soluble factors in combination with defined cell culture substrate properties. This offers a big advantage over standard cell culture techniques when investigating specific cellular responses to varying stimuli.

57.P09
Chondrocyte adhesion and phenotype maintenance in 2D and 3D substrates

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Isolated chondrocytes can be grown in vitro for the repair of articular cartilage defects. Phenotypic changes of chondrocytes in vitro are well characterized, but there is little information about cell adhesion. Previously, we quantified the adhesion of chondrocytes grown in monolayer using a centrifugation adhesion assay, and found that adhesion increases with passage, which correlates with chondrocyte dedifferentiation. This work aims to investigate the adhesion of chondrocytes in 3D culture, which retains the native cell morphology and phenotype. We hypothesize that adhesion of chondrocytes in alginate beads will be lower than cells in monolayer, and that decreased adhesion will correlate with phenotype and morphological maintenance. Chondrocytes were isolated from tibiofemoral calf joints and encapsulated in 2% alginate beads. At each time point, subsets were taken for qRT-PCR, immunohistochemistry, and the centrifugation assay. For the assay, cells were incubated for 1 h in fibronectin coated wells. The well plate was inverted and centrifuged, and imaging was performed before and after spinning to determine percent adherent cells. Compared to monolayer results, adhesion was decreased for cells cultured in 3D. Rather than increase with each time point, the percent adherent cells remained under 11% with no statistical difference between points. These results will be utilized in the further study of the relationship between adhesion and phenotype maintenance.

57.P10
Development of an 3D ex vivo model of bone tissue

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The pathologies of skeleton have a strong socio economic impact in our population. Although therapies have improved the treatment of osteosarcoma and osteoporosis, their efficiency remains still limited. The understanding of the mechanisms during the formation and the remodeling of bone tissue requires the development of an ex vivo model. The aim of this work is to develop a 3D model of bone tissue from human cell precursors cultured on biphasic calcium phosphate (BCP) particles. In this study, human bone marrow mesenchymal stem cells (MSCs) and monocytes were cultivated on particles of BCP (125–200 μm) in order to differentiate into osteoblasts and osteoclasts, respectively. The MSCs seeded on BCP adhered and proliferated producing an extracellular matrix and formed a 3D structure. Light and confocal laser scanning microscopy showed that MSCs created bridges between BCP. Then, at day 14, monocytes were seeded on this structure and cultivated with osteoclastogenic cytokines. At day 28, monocytes merged into multinuclear osteoclasts. The osteogenic differentiation of MSCs was observed using immuno-histology: Alkaline phosphatase, Collagen 1 protein. Alike, Cathepsin K and Tartrate resistant acid phosphatase, indicating osteoclastic differentiation were enhanced in these conditions. Differentiation was confirmed by supernatant analysis. In summary, we develop an ex vivo model that may allow the study of bone physiopathology including bone healing, osteoporosis and osteosarcomas.

57.P11
In vitro tissue disease model for osteoarthritis pathophysiology

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A tissue engineered in vitro disease model for Osteoarthritis pathophysiology was developed using silk fibroin scaffold, coupled with proinflammatory cytokines IL1β, TNFα using human chondrocytes. Chemically modified silk scaffolds anchor cytokines by ionic interactions in a biomimetic manner. Diazonium coupled silk matrix showed constant release of cytokines up to 7 days after adsorption to mimic an OA microenvironment in vitro. SEM analysis showed matrix embedded cytokines induced expression of multiple elongated processes in chondrocytes in vitro, as seen in OA cartilage in vivo. Large scale gene expression profile of in vitro disease model showed striking similarity to OA cartilage tissues (collected from four patients who underwent total knee replacement surgery), while that of healthy tissue engineered constructs (without cytokines) had similar expression profile to healthy cartilage tissue. The common markers of OA including COL, MMP, TIMP, ADAMTS, Metallothionein genes were upregulated at least by 35-fold in in vitro model. Microarray data was validated by RT-PCR. Protein interaction studies indicated that TNFα stimulates IL-1 & 6 that control equilibrium between MMPs and their inhibitors TIMPs, resulting in ECM degradation. TNFα also regulated apoptosis of chondrocytes through MAPK pathway. This in vitro disease model can be modified to assess various signalling mechanisms and their response to selective cell or drug treatments, as a step toward patient specific models.

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57.P12
Developing a novel three dimensional in vitro intestinal mucosa model for drug absorption evaluation

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Human epithelial cell culture models of monolayer Caco-2 cells have been widely employed to assess the absorption of drug molecules across intestinal mucosa. However, cautions should be taken when interpreting the conclusions from those models due to their undesirable phenotype and functionality when compared with the native intestinal tissue. Here, an improved, more physiologicaly relevant three dimensional (3D) culture model of the intestinal mucosa was developed to study drug absorption, in which a co-culture of epithelial cells, Caco-2 cells and HT29-MTX cells, was indirectly seeded on a Transwell filter insert with collagen gel and stromal cells incorporation. This setting-up provided a compatible environment to improve the epithelial cell phenotype and functionality. In comparison with the monolayer culture of Caco-2 cells, the constructed 3D model displayed more physiologically relevant characteristics evidenced by its decreased TEER value and mucus-like layer formation. A decreased expression of P-glycoprotein was also observed in the current 3D culture model, leading to an increased absorptive permeability of the substrates. More importantly, an improved correlation ($r = 0.95$) was obtained between the absorptive permeability in the 3D culture model and the absorbed fraction reported in human body. This reconstructed 3D co-culture model presents a unique, improved opportunity to evaluate drug permeability in vitro.

57.P13
Noninvasive wettability measurement of mucosal epithelial cell sheets

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Introduction: Oral mucosal epithelial cell sheets have been utilized for regenerating corneal tissue. Corneal mucosa has a high wettability for keeping tear. This study noninvasively quantified the wettability of cell sheets in various culture conditions and discussed the relationship between the wettability and gene expression levels.
Materials and methods: For preparing a cell sheet, rat oral mucosal epithelium cells were seeded on a temperature-responsive cell culture insert with Keratinocyte Culture Medium (KCM). Three different conditions were applied for the cell culture; (A) in KCM with Cytochalasin D, (B) in KCM without fatal bovine serum, and (C) in KCM as the control. For the wettability measurement, the width of cell sheet surface exposed by a momentary air-jet application to the medium was measured. Gene expression was quantified by DNA microarray analysis.
Results: The most fragile cell sheet in the case of (A) never broke by air-jet application. A cell sheet wettability measurement was theoretically derived from the residual width of medium after the air-jet application. The residual width in case of (B) was significantly larger than that of (C). MUC4 gene expression in case of (B) was one sixtieth of that of (C).
Conclusions: The cause of the residual width difference between (B) and (C) was speculated to be mucin 4. This method would be useful for screening the wettability of cell sheet before transplant and evaluating tear film breakup time in vitro.

57.P14
Development of an immune responsive 3D human lung model

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Despite enhanced patient care, the morbidity and mortality of patients with lung disease have remained high, for example there is one asthma related death every 20 min worldwide. This is partly due to lack of efficient therapeutic strategies and also that a large proportion of patients do not respond to treatments. There is a lack of predictive preclinical models of asthma and new treatments that enter clinical trials frequently fail, possibly because preclinical animal studies are often limited in their physiological relevance to the human lung. We are developing an immune responsive 3D human lung model that will allow high throughput drug assessment and minimise animal testing leading to more effective preclinical studies. The anatomical and functional properties of the human lung are simulated in our model using a multi cell approach in which human derived cells cultured on relevant polymeric scaffolds. The scaffolds allow cell migration and interaction throughout the interstices of the 3D structure; such interactions are thought to enhance the recovery of the epithelial barrier following wound infliction or allergen challenge. The model is positioned within a Quasi Vivo bioreactor which can enhance 3D culture-growth and better mimic in vivo conditions and may further enhance epithelial barrier recovery following damage.

57.P15
Tissue-engineered psoriatic skin model: differences between involved and uninvolved cells

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Psoriasis can be recognized by the presence of red plaques (involved psoriatic skin) localized close to apparently normal skin (uninvolved psoriatic skin). Previous research had highlighted some characteristics of each skin type. However, the discovery of the triggering factor for psoriatic plaques formation still remains elusive. The aim of this study was to develop pathological skin substitutes produced with involved or uninvolved psoriatic cells, in order to better characterize psoriatic skins. Normal, involved and uninvolved psoriatic skin substitutes were produced according to the self-assembly approach procedure. Results showed that involved substitutes had a thicker epidermis, higher cell proliferation, abnormal cell differentiation and disorganized stratum corneum compared with normal substitutes. When uninvolved cells were used, various results were observed; it appeared that some of the substitutes mimicked those which were produced with normal cells (profile 1), while others shared the same characteristics with involved cells (profile 2). Briefly, uninvolved substitutes of profile 1 had a thin, well-organized epidermis with normal cell proliferation and differentiation such as observed with normal substitutes, while uninvolved substitutes of profile 2 showed an inverse trend such as obtained with involved substitutes. The results suggest that uninvolved substitutes could demonstrate characteristics associated with both normal or involved psoriatic skins.

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57.P16
Tissue-engineered skin substitute as a powerful tool for functional studies of proteins involved in keratinocyte differentiation: the case of the dual leucine zipper-bearing kinase

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A better knowledge of keratinocyte differentiation is necessary to understand skin pathologies showing defects in this process such as psoriasis. The Dual Leucine zipper-bearing Kinase (DLK) is a protein expressed in the stratum granulosum of normal human skin epidermis and in Tissue-Engineered Skin Substitutes (TESS). When overexpressed in cultured keratinocytes using adenoviral vectors, DLK induces keratinocyte terminal differentiation by causing growth arrest, up-regulation of a late differentiation marker (filaggrin) and activation of transglutaminase 1, an enzyme involved in the cornified envelope assembly. To confirm the role of DLK in stratum corneum formation, a TESS model underexpressing DLK was developed. The TESS were transduced at day 0 of culture at the air-liquid interface using lentiviral vectors containing a short hairpin RNA sequence directed against DLK. Biopsies were harvested 14 days later to examine the consequences of DLK depletion. As expected, immunoperoxydase staining showed a reduction of DLK expression in TESS. Consistent with our previous study, immunufluorescence stainings revealed reduced levels of filaggrin and transglutaminase 1. The decrease of DLK was associated with cell detachments and a reduction of desmocollin expression, suggesting defects in desmosome structure. Those results show that DLK play a role in cornified envelope and desmosome assembly which are crucial to the function of the outermost layer of the skin: the stratum corneum.

57.P17
Development of an in vitro wound healing model for fibrosis treatment

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Background: Fibrosis, a threat to implants and wound repair, warrants a therapeutic solution. To study antifibrotic agents we employ an in vitro system in the presence of TGFβ1, a factor which converts fibroblasts to fibrogenic myofibroblasts. However, current protocols do not reflect the pulsatile cytokine exposure prevailing in vivo. Hence, we evaluated the efficacy of short pulses in the creation and maintenance of the myofibroblast phenotype.
Methods: Fibroblasts were pulsed for 0.5, 4 h, 48 h or 2 × 4 h (consecutive days) and maintained in the absence of TGFβ1 for 2 weeks. Periodically, 24 h collagen secretion rate, α-SMA presence and expression of fibrogenic genes were quantified, and their methylation patterns analysed.

Results: A 0.5 h, 4 h, 48 h pulse was sufficient to elevate collagen I secretion rate and α-SMA expression for 7 days, repeat pulses extended this period to 14 days. In all treatment types, fibrogenic genes were up-regulated 24 h post pulse, but no methylation changes were evident.
Conclusion: Customary models of in vitro fibrogenesis use 3–5 days continuous exposure of cells with TGFβ1, a condition far removed from physiology. We show here, that already a single 0.5 h pulse, is sufficient to effect long term changes towards the myofibroblast phenotype, and is potentiated by a second pulse a day later. We believe that our current model is a more realistic reflection of in vitro wound healing model for future fibrosis treatment testing.

57.P18
Optimising glial cell alignment in 3D culture to facilitate the development of neural tissue models for CNS research

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Aim: The overall aim is to develop robust models for neuroscience research, with neural cells arranged in a hydrogel matrix to resemble living nervous system tissue. We create engineered neural tissue by a process of initial glial cell self-alignment within tethered 3D collagen gels and the aligned glia then support and direct neuronal growth to recreate the anisotropy of an organised CNS tract.
Method: A two-stage approach was developed to determine the glial cell seeding density to achieve consistent, predictable alignment regardless of the cell source. (1) Contraction profiles were established using C6 glioma cells at densities from 0.1 to 6 million cells/ml in free-floating round collagen gels, in simple 24-well and 96-well plate assays. (2) Chosen seeding densities were then used to assess the degree of cellular alignment using tethered rectangular collagen gels.
Results: By combining data from the contraction profiles and alignment assays, the relationship between the % contraction of the free-floating round gels and the extent of cellular alignment in the tethered rectangular gels was established.
Conclusion: We have shown that contraction profiles in simple multi-well plate assays, using a small number of cells, can efficiently assess glial cells from different sources and determine the optimal seeding density for generating robust anisotropic engineered neural tissue.

57.P19
Development of a 3D neuronal glial co-culture model for peripheral nerve studies and repair strategies

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3D peripheral nerve models have potential value for studying neuronal-glial development and the design of scaffolds for nerve repair. The aim of this study was to develop an aligned scaffold for this purpose. Electrospinning parameters were carefully devised for producing aligned PCL fibres with diameters of 1, 5, and 8 μm. Neuronal and primary Schwann cell growth was investigated in vitro. Neuronal / Schwann cell co-culture and dorsal root ganglia (DRG) explants were conducted on the fibres in vitro. Neurite extension, Schwann cell responses and a neuronal/Schwann cell co-culture model were examined using 3D confocal microscopy. Neurite guidance was evident on all fibres diameters and the longest neurite growth was detected on 8μm fibres (142.36 ± 9.68 μm). Cell length was measured to assess Schwann cell elongation on the fibres and the length was the longest on 1 μm (116.53 ± 7.85 μm). DRG explants on the fibres showed neurite guidance and co-localization of neurite and Schwann cells. The longest neurite was measured on 1 μm fibres (2.5 mm) and the furthest Schwann cell migration was observed on 1 μm fibres (2.7 mm). We also demonstrated an in vitro co-culture of neuronal and Schwann cells in 3D on the scaffolds, for which co-localisation of both cell types was observed. Aligned electrospun PCL microfibers scaffolds have great potential as a substrate for constructing an in vitro 3D peripheral nerve model. Fibre size influences neurite outgrowth and Schwann cell behaviours.

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57.P20
Astrocyte and fibroblast cross-talk - mimicking in vitro the astrogliosis in a multiple sclerosis lesion

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The inhibition of oligodendrocyte progenitor cell (OPC) differentiation by microenvironmental factors is thought to be one of the main causes of remyelination failure in chronic Multiple Sclerosis (MS) lesions. We aim at developing a microengineered hydrogel-based array to mimic an MS astrogliosis microenvironment to be used for the identification and validation in-vitro of candidate molecules that promote OPC differentiation, towards the development of new therapeutic strategies for the treatment of demyelinating diseases. Firstly, the cross-talk between astrocytes and meningeal fibroblasts (MF), two of the major cellular components of an MS scar, was investigated in vitro. The culture of astrocytes or MF with conditioned medium (CM) from the other cell type resulted in the upregulation of GFAP and Vimentin in astrocytes and of Collagen IV in MF. To investigate the MF-astrocyte cross-talk in a 3D system, alginate (Alg) disks of 0.5, 1 and 2% (w/v) with 4 × 106 astrocyte cells/ml were prepared as a binary composition of high and low molecular weight Alg. Astrocytes remained viable in all Alg formulations tested, with cells being able to spread in 0.5 and 1% (w/v) Alg disks. Furthermore, a significant GFAP up-regulation was detected in relation to the control when astrocytes where cultured in the presence of fibroblast CM. This suggests that astrocyte activation, as in an astrogliosis phenotype, can be achieved by the use of meningeal fibroblast CM in vitro.

57.P21
Study of axonal migration of human induced pluripotent stem cells-derived sensory neurons in a tissue-engineered model

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The developments of tissue-engineered (TE) models recapitulating peripheral nerves migration in vitro are promising tools for studying numerous neuropathies and neuroinflammatory diseases. The primary source of peripheral neurons has historically been the dorsal root ganglion neurons of rodents. Since pluripotent stem cells and neuronal differentiation protocols are now well defined and readily available, these TE models will gain much accuracy to recapitulate neurodegenerative diseases using iPS cells derived from patients. This study is a proof of principle showing that human iPS cells can be successfully differentiated into sensory neurons used to promote axonal migration in a tridimensional TE model. iPS cells were generated and differentiated into peripheral neurons. The phenotype of the differentiated neurons was characterized by staining of TrK receptors, peripherin and TRPV1. The presence of nociceptive peptidegic neurons was quantified by ELISA. The neurons were cultured for 21 days on a TE connective tissue made of a collagen sponge. Axonal migration was shown in the whole thickness of the tissue. Using iPS cells obtained from patients with neuroinflammatory diseases or peripheral neuropathies could be a powerful strategy to recapitulate these disease phenotypes in vitro and better understand the cause and the evolving process of the pathologies. In addition, such models could be very useful to screen new drugs and develop new strategies to treat these diseases.

57.P22
Development of a small calibre artificial blood vessel model

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The treatment of cardiovascular disease often implies bypass grafting. Due to the limited availability of healthy autologous vessels, alternatives are urgently needed. Synthetic large calibre prostheses are well established. However, vascular grafts with diameters smaller than 6 mm are highly susceptible to thrombosis. Therefore, we aim to develop small calibre grafts with characteristics close to in vivo blood vessels to reduce the risk of graft occlusion. Small calibre ePFTE grafts were coated with fibronectin or fibrinogen-thrombin (FT) to facilitate the adherence of human placental endothelial cells (EC) and mesenchymal cells (MSC) on the inner and outer surface of the graft, respectively. The prostheses were exposed to pulsatile flow in a bioreactor for 1 week. Cell identity was evaluated by immunocytochemistry; morphology and efficiency of cell attachment was analysed after H&E staining and by scanning electron microscopy. Fibronectin coated the graft surface homogeneously, while FT showed an irregular covering. EC and MSC adhered to the graft under both coating conditions and showed EC and MSC typical antigen expression. Ultrastructural analysis revealed the existence of pores within the ePFTE tubes, allowing direct interactions of EC with MSC. The combination of fibronectin coating with the co-culture of MSC and EC enables an artificial blood vessel model close to in vivo characteristics. Funded by Styrian Government, Austria

57.P23
Engineering vascularized cardiac tissue in vitro

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Engineering thick tissues can give advance a new myocardial therapy and a new in vitro model for pharmaceutical research. However, the in vitro scaling up of thick tissues is limited due to the lack of vessels supplying oxygen and nutrition, removing waste molecules. We report a new strategy for preserving vital thick tissues using a perfusion bioreactor having collagen-based microchannels. When a multi-layered cardiac cell sheet is incubated within this bioreactor, endothelial cells in the cell sheet migrate and spontaneously vascularise the collagen gel. Fresh culture medium readily flows into the cell sheet through the collagen-base with microchannels, and the cell sheet remains in good condition. Additionally, to successfully exceed the tissue thickness transport limitations, triple-layered cell sheet was repeatedly layered over the original viable cell sheet three different times over 5-day intervals. All layered cell sheets spontaneously integrate completely, and the entire resulting tissue construct exhibits stabilization without necrosis for 20 days of bioreactor culture. Multi-step procedure provides vascularized cardiac tissue with thicknesses of approximately 110 μm. These results confirmed a route to fabricate in vitro engineered thick tissues, vital 3-D cell sheets. Restoring damaged cardiac tissue by implantation and successful production of accurate cardiac tissue models for investigating the effects of pharmaceuticals are enabled.

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57.P24
Fabrication of three-dimensional tubular cardiac tissue using neonatal mouse cardiomyocytes

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The aim of this study was to fabricate pulsatile myocardial tubes using cell sheet based tissue engineering. We have examined the optimization of culture conditions for neonatal mouse cardiomyocyte sheets using temperature-responsive culture dishes, and established a method for tubular structure formation by wrapping cell sheets. When purified mouse cardiomyocytes were cultured on a temperature-responsive dish, it was difficult to make contiguous cell sheet due to weak cell-to-cell junction. Therefore, we cultured cardiomyocytes with mouse neonatal cardiac fibroblasts for enhancing cell-to-cell junctions. The homogenous and intact cell sheets were successfully fabricated at the co-culture ratio of 4:1. Then, the square co-culture cell sheet (20×10 mm) was wrapped around a silicon tube. After this tube was removed, the wrapped cell sheet was connected to a novel perfusion bioreactor. After 4 days perfusion culture, the tubular tissue was remove from the perfusion system, fixed, and stained with HE. The histological analysis showed the multilayered structure of the engineered tissue, which was similar to that of native myocardial tissue. The co-culture of cardiomyocytes and cardiac fibroblasts was thought to have the enough amount of attached cell-to-cell connection compared to mono-cultured cardiomyocytes, resulting in the successful fabrication of tubular tissue. Three-dimensional tubular cardiac tissue may contribute to cardiovascular research as a new model of heart tissue.

57.P25
Studying the drug-responsiveness of breast cancer cells cultured within human osteoblast-derived matrices

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Bone metastases frequently occur in advanced breast cancer (BC) and at that stage the disease is incurable with current treatment options. To date, cancer research strategies still rely on 2D cultures despite the increasing awareness of the role that the extracellular matrix (ECM) plays in regulating cancer cell behaviour, including proliferation, invasiveness and resistance to drug-induced apoptosis. To mimic stromal ECM, cell-secreted matrices are promising tools for cancer research and have been shown to influence cancer cell response to cytotoxic agents. In our previous work we have established human primary osteoblast-derived matrices (OBM) as a model system to study interactions occurring between bone metastatic cancer cells and the bone matrix. OBM is highly mineralised, composed of fibrillar structures, and contains proteins and growth factors characteristic of bone ECM. In the presented project we set out to investigate the effects of the bone matrix-mimicking OBM on the drug-responsiveness of four BC cell lines to Paclitaxel in comparison to tissue culture plastic. Preliminary results indicate that culture on OBM does not significantly modulate the cell's drug resistance when compared to tissue culture plastic. In our future work we plan to compare the drug-responsiveness of BC cells grown on osteoblast- versus fibroblast-derived matrices to gain further insights into ECM components that can mediate drug resistance.

57.P26
Hormone-responsive 3D multicellular culture model of human breast tissue

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A hormone-responsive 3D human tissue-like culture system was developed in which human primary mammary epithelial cells (MECs) were co-cultured with two types of predominant mammary stromal cells on silk scaffolds. Silk porous scaffolds incorporated ECM provided a compatible environment for epithelial structure morphogenesis and differentiation. The presence of stromal cells promoted MEC proliferation, induced both alveolar and ductal morphogenesis and enhanced casein expression. In contrast, only alveolar structures were observed in the monocultures. The alveolar structures generated from the heterotypic cultures in vitro exhibited proper polarity similar to human breast tissue in vivo. Consistent with their phenotypic appearance, more functional differentiation of epithelial cells was also observed in the heterotypic cultures, where casein- and mRNA expression was increased significantly. Additionally, this 3D multicellular culture model displayed an estrogen-responsive capability in a physiologically relevant manner, evidenced by the enhanced cell proliferation, aberrant morphology, changes in gene expression profile and few polarized lumen structures after estrogen treatment. This culture system offers an excellent opportunity to explore the role of cell–cell and cell–substrate interactions during mammary gland development and their alteration during neoplastic transformation in human breast tissue.

57.P27
3D in vitro tumor test systems of nerve sheath tumors

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Neurofibromatosis type 1 (NF1) is a hereditary disease associated with neurofibromas, which can transform to highly aggressive and life-threatening malignant peripheral nerve sheath tumors (MPNST). Therapy options for those patients are very limited, still there are promising drug therapies under study. The drug testing, however is predominantly done in 2D culture systems which do not mirror the complexity of such tumors. Therefore, a 3D in vitro tumor test system is being developed in this study. For establishing a 3D test system, two MPNST cell lines (NSF-1, S462) were cultured on an acellularized biological scaffold from porcine gut. Additionally, we introduced primary fibroblast cells. The growth pattern and gene expression of selected markers were analyzed in 2D and 3D culture systems. Both MPNST cell lines showed a distinct growth in 2D as well as on the 3D scaffold. After 14 days in culture cells grew homogenously on the 3D scaffold surface. Compared to the NSF-1 cell line, S462 cells invaded into deeper regions of the matrix with a higher rate of proliferation which suggests a more malignant phenotype of these cells in the 3D system. Fibroblasts altered the cell growth and migration potential of tumor cells indicating a strong modulatory role of tumor-associated cells. In conclusion, we were able to show that our model reflects a much more realistic in vivo-like environment and therefore might be applicable in basic research or future drug screening studies.

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57.P28
A model system for screening cancer drugs and evaluating chemosensitivity

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A major challenge facing the field of in vitro drug screening is the lack of reliable tumor model systems for evaluation of drug potency and patient-specific chemosensitivity. In this study, we developed an in vitro tumor growth system that uses a simple approach to quantify the chemosensativity and the optimal drug dosing for malignant tumors. The system works by cultivating a tumor biopsy in a PEG-fibrinogen (PF) hydrogel matrix for up to 6 days and documenting tumor cell migration. The PF hydrogel was capable of supporting the progressive expansion of the tumor biopsy in vitro in a 3D milieu that is specifically designed to selectively enhance malignant cell migration from the tumor biopsy, while limiting mesenchymal cell outgrowth. In addition, the matrix is transparent, enabling straightforward monitoring of in vitro malignant cell progression using simple, non-invasive optical techniques. Quantitative cell outgrowth data showed a linear relationship between tumor cell outgrowth and culture time, with significantly different outgrowth kinetics for tumor cells compared to mesenchymal cells. The presence of anticancer drugs in the culture medium, including doxorubicin and cisplatin, inhibited the tumor cell outgrowth kinetics in a dose dependent manner, thus enabling straightforward verification of chemosensitivity. Taken together, these results confirmed that our tumor culture system enables the effective chemosensitivity assaying of tumor biopsies.

57.P29
Establishment of an in vitro assay for high-throughput screening of pro-adipogenic agents

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Introduction: The identification of adipogenic agents that can be used effectively in vivo to overcome fat graft resorption is an unmet need for enhancing reconstructive therapies. A highly reproducible assay is needed to both effectively screen agents and approximate normal human physiological conditions in an in vitro setting.
Material/Methods: Adipogenic stimulation of murine 3T3-L1 cells was assessed over 14 days with 'in-house' differentiation media and quantified by intracellular lipid droplet accumulation using both AdipoRed and Lipidtox reagents. Bright field and fluorescent images were recorded for confirmation of fluorescence data.
Results: Differentiation media percentages of 1% yielded adipogenic responses approximately 10–30% above baseline (i.e. absence of differentiation media) and was consistent with adipogenesis using a manufactured differentiation media (ZenBio). Nuclear area decreased linearly in a dose-dependent manner above a cut-off value of 10% differentiation media. With 100% differentiation media treatment, 70% of 3T3-L1 cells exhibited 'strong' differentiation based on an algorithm we developed.
Conclusion: We are establishing a high-throughput assay that can screen for adipogenic agents based on varying thresholds of differentiation (weak, moderate, strong) and can confirm fluorescence based data. Importantly, conditions for this assay control for physiological concentrations of key adipogenic factors such as insulin, IBMX and PPAR-gamma.

57.P30
Collagen-based scaffolds as models for the study of human breast cancer tumours

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Collagen-based scaffolds exhibit excellent biocompatibility and a 3D structure making them favourable for mimicking the microenvironment of breast cancer tumours. We hypothesise that 1) collagen scaffolds may be used as 3D cell culture in vitro 'tumours' that mimic characteristics of in vivo primary tumour progression while 2) collagen-nanohydroxyapatite scaffolds may serve as models for the study of breast cancer bone metastasis that often results in secondary tumour growth. Hs578Ts(i)8 human breast cancer cells were seeded on collagen-based scaffolds. Cell-seeded scaffolds were assessed for attachment, proliferation and mineralisation potential using DNA assays, calcium quantification assays and histology. Collagen-based cell-seeded scaffolds demonstrated similar cell attachment and proliferation profiles. Live/dead staining confirmed these results showing primarily live cells attached to scaffold surfaces. Cells cultured on the collagen-based scaffolds under osteogenic conditions demonstrated enhanced calcium deposition in a time-dependent manner compared to growth media. Collagen-based scaffolds demonstrated the ability to support the attachment, proliferation and mineralisation of breast cancer cells. Enhanced mineralisation was observed with osteogenic media treatment demonstrating the response of the breast cancer cells to their microenvironment. These results demonstrate the utility of these scaffolds as platforms that permit the study of cancer biology.

57.P31
Three-dimensional co-culture cancer model with vascular endothelial cell for anti-cancer drug testing

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Introduction: Angiogenesis is essential in cancer microenvironment. To mimic interaction between vasculature system and cancer cells in vivo, a 3D co-culture model of human endothelial cells and cancer cells was developed.
Methods: Human umbilical vein endothelial cells were cultured on the Matrigel, a basement membrane preparation, then co-culture was created by adding another layer of Matrigel containing human cancer cell line Adr/NCI. For anti-cancer drug testing, Paclitaxel and Cisplatin, two widely accepted anti-tumour drugs were used. Two-dimensional (2D) culture and three-dimensional (3D) cultures were compared by total cell viability, colony size was used to compare mono-culture and co-culture in 3D, and different effects of two drugs were evaluated by angiogenesis parameters like tubule length distribution and branch point number only in 3D co-culture.
Results: The optimum ratio of HUVECs to Adr/NCI cells in the co-culture system was found to be 3:1. The co-culture system can be maintained for 10 days and was observed to feature vascular network and tumour-like structures. Compared with 3D mono-culture of cancer cells, the co-culture model was shown to have larger tumour-like colony structures and higher drug resistance to Paclitaxel and Cisplatin.
Conclusions: In summary, this model has shown that cancer cells may be affected by co-culturing with endothelial cells in 3D. The results can potentially be useful for pre-clinical drug testing to screen drug candidates.

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57.P32
Evaluation & validation of an in vitro system for predicting the acute inflammatory reaction elicited by biomaterials

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Leukocytes are a fulcrum in inflammation, possessing an armament of antimicrobial & proteolytic molecules. Therefore unfavourable interactions between these cells & biomaterials significantly reduce longevity & efficacy. In this study we evaluated a chemiluminescent screen for quantifying biomaterial stimulated leukocyte reactive oxygen species (ROS), & validated this in a rat model. Materials; Synthetics: polypropylene (PP), polyester terephthalate (PET) & polyglycolic acid (PGA) varied in polymer composition & fibre conformation. Biologics: human & porcine dermis & small intestinal submucosa (SIS) varying in decellularisation & cross linking. In vitro: Materials were incubated under continuous luminsecent recording with human blood from healthy donors ($n = 5$) & pholasin. In vivo: implants delivered SC into 6 week old, male wistar rats adjacent to dorso-lumbar musculature. Synthetics demonstrated PGA was the most ROS stimulating polymer & material complexity & surface area played an over-riding role in leukocyte activation vs. polymer chemistry. Biologics showed SIS was significantly more activating than dermis and that SDS was a particularly pro-inflammatory decellularisation reagent. In vivo observations supported these findings showing aggressive & prolonged neutrophil response toward the SIS materials. The data also concluded inter-patient variation in ROS response to specific materials suggesting this technique as a screen to maximise patient/material compliance.

57.P33
Establishment of standard test method for cell migration ability for bioactive ceramics

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Porous bioactive ceramics are widely used as bone fillers and scaffolds. However, no international standards for cell migration ability are proposed. In the present study, cell migration test method for international standardization was confirmed and proposed. Five types of porous bioactive ceramics and a porous composite were used as test materials. Prior to cell culture, specimens were soaked in the MEM and applied vacuum to let medium infiltration into their pores. The specimen was placed on the confluent MG-63 or MC3T3-E1 layer on a 6-well plate. To inhibit shifting of the specimen, SUS316 ring weight was placed on the specimen and/or collagen gel was coated on the well before cell culture. After 3 days, the specimen was harvested, fixed, cut into half, stained with Giemsa and observed with stereomicroscope. The same specimen was implanted into cranial bone hole and observed with μ-CT. The cells went up to pores in all specimens. In the case of MG63, the migration distances were; A: 430, B: 275, C: 336, E: 462, G: 486 and H: 706 μm, and this trend was the same as in the case of MC3T3-E1. Weight and/or coating allowed even cell migration; however, the trend was mostly the same. The in vitro cell migration and in vivo bone formation showed the same trend except for H due to its anisotropic porous structure. This test method could be a good for testing cell migration ability in porous bioactive ceramics.

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57.P34
Gelatin-chondroitin-hyaluronan tri-copolymer scaffold improves cytochrome P450 induction by delivery transcriptional regulators in human dermal fibroblasts

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Reliable and convenient in vitro systems for determining drug metabolism and predicting risk of drug-induced hepatotoxicity are of tremendous value but are still lacking. In this study, we selected 10 hepatic transcription factors and nuclear receptors to deliver into human dermal fibroblasts (HDFs) to induce their expression and activities of cytochrome P450 (CYP) enzymes 3A4, 1B1 and 2C9 to the levels much higher than those in 3 hepatoma cell lines. These induced HDFs were further cultured into spheroids and seeded into tri-copolymer scaffolds. Scanning electron microscopy and confocal microscopy disclosed well accommodation of these spheroids inside the scaffolds. The induced CYP activities in these tissue constructs were increased further. CYP3A4 activities in these constructs persisted for as long as 2 weeks. This present study demonstrated a novel strategy to induce high expression and activities of important CYPs in HDFs to a long-enough period of time, and this approach has potential to establish convenient in vitro assay systems based on easily obtainable HDFs with various genetic backgrounds to study drug metabolism and to predict human risks of drug toxicities.

57.P35
Endotoxins – the real reason for the bioactivity in your in vitro cell assays?

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In order to provide innovative biomaterials for the treatment of orthopedic tissue injuries, an increasing number of non-chemists and non-biologists have entered the field of biomaterials research. This results in the lack of profound knowledge to understand the complex interaction mechanisms of natural substances and to assess the risk for contaminating biota. It is this deficit in knowledge combined with sensitive cell-based assays that can lead to inaccurate evaluation of biomaterials. Hence, there should be both an active effort to assemble multi-disciplinary teams and a genuine concern for the effect of contamination on in vitro assays. Here we show that the presence of low levels of endotoxins in chitosan derivatives can significantly improve osteogenic differentiation and deposition of calcium hydroxyapatite crystals without affecting cell viability. However, after appropriate endotoxin removal, chitosan derivatives are essentially rendered ineffective and thus less appropriate for the use as biomaterial. We conclude that the presence of endotoxins in a natural substance used in biomaterials research may result in false positive results, profoundly altering product performance in vitro. False positive results through uncritical use of natural substances in vitro can be avoided by proper endotoxin testing and careful evaluation of cytokine secretion patterns.

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57.P36
Design of an ex-vivo culture system for studying early arterialization phenomena occurring in saphenous vein graft disease

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Introduction: Saphenous vein (SV) graft disease represents an unsolved problem in coronary artery bypass graft (CABG). After CABG, progressive structural modifications of the SV wall, due to intima hyperplasia, lead to the occlusion of the graft lumen. In this scenario, the design of an ex vivo vein culture system (EVCS) is presented. The device is aimed at replicating ex-vivo arterialization conditions experienced by SV after CABG for studying the biological mechanisms activated by SV exposure to arterial-like conditions.

Methods: The EVCS chamber consists of the SV housing hosted in a falcon tube acting as reservoir, and an external stimulation system connected to the chamber and dimensioned according to preliminary compliance measurement of SVs. The stimulation phase consists of a loading step (0÷80 mmHg), followed by the application of a cyclic CABG-like pressure wave (80÷120 mmHg), and a final unloading phase (80÷0 mmHg). Stimulation phase is cyclically alternated with a recirculation phase via I/O hardware and dedicated user software interface. Preliminary experiments (sterility maintenance and SVs survival over time) were performed on human SVs using the EVCS.
Results & Conclusions: The EVCS provided a sterile environment suitable for stimulation experiments and assured the survival of excised human SVs up to 8 days. Stimulation experiments are ongoing and will permit to study the SV responses to arterial-like conditions in a controlled environment.

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58. Innovative Microscopic Techniques

58.P01
Discriminative performance of biomaterials and regenerative medicine products for soft tissue repair, by quantitative histopathology

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Biomaterials and Regenerative Medicine products (hereinafter referred as implants) for soft tissue repair become even more innovative with increased efficacy potential for the treatment of soft tissue defects. Their in vivo performance is generally analysed by histology, but showing often limitations in discriminating their performance. It may be completed by series of (i) immunohistochemistries & (ii) gene expression analyses. There, we developed an alternative approach for an improved histology analysis, based on two specific stainings for the quantification of collagen formation and cell. Total collagen content, Type I and III collagens and cells were quantified by image analysis. Digital cell grouping were further achieved, based on image treatment of several parameters: optical density, size, nucleus circularity. This allowed cell separation & quantification into several types, involved in the wound healing process. This methodology was first validated by comparing manual vs. digital cell groupings and has been used for implant evaluations, in a hernia animal model. It showed its value (i) in performing multiple analyses on the same tissue sections & (ii) in better discriminating the performance of implants versus standard histology. This quantitative histology approaches permits simple, more objective & concise evaluations of the performance of new implants, by enabling concurrent multiple analyses of inflammation and wound healing markers.

58.P02
Noninvasive discrimination of undifferentiated iPS cells and malignant tumor cells among cultivated cells for transplantation by the measurement of laser phase shift

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We previously reported that the laser phase shift of adherent animal cells, which is a product of cell height and cell refractive index, could be noninvasively determined by phase-shifting laser microscopy (PLM) in a short time. Aiming the discrimination of remaining undifferentiated iPS cells and detection of generated malignant tumor cells from the view point of quality control of regenerative medicine, the noninvasive discrimination of these cells among cultivated cells for transplantation was investigated using the measured value of phase shift of cell transmitted laser, respectively. A human prostatic carcinoma epithelial cell line (PC-3) showed markedly lower phase shift measured by phase-shifting laser microscopy (PLM, FK Optical Institute, Japan) than that of human normal prostate epithelial cells (PREC), although there was no apparent difference in cell morphology between them. The difference in the phase shift was due to lower actin density and lower cell height of malignant tumor cells. Human iPS cells (253G1) were respectively cultivated in single cells on the dish coated with Matrigel and in colonies with feeder layer of MEF cells, stained with ALP, and observed under PLM to measure the phase shift. The phase shift of ALP positive cells was markedly higher than that of ALP negative cells in both culture types. The higher phase shift of ALP positive cells was due to higher refractive index.

58.P03
Imaging and evaluating the degradation kinetics of resorbable bone substitute scaffolds using two-photon laser scanning microscopy

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Large bone defects need to be filled with a grafting material acting as an osteoconductive scaffold for new formation of bone. β-tricalcium phosphate (β-TCP) is a widely used substitute in bone tissue engineering that is degradable over an appropriate period, and consequentially replaced by newly formed tissue. In theory osteoclasts are able to resorb bone by changing the environmental pH below 7, increasing locally the solubility of the biomaterial that is consequentially dissolved. We hypothesize that macrophages and/or activated macrophages are able to phagocytize calcium phosphate particles, and moreover they are able to resorb calcium phosphate based scaffolds. Using an innovative imaging technology, we compared different cell-ceramic scaffold combinations behavior, imaging and evaluating the amounts of resorbed material for every study sample. Scaffolds of β-TCP with 10 mm diameter, 1 mm height, and a porosity of 30% were incubated with (1) RAW macrophages, (2) interleukin-4 activated macrophages, and (3) osteoclasts for up to 21 days. Interestingly, β-TCP degradation kinetics was not statistically different after the incubating period for macrophages and osteoclasts, therefore corroborating our hypothesis. Here, we show for the first time the possibility of ceramic particles to be phagocytized by macrophages or activated macrophages, by imaging with a two-photon laser scanning microscope.

58.P04
Noninvasive monitoring methodology for maturation process of tissue-engineered cartilage

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Chemical and histological examinations applied to quality evaluation of chondrocytes cultured in collagen gel are quite invasive, and cannot be performed repeatedly during culture period. In contrast, second harmonic generation (SHG) microscopy visualizes collagen without any preparation because SHG light is selectively emitted from collagen fiber. Hence, SHG microscopy has a potential in estimating the quality of tissue-engineered cartilage noninvasively. In this study, chondrocytes isolated from rabbit articular cartilage were cultured in atelocollagen gel. Cell seeding densities were normal and lower condition that causes cell dedifferentiation. We observed structural changes of the gel by SHG microscopy, and evaluated the cells by immunostaining of type II collagen. Our results showed that aggregations of round-shaped cells were

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detected as clear holes of SHG image only in the gel with normal cell density, and these cells produced type II collagen, which is essential protein in cartilage. Moreover, the size of the holes associated with the number of cells in the aggregations. These results indicate that we can infer the distribution and the number of cells producing type II collagen. In culture process, it is valuable to monitor the number and the distribution of cells producing matrix since it is the most important index of the maturation of tissues. Thus, SHG microscopy can be a helpful tool for management of process quality in tissue-engineered product.

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59. Nanotools

59.P01
Application of the nanoprobes derived from carbazole diiodide for detecting cell transformation

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The plastic nature renders cells to be changed by external stimuli. Multistep and multifocal processes are required for cell transformation. To establish a method for detecting cell transformation significantly benefits cell lineage tracing, cell identification, and cancer therapeutics. We developed a novel fluorescent nanoprobe, 3,6-bis(1-methyl-4-vinylpyridinium) carbazole diiodide (BMVC) to detect cell features of transformation and differentiation. BMVC probes can bind to quadruplex DNA structure and have been successfully used for differentiating cancer from normal cells. It can also distinguish stem cells from differentiated cells in various cellular types. It can be applied to living cells, and its signals are easily identified by conventional fluorescent microscopy. In this study, several cell transforming models were used to evaluate the utility of BMVC nanoprobes. These transformed cells demonstrated various alterations both in morphological phenotypes and subcellular organization. When BMVC probes were applied, the number of BMVC-positive cells increased in accordance with the changes of the transformed cells. The ratio of BMVC expression was correlated with the foci formation, increased cell motility, cell proliferation and apoptosis, anchorage-independent growth, and increased invasiveness. These results provide evidence of the potential of BMVC nanoprobes, and show the possibility of BMVC probes to be applied to monitor cell transformation.

59.P02
Self-assembled microcapsules with tunable and sustained release of macromolecules for tissue engineering

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Layer-by-layer (LbL) has been presented as a tool capable of constructing tunable and sustained release reservoirs for therapeutic and proliferation/differentiation agents. We report the conception of biocompatible stimuli-responsive microcapsules fabricated using LbL containing BSA as model protein. The capsules were constructed by the sequential adsorption of chitosan and a temperature-responsive elastin-like recombinamer (ELR) containing RGD into self-assembled layers and onto inorganic CaCO3 microparticle templates. By increasing the temperature (25–37°C), a considerable shrinking was observed (5.5–3.2 μm) as well as higher retention of the encapsulated BSA. Such variations were observed for the first time at a relevant physiological temperature, contrary to similar LbL systems requiring temperatures ranging 50–70°C. Different number of bilayers allowed tuning further the permeability: more layers resulted in a more effective barrier to the protein diffusion. Cell viability tests using L929 cells also demonstrated the non-cytotoxicity of these structures. The developed nanostructured reservoirs exhibited tunable and sustained permeability by simply varying the temperature and the number of layers. Exploiting both permeability mechanisms, it would be possible to control the release not only of a pharmaceutical in traditional drug delivery applications but also of agents in tissue engineering that influence the proliferation and differentiation of cells.

59.P03
Enhanced mesenchymal stem cell chondrogenesis using controlled nano-topography patterned on polycaprolactone scaffolds

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While previous reports have shown the importance of matrix materials and soluble factors on MSC chondrogenesis, the influence of nano-topography environment, which have been known to exert a critical effect on cell proliferation and differentiation in-vivo, have not been investigated in the chondrogenic differentiation of mesenchymal stem cells (MSC). In this study, we carried out a systematic study on the influence of nano-topographic matrix material (chondroitin sulfate) on MSC chondrogenesis. Distinct spatially-controlled nano-topography in the form of nano-pillar, nano-hole and nano-grill structures were directly patterned onto polycaprolactone (PCL) film acting as scaffold, followed by surface coating with chondroitin sulfate (CS). The effect of nano-topography on cell-proliferation, cell morphology, cell elasticity, F-actin distribution and chondrogenesis differentiation were studied and compared against non-patterned PCL film. Our results indicate that nano-topography, especially nano-pillar and nano-hole, exerted a significant enhancement on MSC chondrogenesis which corresponded to specific change in cell morphology and cytoskeleton reorganization and increased cell stiffness at early stage, compared to other topographic surfaces. This study indicates the sensitivity of MSC differentiation and development to surface topography and highlight the importance of incorporating topographical design in scaffolds for cartilage tissue engineering.

59.P04
Hepatocyte behaviors on single-walled carbon nanotubes

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Nanomaterials, such as nanoparticles, nanofibers, and nanotubes, advocated as useful material for cell scaffolding that mimics tissue architecture. Especially, carbon nanotubes (CNTs) have attracted attention as one of the most useful scaffolds for biological and medical applications due to their unique structural, electrical, and mechanical properties. In this study, we focus on the morphological and functional behaviors of

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rat hepatocytes on single-walled CNT-coated surfaces. Although the hydrophobic characteristics of CNT-coated surfaces increased with increasing CNT density, hepatocyte adhesion decreased, indicating that the interaction between hepatocytes and CNTs is weak. The hepatocytes on a CNT-coated surface gradually gathered together and formed spheroids (spherical multi-cellular aggregates). In contrast, collagen treatment in conjunction with the CNT-coated surface improved hepatocyte adhesion, and the cells maintained a monolayer configuration throughout the culture period. The albumin secretion and ammonia removal activities of hepatocyte spheroids were maintained at elevated levels for at least 15 days of culturing as compared with hepatocyte monolayer. Furthermore, connexin-32 expression was higher in the spheroid than in the monolayer, indicating that the spheroids develop cell–cell communication. These results indicate that CNTs can be used for the formation and long-term culture of hepatocyte spheroids.

59.P05
Monitoring microconstructs: cell and nanosensor encapsulation in alginate micro-beads

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Monitoring and controlling the microenvironment of cell cultures is an ongoing challenge for many researchers. Much research has been conducted characterising individual aspects such as 3D architecture, mechanical properties, biochemicals, etc. The biggest deficits in existing models for monitoring analytes within the cellular environment is the lack of appropriate means for non invasive, real-time and integrated monitoring of the cellular responses. Nanosensors can overcome these issues: they are porous polymeric nanoparticles that are sensitive to a range of analytes including pH and O_2. Microfabrication techniques are innovative tools to obtain controlled microstructures with a defined 3D architecture. In this work uniform sized 3D micro-scaled hydrogel constructs of approximately 300–400 μm diameter were fabricated. Through dual incorporation of cells and nanosensors within these constructs we aim to have a real-time, non invasive method to measure microenvironmental pH value and O_2 content. Ratiometric fluorescent output from the microenvironment is used to monitor O_2 and pH during cell culture. Measurements show that 3D micro-scaled constructs are suitable for cell growth and proliferation. Moreover O_2 content and pH within hydrogel cellularised microspheres are shown to have physiological values which enable the maintenance of the hepatic phenotype.

59.P06
Nano-reinforced poly(hydroxyethyl acrylate) hydrogels

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The application of hydrogels as scaffolding material in tissue engineering is sometimes prevented by their excessive compliance and lack of sufficient mechanical resistance. Different hybrid polymer composites containing an inorganic nano-reinforcement have been proposed in the literature to improve mechanical properties of hydrogels. Amazing properties can be obtained by precise organization of the organic and inorganic phases in the composite. The strategy proposed in this work to obtain a double micro and nano-reinforcement of a hydrogel matrix consists in a two-step synthesis being the polymer network formation the first step. Polymeric microstructure drives the absorption of a silica precursor solution and the further distribution of the inorganic phase which is formed 'in situ'. Poly(hydroxyethyl acrylate) micro / nano-composites were obtained with silica content up to 60% by weight. Silica phase is continuous, samples maintain their integrity after eliminating the organic phase by pyrolisis. Water absorbed in the gel is able to crystallize, at least in part, when the silica content is below 30% by weight, interestingly for higher silica content the glass transition of the polymer phase is suppressed as well. Increase of the elastic modulus with silica content is continuous. Improvement of bioactivity of the material in simulated body fluid was also assessed. The synthetic route proposed allows obtaining a family of composite hydrogels with adjustable properties.

59.P07
Hyaluronic acid/poly(amidoamine) dendrimer nanoparticles for central nervous system applications: In vitro and in vivo studies

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Central nervous system (CNS) disorders are among the diseases with less efficiency in treatment. In order to reach its target and exert its effect within the brain parenchyma, drugs must overcome the blood brain barrier (BBB) and the blood-cerebrospinal fluid-brain (BCSFB). The aim of this work was to develop a novel nano-based dendrimer which could serve as a nanocarrier with the ability to cross these barriers. Hyaluronic acid/poly(amidoamine) dendrimer nanoparticles (HA/PAMAM NPs) were synthesized and a detailed physicochemical characterization was performed. Results from DLS analysis showed that these HA/PAMAM NPs possessed a mean diameter of 61.23 nm. The HA/PAMAM NPs were negatively charged and had a low polydispersity factor, which indicates a narrow size distribution. Moreover it was also possible to bind them to fluorochromes, such FITC, in order to trace them in biological environments. In vitro biological assays revealed that HA/PAMAM NPs did not cause any cytotoxic effect on the viability and proliferation of neuronal and glial primary cell cultures. It was also possible to observe that the fluorescent-labeled NPs could be in vitro internalized by glial, neuronal and endothelial cells. Finally, in vivo assays revealed that these nanoparticles could be found in the brain parenchyma upon intrathecal injections. Further studies will focus on testing these systems in relevant models of CNS injury and degeneration.

59.P08
Characterization of polymer surfaces to optimize cell behavior

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Polymeric nano-surfaces can imitate the extracellular matrix, whose topography interacts in vivo with cells and regulates their activity and morphology. The possibility to optimize such tools for tissue engineer-

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ing and regenerative cell cultivation will be highly interesting for both the biomedical and polymer processing research and industry. To gather basic information about polymeric substrates we examined at first selected unstructured polymers with diverse treatments regarding hydrophilicity (surface energy), surface chemistry (XPS) and topography (AFM). The results are presented in this work. It can be said that due to surface treatments surface energy increases, which is equivalent to an increase of hydrophilicity. Hydrophilicity is known to enhance cell adhesion. Furthermore the compared commercial products can be outreached concerning hydrophilicity by choosing appropriate treatment parameters.XPS demonstrated that as result of the oxidation reactions due to surface treatment polar functional groups are implemented in the surface. These groups seem to be important for cell adhesion and furthermore cell viability. AFM investigations showed a notable topography impact (roughening, characteristic formations) because of surface treatment, but there was no topography impact on cell adhesion and viability. In future the outcome of this work will give base knowledge for investigating the influence of nano-structures on cell behavior.

59.P09
Evaluation of novel magnetic Fe-hydroxyapatite nanoparticles for bone applications

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The use of magnetic nanoparticles (MNPs) for biological and medical purposes has been enormously increasing in the last years and recently the interest has focused in their use also in tissue engineering. Here, novel biocompatible and bioresorbable superparamagnetic nanoparticles, which avoid coating and the presence of poorly tolerated magnetic secondary phases, were investigated in vitro and in vivo. Magnetic biomimetic Fe-Hydroxyapatite (Fe-HA) powder and granulate were prepared by doping HA with $Fe2^{+}$ and $Fe3^{+}$ ions. Saos-2 human osteoblast-like cells were tested with different concentrations of Fe-HA MNPs from 200 μg/ml to 2000 μg/ml, with and without the application of a magnetic field. Assays for cell proliferation, cell viability, cell morphology and alkaline phosphatase activity did not show any differences between Fe-HA and HA control groups, indicating good biocompatibility. Fe-HA granulate has been implanted in vivo in a critical bone defect rabbit model and after 12 weeks good biocompatibility and osteointegration has been observed. This study shows that the novel Fe-HA MNPs are biocompatible and comparable to commercially available HA formulations. We anticipate that Fe-HA MNPs could be guided to a desired bone site to increase cell proliferation and enhance bone formation using an external magnetic field, moreover, they could be functionalized with several growth factors and/or drugs to induce tissue regeneration and reduce recovery time.

59.P10
Mechanical characteristics and release properties of magnetic nanoparticle encapsulating biodegradable hydrogels

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Magnetic nanoparticles (MNPs) currently have broad applications in biology and medicine. MNPs have been recently encapsulated within cell encapsulating hydrogels which can be manipulated and assembled in magnetic fields. Here, we studied mechanical and the release properties of MNPs from gelatin methacrylate (GelMA) hydrogels. We performed non-confined compression tests and analyzed the results as Young's modulus, ultimate stress and failure strain. We observed that 1% and 5% MNP encapsulating hydrogels had two failure levels compared to GelMA hydrogels as control. MNP concentrations didn't significantly affect to the mechanical properties of hydrogels. The effect of MNP concentration on porosity ratio and swelling behavior of hydrogels was also evaluated. In swelling behavior of hydrogels, there was a statistically significant decrease in MNP encapsulating hydrogels compared to the control. There was also significant difference observed between the control and 5% MNP encapsulating hydrogels in porosity ratio of hydrogels. We analyzed MNP release from hydrogels by using inductively coupled plasma atomic emission spectroscopy and hydrogel degradation to evaluate the effect of cells and MNPs on hydrogels. Hydrogel degradation increased when high MNP concentrations were used. MNP release also correlated with hydrogel degradation. Results indicated that MNP encapsulating hydrogels can be potentially used in tissue engineering, regenerative medicine and on-demand drug delivery.

59.P11
Electrophoretic characterization of magnetic nanoparticles coated with poly(ethyleneglycol) (PEG) as an antieneoplasic drug carrier

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In recent years therapeutic nanoparticle approaches attracted a great interest because of the new applications in biological systems. In this scenario iron oxide nanoparticles are particularly attractive because of their magnetic properties, which allow them to be used in diagnostic protocols and treatments. Additionally their potential to reduce circulating concentrations of free drug, to promote tumor-specific deposition and to restrict drug distribution to noncancerous tissues make them suitable candidates in drug delivery applications [1,2]. In the present work, we describe how superparamagnetic iron nanoparticles stabilized by an Fe_3O_4 shell can be used as nuclei for the engineering of composite particles with an external poly(ethyleneglycol) (PEG) coating capable of loading doxorubicin. Iron nanoparticles were synthesized according to Sheng Peng's thermal decomposition method [3]. After the iron nanoparticles synthesis, we made the characterization of their size, magnetic properties, surface charge and composition. Finally, the designed nanostructures were used to incorporate an antineoplasic drug, doxorubicin, on the surface of the nanoparticles, after they were coated with PEG (molecular weight 2000). Electrokinetics and absorbance spectra demonstrated that the coating was produced. Absorbance in UV-VIS range was used to assess the drug adsorption on the nanoparticles surfaces. Preliminary in vitro tests show that nanoparticles can be introduced into the cells.

59.P12
Effects of components of the blood on magnetic targeting of stem cells for therapy

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Stem cell therapy may provide solutions for chronic musculoskeletal disease. One challenge in therapy development is localisation to the site of action without acute inflammation. Magnetic trapping may provide a means to deliver stem cells to the site of repair and we have been investigating the use of magnetic nanoparticles for this process. In

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this study we investigate some of the blood parameters which will modulate the movement of cells in circulation and the magnetic field effect. Specifically, the effect of the large circulating protein albumin on stem cell targeting has been determined. Mesenchymal stems cells were cultured with superparamagnetic iron oxide particles and placed in an in vitro circulatory flow model with external magnets, in media and with Bovine Serum Albumin (BSA) added at varying human physiological concentrations. After a period of 24 h the suspension medium and section of tubing overlying the magnet were collected and subjected to analysis of DNA concentration using PicoGreen Assay. The ratio of DNA concentration adherent to the tubing, compared to DNA concentration remaining in the suspension was analysed and a dose response was observed. Our results show that magnetic trapping is affected as a result of BSA added to the media. This technique allows us to assess in vitro the factors which influence magnetic targeting in vivo and will be used to determine effects of circulating blood cells on cell distribution or delivery to the target tissue.

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60. Cryopreservation

60.P01
Cryoprotective cell scaffold for tissue engineering

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Cryopreservation of regenerated tissue is the one of the key techniques to spread of the use of the production of tissue engineered products for clinical application. However, cryopreservation of regenerated tissues including cell sheets and cell constructions is not easy compared to cell suspensions because of the weakness of cell–cell interaction and their inhomogeneous structures. Recently we showed that several kinds of polyampholytes have a cryoprotective effect on cells in solution without any other cryoprotectant. This interesting phenomenon is characteristic of polymers with high electron charge, especially polyampholytes. Cells are killed because of the damage caused by the intracellular crystallization of water during freezing. Therefore, a membrane-permeable chemical such as dimethyl sulfoxide (DMSO) is usually added in order to cryopreserve the cells. However, the cryoprotective effect of polymers such as polyampholytes that do not penetrate the membrane cannot be explained by the same mechanism of DMSO. We revealed that polyampholyte chains trap the sodium ions and restrain their movement at low temperatures. This phenomenon might decrease cell damage caused by an abrupt increase in osmotic pressure during freezing. The result suggests that extra cellular environment might affect the cell viability after cryopreservation. In this study, we attempted to make cell scaffolds using polyampholytes for the development of the novel cryoprotective cell scaffolds.

60.P02
Cryopreservation of a human cornea equivalent: conservation of the barrier function for transcorneal drug adsorption studies

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Currently, cryopreservation is the only method for long-term storage of living cells and tissues. However effective cooling and thawing of native and engineered tissue pose a specific challenge. Aim of this project is to develop an effective transport and storage protocol for human hemi-cornea equivalents (HCC) used as *in vitro* model for transcorneal drug absorption studies. Effective cryopreservation requires optimization of freezing parameters for every cell type. We showed for suspended human corneal epithelial cells (HCE-T cells) that a cooling rate of 0.2 K/min led to the highest survival rates, whereas human keratocytes (HCK-Ca) reached optimal survival with 5–10 K/min. Despite this discrepancy acceptable cell survival could be achieved by adaption the composition and concentrations of cryoprotective agents (CPA). The findings are transferred to the procedure for 3D HCC. With the applied protocol the main barrier characteristics of the *in vitro* model could be maintained. This is evaluated by measuring the transepithelial electrical resistance (TEER) and of Na-fluorescein permeation. The data show promising results to develop an effective cryopreservation strategy for HCC. Furthermore this will offer new options for corneal graft banking in the future.

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60.P03
Cryopreservation of mesenchymal stromal cells in alginate microbeads by conventional freezing and vitrification

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The aim of this study was to investigate the viability, metabolic activity and differentiation properties of human adult bone marrow mesenchymal stromal cells (MSC) after cryopreservation by slow cooling or vitrification within alginate microbeads. MSC were isolated from adult human bone marrow, expanded *in vitro* and then encapsulated into alginate microbeads (AM). Cell seeded AM were cryopreserved by slow (1 °C/min) cooling protocol under protection of 10% Me_2SO and 20% FCS, or by vitrification under protection of 10% Me_2SO, 20% ethylene glycol, 20% propanediol and 0.5 M sucrose. Cells viability was assessed by FDA/EB staining. Metabolic activity – by Alamar blue and MTT assays. nn MSC within AM maintained spherical shape during *in vitro* culture and did not adhere and proliferate. However, MSC were able to differentiate into adipogenic and osteogenic cell lineages in response to specific induction stimuli. The cryopreservation of MSC within AM by slow cooling allowed to preserve cells survival and metabolic activity at 87% ± 2% and 69% ± 4% of control levels, correspondingly. The viability of encapsulated MSC cryopreserved by vitrification varied depending on time of equilibration with vitrification solution. The maximal viability and metabolic activity of vitrified cells comprised 73 ± 6 and 55 ± 5 correspondingly. Encapsulated MSC preserved multilineage differentiation potential after both conventional and vitrification cryopreservation protocols.

60.P04
Cryopreservation of bone marrow-derived stem cells using natural cryoprotectants

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Efficacy of cryopreservation of human bone marrow-derived stem cells (BMSCs) in clinically safe, animal product free medium containing natural cryoprotectants was evaluated. We used disaccharides, antioxidants and caspase inhibitors for cryopreservation of BMSCs in combination with a reduced concentration of dimethylsulfoxide (Me_2SO). The thawed cells were tested for viability with MTT assays and a growth curve was created to measure population doubling time. In addition, we performed flow cytometry analysis for cell surface antigens, and RT-PCR for mRNA expression of stem cell markers. The solutions containing trehalose and catalase with 5% or 2.5% (v/v) Me_2SO produced results similar to those for the control (10% (v/v) Me_2SO and 30% FBS) in terms of cell viability, culture growth, expression of

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cell surface antigens and mRNA expression of stem cell markers in BMSCs cryopreserved for a minimum of 3 weeks. Thus, BMSCs can be cryopreserved with a reduced concentration of Me_2SO with the addition of disaccharides, antioxidants and caspase inhibitors. Our results demonstrate that human bone marrow-derived stem cells could be successfully cryopreserved for banking and clinical applications in clinically safe protective reagents. [Supported by the grants of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091224) and Regenerative Medicine R&D Project from Daegu Metropolitan City.]

60.P05
Development and cryopreservation of bone and cartilage constructs for orthopaedic tissue engineering

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In recent years the development of bone and cartilage tissue engineered constructs have been gaining importance for their use in treating patients suffering from several osteochondral defects caused by tissue degeneration, traumatic injury and orthopaedic surgery. This paper describes the development of nano and micro structured composite scaffolds from a variety of silk and chitosan biopolymers with supramolecular coating and addition of filler materials. The scaffolds were seeded with hMSCs. The resultant cell-scaffold constructs were grown and differentiated to develop bone and cartilage constructs. However, the successful cryopreservation is the key challenge for long term preservation of these constructs for their commercial utilization. For this purpose, the cryopreservation study has been carried out in our laboratory to standardize the preservation protocol. In this study, we investigated several natural osmolytes and selective antioxidants in combination to see their effects on post-thaw cell viability and early apoptosis of the cryopreserved cells and constructs. So far, the study has shown encouraging results towards the maintenance and survival of cells and cell scaffold constructs under extreme stressed conditions during and after freezing by using the combination of natural extracellular CPA, intracellular CPA and antioxidant.

60.P06
Isolation, characterization and preservation of MSCs derived from umbilical cord blood

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Mesenchymal stem cells (MSCs) are considered as a potential source for the treatment of various tissue related diseases such as bone, cartilage, neuron, cardiac and skin. However, the isolation and preservation of MSCs is of paramount importance for their commercial use. In the present work mononuclear cells were isolated from human umbilical cord blood (hUCB) using Ficoll Hypaque density gradient technique and cultured. MSCs were identified and characterized based the expression of anti CD markers (Positive for CD 44, CD 79, CD 90, CD 105, Negative for CD34, CD45 and HLA DR). MSCs of passage 4 were cryopreserved using controlled rate freezing method. The effects of a variety of osmolytes (individually and in combination) with different antioxidants on post thaw viability of cryopreserved MSCs were investigated. The combination of osmolytes and antioxidants showed better cell viability than the use of conventional DMSO. There was no significant change was observed in the G0/G1 cell cycle phase (BrdU assay) when the cryopreserved MSCs were cultured. Furthermore, the ROS activity was significantly reduced due to addition of antioxidants. Thus, it is demonstrated that the combination of osmolytes and antioxidants may be an effective medium for cryopreservation of hUCB derived MSCs.

60.P07
Analysis of human umbilical cord cells for the establishment of a vascular cell bank

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Introduction: Characterization of human umbilical cord artery derived cells (HUCAC) and Wharton's jelly cells (WJC), i.e., their proliferation and differentiation potential, is not only essential for fabrication of tissue engineered constructs but should be used also an important element of quality control for the establishment of an individual cell bank.
Methods: WJC and HUCAC were isolated, expanded and characterized. Cell viability, growth potential and the expression of specific markers from fresh and cryopreserved cells were studied over several passages. Differentiation potential into chondrogenetic, osteogenetic and adipogenetic lineages was analyzed and compared to the differentiation potential of mesenchymal cells from human bone marrow.
Results: Viability of directly thawed and recultured cells increased with rising passage number. Growth potential of cryopreserved, recultured cells was similar to that of fresh cultivated cells. Fresh cultivated and cryopreserved WJC and HUCAC revealed positive staining for several mesenchymal cell markers. Differentiation potential of WJC and HUCAC revealed only partial differentiation of a few cells into adipogenic, chondrogenic and osteogenic lineages compared to bone marrow cells.
Conclusion: Viability, proliferation and the cellular marker concentration of all fresh and cryopreserved cells were similar. Compared to mesenchymal bone marrow cells WJC and HUCAC have only a minor differentiation potential.

60.P08
Natural cryoprotectants to do cryopreservation of amniotic fluid-derived stem cells

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Natural cryoprotectants are most desirable chemicals in the field of Tissue Engineering. Amniotic Fluid-derived stem cells (AFSCs) are a potential cell source for therapeutic applications. They can be easily mass produced, cryopreserved and shipped to clinics for immediate use. However, one major obstacle to the manufacturing of clinical grade stem cells is the need for current good manufacturing practices for cryopreservation, storage, and distribution of these cells. Most current cryopreservation methods used for stem cells include the potentially toxic cryoprotectant (CPA) dimethylsulfoxide (Me_2SO) in the presence of animal serum proteins that prevent direct use of these cells in human therapeutic applications. It will be essential to develop non-toxic CPAs or reduce CPA concentration in the freezing media. Here we assessed the use of disaccharides, antioxidants and caspase inhibitors for cryopreservation of AFSCs in combination with a reduced concentration of Me_2SO. The thawed cells were tested for viability with MTT assays and a growth curve was made. We also performed FACS analysis for cell surface antigens, RT-PCR for mRNA expression of markers and assays to determine the myogenic differentiation potential. Statistically significant increase in post-thawed cell viability in solutions containing trehalose, catalase and ZVAD-fmk with 5% Me_2SO was observed. The solutions containing trehalose and catalase produced similar results. So, AFSCs can be cryopreserved this way.

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61. 3D Scaffold/Cell Printing

61.01
Bioprinting anisotropic stem cell microenvironment

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Current cell/tissue scaffolding methods present challenges due to lack of control over spatial cell seeding and extracellular matrix (ECM) composition. Microdroplet-based hydrogel bioprinting can be used to engineer complex tissue anisotropies by producing scaffolds with controlled microscale spatial heterogeneity in ECM and cellular compositions. We showed that microdroplet-based hydrogel bioprinting approach will facilitate engineering of complex tissue anisotropies. We achieved a biochemical gradient with microdroplets encapsulating human Mesenchymal Stem Cells (hMSCs) and to assess the hMSC differentiation at the engineered fibrocartilage phase. Microdroplets were created by cell-encapsulating droplet generation system and cell-laden collagen droplets were printed on collagen coated substrate. hMSCs were patterned in microdroplets with BMP-2 and TGF-β1 representing the fibrocartilage phase. Quantitative RT-PCR array was used to assess the differentiation of hMSCs to bone and cartilage following 16 days of culture. PCR analysis showed that the hMSCs displayed a potent upregulation of osteogenesis and chondrogenesis related genes. MSC related genes as well as other tissue specific (e.g., adipose and tendon) genes were down regulated at the micropatterned interface. These results indicated that microdroplet-based bioprinting can generate biochemical anisotropy to facilitate controlled hMSC differentiation in bone-fibrocartilage phase.

61.02
Development and validation of an integrated organ printing system

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Although a variety of tissue constructs can be generated with gel materials, these often present with limitations in maintaining structural integrity due to their low mechanical strength, which makes handling and surgical suturing difficult. As such, we developed a novel organ printing system that integrates solid freeform fabrication and cell printing technology. The integrated organ printing system consisted of four delivery modules that can process multiple types of materials, including synthetic polymers and a cell/gel mixture. The materials are precisely dispensed by the control of air pressure. A heating unit was used to obtain dispensable synthetic polymers. We investigated the feasibility of delivering viable cells while fabricating rigid synthetic scaffolds simultaneously to create durable tissue structures. NIH 3T3 cells/Pluronic F-127 mixture, gelatin, and poly(ϵ-caprolactone) (PCL) were delivered to construct 3D structures. Live/dead staining and MTS assays were performed to evaluate cell viability within the 3D configuration. The results show that the cells were successfully placed into the desired position with approximately 80% cell viability. We have successfully designed and constructed an integrated organ printing system that is able to print 3D-organ structures with precision. This system provides a major leap in the advancement of organ printing technology.

61.03
Monitoring analyte concentration using self reporting scaffolds a 3D culture model

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Within a 3D cell culture construct it is favourable to have a homogenous cell culture environment that is free from analyte gradients such as those created from inefficient nutrient exchange or metabolic waste removal. Assessment of analyte concentration within a cultured 3D environment can be performed by physically probing the construct; however this can disturb cell viability and function and ultimately led to sacrifice of the tissue model. We have developed novel self-reporting scaffolds that can be utilised as an analytical tool to monitor local environmental analyte concentrations within 3D cell culture models. The self-reporting scaffolds incorporate optically responsive nanosensors within the polymer fibres to produce scaffolds capable of non-invasive *in situ* assessment of local environmental analyte concentrations such as oxygen and pH. The nanosensors report the analyte concentration by ratiometric fluorescent output from dyes incorporated into a biocompatible matrix. Culture models positioned within a bioreactor can prevent 3D culture-associated cell death but may not entirely eliminate analyte gradients. Coupling the use of self-reporting scaffolds and perfusion bioreactors can in the future provide on-line assessment of dynamic 3D culture.

61.04
Laser fabrication of multi-scale elastic 3D scaffolds

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Until recently 3D cell-culture matrices were mainly considered from a standpoint of support and guidance of cell proliferation and tissue development. The early designs of tissue engineering constructs focused on bulk properties, while disregarding individual cell environment. Current findings show that the role of the extracellular matrix (ECM) extends beyond a simple structural support to regulation of cell and tissue function. Mechanical stimulation and cell interaction with the topographic cues is an important part of this regulation. Systematic studies of its underlying mechanisms *in vitro* rely on methods capable of creating such 3D cell-culture matrices with high reproducibility and in accordance to a defined design. Two-photon polymerization (2PP) is a method based on localized cross-linking of the photopolymers, induced by femtosecond laser pulses. High resolution of the 2PP facilitates fabrication of 3D scaffolds, which contain features at several length scales, in a single step. In this contribution our recent results on fabrication of 3D scaffolds by 2PP of novel biodegradable photoelastomers are presented. The scaffolds were seeded with different cell types, and cultured under mechanical stimuli and in static conditions. Our results emphasize the potential of the proposed method for realization of rationally engineered multi-scale 3D scaffolds.

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61.05
Self-assembly-based biofabrication

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We introduce an automated rapid prototyping method (organ printing) that allows the engineering of fully biological three-dimensional custom-shaped tissue and organ modules. In this technology bio-ink units (multicellular aggregates) composed of single or several cell types are deposited into a support structure by special-purpose printers. Printing of the bio-ink units (controlled by architectural software) is carried out according to a design template, consistent with the geometry and composition of the desired organ module. Structure formation occurs by the post-printing fusion of the discrete bio-ink units. When the bio-ink units contain more than one cell type, fusion is accompanied by sorting of the cells into the physiologically relevant pattern. Thus structure formation takes place through self-assembly processes akin to those utilized in early embryonic morphogenesis. We demonstrate the technology by detailing the construction of vascular and nerve grafts. Spherical and cylindrical bio-ink units have been employed to build fully biological linear and branching vascular tubular conduits and multiluminal nerve grafts. Upon perfusion in a bioreactor the constructs achieved desirable biomechanical and biochemical properties that allowed implantation into animal models. Our results show that the biofabrication by printing of conveniently prepared cellular units is feasible and may represent a promising tissue and organ engineering technology.

61.06
Design, physical prototyping and initial characterization of 'lockyballs': enabling technological platform for bottom-up modular 3D tissue biofabrication

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Directed tissue self-assembly or bottom-up modular approach in tissue biofabrication is an attractive and potentially superior alternative to a classic top-down solid scaffold-based approach in tissue engineering. There is a growing consensus that a strategy based on the integration of a directed tissue self-assembly approach with a conventional solid scaffold-based approach could be a potential optimal solution. We hypothesize that tissue spheroids with 'Velcro®-like' interlockable solid micro-scaffolds or simply 'lockyballs' could enable the rapid *in vivo* biofabrication of 3D tissue constructs at desirable material properties and high initial cell density. Recently, biocompatible and biodegradable photo-sensitive biomaterials could be fabricated at nanoscale resolution using two-photon polymerization (2PP). Here we report design studies, physical prototyping using 2PP and initial functional characterization of inter-lockable solid micro-scaffolds or so-called 'lockyballs'. The lockable skeletal tissue spheroids (chondrospheres and osteospheres) biofabricated using the described lockyballs as solid micro-scaffolds are characterized by attractive new functionalities such as lockability and tunable material properties of the tissue engineered constructs. The bottom-up lockyball based modular approaches creates the basis for a development of *in vivo* rapid skeletal tissues biofabrication and bioprinting.

61.P01
Feasibility of 3D printing and stereolithography for fabrication of custom-shaped poly (lactic acid): hydroxyapatite composite biomaterial scaffolds

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Orthopaedics craniofacial defects would benefit greatly from fabrication of custom-shaped scaffolds. Solid freeform fabrication methods (including robocasting and stereolithography) show great promise, but the focus of work to date has been on the preparation of scaffolds using single polymers. Hydroxyapatite is widely employed in bone repair due to its osteoconductive properties. The aim was to evaluate the potential of using SFF methods in manufacturing predefined PLA/HA porous scaffolds. We compared robocasting with projection microstereolithography (μSL) for fabrication of macroscopic scaffolds. With robocasting we produced scaffolds via high pressure dispensing of HA: PLA paste diluted in solvent from a 100 μm inner diameter needle onto a substrate, while translating the dispensing needle with a computer-aided xyz-stage. In projection μSL, we projected a 2D image into a liquid photocurable HA/PLA composite resin, which cures under irradiation. A 3D object can be built up layer-by-layer via projecting consequent images while translating the substrate in one direction. The techniques proved to produce predefined 3D macroscopic (cm-sized) objects with ~100 μm resolution. Preliminary biocompatibility studies indicated that these scaffolds were capable of supporting cell attachment and growth. It was concluded that both techniques were suitable for preparation of composite scaffolds, although 3D printing had the advantage of using non-photocurable polymers.

61.P02
3D micropatterning of biological structures using holographic optical tweezers

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In the human body the points of contact between cells and matrices, as well as intercellular adhesion points, are specifically controlled, influencing cell architecture and morphogenesis. In order to accurately investigate and replicate these complex systems *in vitro*, precise three dimensional control of cell patterning at the cellular level is of key importance. Here we demonstrate the use of holographic optical tweezers to simultaneously position multiple cells into 3D patterns. Individual mouse embryonic stem cells were positioned into defined aggregate architectures, directing subsequent embryoid body development and differentiation. Furthermore, the technique has been used to control the formation of primitive vascular structures by patterning human endothelial cells, thereby controlling the position of tubule formation. The data gathered in this study clearly demonstrates the significant potential of this method for generating sophisticated *in vitro* model systems.

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61.P03
Chemically modified hydrogels for two-photon polymerization (2PP)

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Two-photon polymerization (2PP) is a promising approach to fabricate micro- and nanostructured three-dimensional scaffolds for tissue engineering. As a scaffold material, a variety of synthetic polymers, but especially naturally occurring hydrogels like gelatin, hyaluronic acid and chitosan are qualified to be biocompatible. The application of the 2PP-technique to such natural materials requires their certain chemical modification to enable photopolymerization. While gelatin is a mixture of proteins, hyaluronic acid and chitosan are natural polysaccharides, they all provide molecular sides for further chemical functionalization with photosensitive groups such as methacrylate, vinyl or azide. In this work, we discuss the preparation of methacrylamide-modified gelatin, chitosan and hyaluronic acid, as well as the utilization of these hydrogels for photocrosslinking and 2PP structuring. The mechanical properties of the generated materials depend significantly on the amount of incorporated methacrylamide moieties which was determined using a picrylsulfonic acid essay for primary amines detection. To create hydrogel-hybrid materials with tunable properties, photocurable chitosan hydrogels were alternatively prepared by covalently bonding of the free amino groups to the acid group of a photosensitive hetero-bifunctional crosslinking agent (4-azidobenzoic acid). Such functionalization allows the material to be cross-linked with further biocompatible polymers such as polylactic acid.

61.P04
Cure-depth control and biocompatibility in microstereolithography

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There is a need for three dimensional, biocompatible microstructures in tissue engineering and cell culturing applications. Microstereolithography is a powerful technique to additively build these structures from photocurable materials under UV or blue visible light. To achieve the desired accuracy a high degree of cure-depth control is necessary, and this is particularly important in transparent biomaterial compositions such as hydrogels. The process hence often requires a dye that efficiently absorbs light of the applied wavelength, minimises cell toxicity and is available at a reasonable material cost. Tartrazine is a well known food dye that qualifies as such a processing additive, while many biomedical stains are too cost-intensive in relation to the material volumes required in stereolithographic processing. This study demonstrates that the Tartrazine concentrations required to achieve good cure-depth control can be as low as 0.1 wt%. It was furthermore observed that Tartrazine does not negatively affect cell viability at dye concentrations of 0.05 wt% and enhances cell growth at 0.1 wt%. These results justify the further investigation and a potentially novel application of Tartrazine in photopolymer processing of water-containing biomaterials under blue light

61.P05
Laser microfabrication of hydrogel scaffolds

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Additive manufacturing techniques have found increasing interest to fabricate 3D hydrogel scaffolds. The constructs can be produced reproducibly and in accordance to a CAD model. The cells can be introduced by two different ways into the construct: seeding of cells onto the surface after the fabrication and incorporation of cells into the fabrication process. The latter might allow higher cell densities and better control over the distribution. Two-photon polymerisation (2PP) is a rapidly emerging platform for the 3D microfabrication of biocompatible scaffolds. Most biological tissues exhibit a window of transparency at the wavelength of the applied femtosecond laser. Hydrogels based on synthetic (PEGDa) and natural monomers (Gelatine Hydrolisate, Bovine Serum Albumin) are presented. Using novel water-soluble 2PP initiators (4, 4'-dialkylamino bis(styryl)benzene core), complex 3D constructs can be fabricated reproducibly in environments with high water contents. The biocompatibility of the presented compounds is evaluated and reported. We demonstrate the feasibility and potential of two-photon polymerisation for the bio-fabrication of 3D tissue constructs directly in the presence of living cells.

61.P06
Towards *in vivo* bioprinting: design and implementation of *in vivo* bioprinter

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Organ printing is a variant of robotic computer-aided additive biofabrication technology with using self-assembling tissue spheroids as building blocks. Bioprinting *in vivo* using robotic dispensing tissue spheroids such as chondrospheres or osteospheres is one of most promising directions in clinical translation of rapidly emerging bioprinting technology. Here, we report about design and development of first clinical *in vivo* robotic bioprinter. Previously, we have developed robotic biofabricator for scalable biofabrication of tissue spheroids of standard size and novel robotic bioprinter with three nozzles (Two Nordson sprayers and one Fishman dispenser). However, design of cartridge for clinically relevant number of tissue spheroids for bioprinter was one of unsolved problem. The development of magnetic forced driven harvester of tissue spheroids labelled with magnetic nanoparticles solves this problem. It has been shown that magnetic levitation-based harvesting of tissue spheroids from robotic tissue spheroids biofabricator with their transfer directly into bioprinter with sequential delivery into damaged tissue is feasible. Working prototype of first *in vivo* clinical bioprinter has been designed and implemented. It has been demonstrated that *in vivo* bioprinting of 3D skeletal tissues such as cartilage and bone using tissue spheroids is technically feasible and that this open realistic opportunities for clinical applications of bioprinting in rapidly emerging surgical robotics.

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61.P07
Developing biodegradable 3D hydrogels scaffolds: from synthesis to two-photon polymerization

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Developing 3D hydrogel scaffolds capable of promoting cell viability and cell-extracellular matrix (ECM) interactions is of great importance for Tissue Engineering (TE) applications. To mimic the biochemical and structural complexity of ECM, two-photon polymerization (TPP) shows the greatest promise to fabricate ECM-biomimetic hydrogels because it allows fabrication of complex user-dictated shapes with micrometer-scale resolution. Poly(ethylene glycol) diacrylates have been widely used for TPP fabrication of hydrogels due to high reactivity. However, unreacted acrylate groups are irritant and potentially cytotoxic. For most TE applications, monomers with low cyto-toxicity are in demand. Our previous work proved that vinyl esters (VEs) are much less cytotoxic than acrylates references. Although VEs are generally not as reactive as acrylates, the thiol-vinyl ester photo-click reactions are robust enough for efficient TPP applications. Since it is hard to assemble ECM-biomimetic hydrogels using synthetic monomers alone, proteins with Thiol and VE functionalities are developed for their potential use as Thiol-ene photo-click hydrogels. Presented is the synthesis of vinyl ester derivative of gelatin hydrolysate, preparation of thiolated bovine serum albumin, and 3D fabrication of gelatin/albumin based hydrogel scaffolds via TPP click reactions. We suppose the robust thiol-vinyl ester photo-click reactions are responsible for the reasonable writing speed and broad processing window.

61.P08
Water soluble initiators for two-photon polymerization

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Two-photon polymerization (2PP) in aqueous environment has attracted increasing attentions due to its versatility in biological applications, especially in the microfabrication of biocompatible 3D scaffolds for tissue engineering with nano-level resolution. Efficient water-borne two-photon curable formulation demands reactive water-soluble photoinitiators (PIs) with low toxicities. Although some commercial hydrophilic dyes have been used as initiators in 3D biological fabrication, high laser intensities and long exposure time are required due to their small two-photon absorption (TPA) cross sections. Here, a novel water-soluble TPA PI 2, 5-bis-[4-[(2-sodiumcarboxylate-methyl)(methyl)-amino]-benzylidene]-4- methylcyclohexanone (G2CK) was prepared via introducing hydrophilic sodium carboxylate groups into the efficient TPA PI 2,5-bis-[4-(dimthylamino)-benzylidene]-4-methylcyclohexanone, which showed large TPA cross section combined with high photoinitiation activity. In 2PP structuring tests using formulations with water contents of up to 80%, G2CK exhibited broad ideal processing windows and nicely shaped structures were obtained at low laser intensities, as well as high writing speeds. Finally, the results of time resolved cytotoxicity tests with this novel water-soluble PI are presented.

61.P09
Controllable and tunable 3D scaffolds fabrication using laser based techniques

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In tissue engineering, the development of a reproducible and controlled method of micro/nano fabrication of 3D scaffolds is of great importance. Ultrafast pulsed lasers micro/nano engineering offers unique features; nanoscale spatial resolution, nonthermal and nondestructive engineering. Here, we report on the fabrication of high resolution 3D scaffolds deploying three laser based techniques: (i) two-photon polymerization (2PP), (ii) ultrafast laser micro/nano structuring (ULMNS) (iii) single pulse UV laser irradiation of biopolymers. The 2PP technique relies on two-photon polymerization enabling 3D bioactive structures fabrication with submicron resolution. The ULMNS technique is an effective direct-write method to fabricate hierarchical micro/nano structures on Si surfaces with superior control over structure geometry and pattern regularity. We report Si micro/nano spike forests fabrication and replication of the initial spiked morphology on soft polymeric materials. The third technique relies on the generation of a foam layer on biopolymers. Following scaffolds' fabrication, tailoring of their surface properties has been advantageously achieved. The ability of different cell types to be grown and differentiated onto the specific scaffolds has been investigated. The ultimate goal is to explore the influence of the synergy of surface topography and chemistry on cell behaviour, related to viability, motility, adhesion, morphology, cytoskeletal arrangement and differentiation.

61.P10
Application of riboflavin as water-soluble photoinitiator for fabrication of tissue engineering scaffolds via two-photon polymerization

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In recent years, two-photon polymerization (2PP) became a powerful tool for fabrication of different biomedical micro-devices ranging from microneedles for transdermal drug delivery and ossicular prosthesis for middle ear to scaffolds for tissue engineering (TE). Applying 2PP, precisely defined complex 3D scaffolds for TE can be fabricated from variety of materials such as completely synthetic and non-biodegradable acrylates, PEG-hydrogels and modified natural proteins. However, cytotoxity of the photoinitiators is the factor which limits the use of 2PP technology for fabrication of TE scaffolds. One potential source of biocompatible photoinitiators is free radical generating molecules that are naturally occurring in biological systems. Riboflavin (vitamin B2) is one chemical that has potential to be used as a more biocompatible photoinitiator. In addition to a biocompatible photoinitiator, a biocompatible polymer that can be cross-linked by free radicals is needed for making tissue engineering scaffolds by 2PP. We have demonstrated, for the first time, the ability to perform two-photon polymerization using riboflavin as a photoinitiator. TE scaffolds produced out of PEGda with riboflavin as a photoinitiator were found to support cell growth. Toxicity testing indicated that PEGda crosslinked using riboflavin has superior biocompatibility, much greater than when crosslinked using conventional photoinitiators, such as Irgacure 369 and Irgacure 2959.

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61.P11
Utilization of photopolymerization of new low toxic vinyl monomers in bone tissue engineering

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The fabrication of 3D scaffolds by lithography-based additive manufacturing technology (AMT) represents an appealing approach in bone tissue engineering. As state of the art poly(lactic acid) cannot be processed by high resolution photopolymerization-based techniques, it has so far been necessary to use (meth)acrylate-based photopolymers. Although (meth)acrylates have excellent photoreactivity and afford photopolymers with good mechanical properties, their cytotoxicity and degradation products disqualify them from medical use. Therefore, (meth)acrylate-based monomers were replaced by vinyl esters and vinyl carbonates with exceptional low cytotoxicity. *In vitro* cytotoxicity studies with osteoblast-like cells proved that these monomers are 1–3 orders of magnitude less cytotoxic than (meth)acrylates. Although the photoreactivity is lower than that of acrylates, they are sufficiently photoreactive to be structured by lithography based AMT. By addition of thiols to the photocurable formulation, curing speed can reach the value for acrylates. The mechanical properties and rates of degradation can be tuned over a broad range. All polymers were significantly stiffer than PCL, being almost as stiff as PLA. Degradation of the polymers results in the formation of non-toxic degradation products of low molecular weight that can be easily transported within the human body. Finally, *in vivo* testing proofed an excellent biocompatibility of these materials.

61.P12
Submerged biofabrication into a hydrophobic high-density fluid

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Over the last decade, biofabrication technologies appeared as important tissue engineering strategies for regenerative medicine and organ transplantation. Several combinations of materials serving as matrices for cell printing and different printing techniques, such as inkjet and laser guided printing were attempted worldwide. The major drawback of these past approaches was the poor or inadequate material-printing device and substrate combinations as well as the relatively small size of the printed construct. Here, we show a promising alternative for printing living cells, submerged into a hydrophobic high-density fluid substrate. Human MG-63 cells and human mesenchymal stem cells were encapsulated into agarose hydrogels and afterwards submerged printed. Constructs with variable shapes and sizes were achieved and remained stable for more than 6 months. Live/dead staining showed viable cells after the printing process, as well as after incubation for 21 days. Further histological analyses after 2 and 3 weeks of *in vitro* culture revealed vital mesenchymal cells with marked matrix production and focal mitotic activity as a sign of proliferation. Compressive strength values of printed gels consecutively increased over the 3 weeks in culture. Our concept appears as a simple, reproducible, precise and 'printing-supporting' approach allowing for the fabrication of polymeric constructs on a 3D freeform basis. Our first results are encouraging for future regenerative applications.

61.P13
Mosaic hydrogels: Dynamic tessellation and coding of cells

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Patterning of biological molecules and cells in soft materials using traditional top-down deposition or layer-by-layer fabrication lack spatiotemporal control and presents a significant challenge in the engineering of 3-D constructs. Here we show the one-step preparation of planar hydrogels with precise control over its local composition and mechanical properties for creation of 3-D tissue-like structures. A scaled-out microfluidic device with on-chip reservoirs enables the incorporation of up to seven distinct biopolymer into a continuously flowing base biopolymer sheet. The obtained planar hydrogels had directionally dependent properties, with tessellations of different hydrogels leading to mosaic stiffness and diffusivity patterns. We demonstrate the local storage or the timed release of an embedded colloidal, biomolecular, or cellular payload within the patterning biopolymer. The simultaneous and controlled incorporation of endothelial cells, fibroblasts, and cardiomyocytes within hydrogel sheets in combination with a barcode system enables to track and record the various conditions patterned throughout the culture time. We envision the routine fabrication of heterogeneous soft materials with higher degrees of structural and compositional complexity. The ability to assign a biomolecular or cellular payload to regions of a hydrogel promises systematic investigation of cell–cell and cell–matrix interaction, cellular patterning, and co-culture, all in a 3D environment.

61.P14
Fabrication of 3D hydrogel scaffold for cartilage tissue engineering

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Recently, there has been a growing interest in fabrication of tissues and organs using cell printing technique. Cell printing technique is based on Solid Freeform Fabrication (SFF) technology, which can overcome the limitation of the conventional scaffold fabrication method. Using cell printing technology, cells, biomaterials and biochemical factors will be integrated to construct artificial tissues and organs most similar to the human ones. In this paper, we fabricated a lab-made 3D cell printing system and 3D cell-laden hydrogel scaffolds. The 3D hydrogel scaffolds have good environmental advantages for cell growth. We fabricated interconnected porous 3D alginate scaffolds using our cell printing system. The chondrocytes are encapsulated in the alginate scaffolds. Experiment results show that chondrocytes encapsulated scaffold with pores has better viability than without pores. In other to promote the proliferation and differentiation of the chondrocyte, we add 1%, 3%, and 5% of gelatin to the alginate. The experimental results show that the 3D alginate-gelatin scaffold promotes the proliferation and differentiation of the encapsulated chondrocytes.

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61.P15
3D bioprintable hydrogels with tunable physical and mechanical properties for encapsulation of heart valve cells

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3D bioprinting of living aortic valve conduits with native anatomical shape and mechanical and cellular heterogeneity have potential for pediatric heart valve regeneration. It is important to investigate the phenotype of heart valve cells (smooth muscle cells (SMC) from heart valve root and valve interstitial cells (VIC) from leaflet) in bioprintable hydrogel with tunable stiffness. In this study, SMC and VIC were encapsulated within photocrosslinkable poly(ethylene glycol) diacrylate (PEGDA)/methacrylated-gelatin/alginate hydrogels. The mechanical properties were tunable and well controlled by varying the molecular weight of PEGDA and the mixture ratio. We characterized different hydrogel networks including the swell ratio, tensile test, encapsulated cell (SMC and VIC) viability, cell growth rate and cell phenotypes. SMC showed significant higher growth rate in stiff hydrogels and expressed more smooth muscle actin, but less vimentin compared to compliant counterparts. But the encapsulated VIC did not show any difference between hydrogels with different stiffness. We have also employed our extrusion based 3D bioprinter to bioprint a 3D grid pattern structure using stiff hydrogel encapsulated with SMC and compliant hydrogel with VIC. Both cell types survived 3D bioprinting and photocrosslink process. By mimicking the native valve structure and biomechanics, the hydrogels with tunable properties can accommodate VIC and SMC for bioprinting of heterogeneous aortic valve conduit.

61.P16
The influence of freeze-drying methods on porous gelatin scaffolds structure

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In this present work gelatin scaffolds were manufactured by several freeze-drying methods. This study assessed the structure of scaffold, which is prominent factor to preparation porous gelatin scaffold. Results indicated that porous gelatin scaffolds made by bidirectional, unidirectional and traditional freeze-drying are strikingly different. It has shown that gelatin scaffolds made by bidirectional freeze-drying are interconnected more than unidirectional and traditional method. In addition, scaffolds properties such as porosity and water adsorption ability were studied. Toxicity of porous gelatin scaffolds was examined by *in vitro* cell culture. Porous structure was characterized by scanning electron microscopy.

Keywords: freeze drying method, porous structure, gelatin scaffold

61.P17
How structure and chemical composition influence mechanical and biological properties of 3D tissue engineering scaffolds fabricated using rapid prototyping method

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The aim of the study was to create and evaluate 3D scaffolds made of polymer (polycaprolactone) and composite (polycaprolactone + 5% tricalcium phosphate) which could be used for bone tissue engineering (BTE). Cylindrical porous scaffolds with 3D orthogonal periodic porous architectures were fabricated using rapid prototyping method and Bioscaffolder. The scaffolds structure was investigated using scanning electron microscopy (HITACHI SU8000). X-ray microtomographical analysis and the mechanical testing of the porous structures were performed using SkyScan 1172 machine, equipped with a material testing stage. Fatigue strength was evaluated using dynamical electromechanical testing machine (BOSE). After Gamma sterilisation, the scaffolds were seeded and cultured with human umbilical cord stromal cells (UCSC) (1.4×106/cm3) for 6 weeks under osteogenic stimulation conditions (MesenCult, Stem Cell Technologies). Calcium deposits were stained with Alizarin Red. The obtained scaffolds had structures with high porosity (50%–60%) and interconnected pores ranging from 400 to 500 μm. Addition of the 5% TCP particles did not affect significantly the mechanical properties of the composite scaffold. Higher surface development has been observed for the composite samples what has positive influence on cell proliferation. Both the adhesion rate (seeding phase) and expansion rate of UCSCs and osteogenic differentiation were significantly higher (over 30%) for composite scaffolds.

61.P18
Designing *in-situ* architectures using microscopic laser photolithography in 3D cell culture hydrogels

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An advantage of hydrogel scaffolds is their ability to be spatially structured with mechanical cues that guide cells. However, creating unique stiffness landscapes within a hydrogel using photolithography has proven difficult in materials that support 3D cell culture. For this purpose, temperature responsive bioactive hydrogels were made from fibrinogen and Pluronic®F127 (FF127). These materials form a physical hydrogel at physiological temperatures and can be further photopolymerized (fully or locally) in the presence of an initiator and UV light (i.e., chemical crosslinking). Particle tracking microrheology was used to study the local rheological properties of these heterogeneous hydrogels. The beads' displacements in the chemically crosslinked regions was lower than in the physically crosslinked ones. Patterned UV illumination of FF127 constructs made with rhodamine-labeled F127-DA pinpointed the regions of chemical crosslinking in the physically crosslinked constructs. Cellularized constructs where similarly patterned to reveal a difference in cellular morphology between chemically and physically crosslinked regions. Although equally viable, mesenchymal cells were less spindled in the stiffer regions. Interestingly, the cells favorably oriented at the interface between stiff and compliant regions. These results emphasize the importance of mechanical heterogeneity in cellular morphogenesis, providing validation for cutting-edge tools than can be used to explore this topic.

61.P19
Biodegradable microparticles for cell aggregation and proliferation

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Microparticles (MPs) can be used as matrices to organize three-dimensional (3D) assembly of cells for tissue engineering. Specific interaction using a ligand was incorporated to support aggregate formation between microparticles and cells. We have modified poly(glycerol adipate) (PGA) polymer by stearoyl chloride (C18) substitution (40% C18-PGA)to increase drug-polymer interaction. Furthermore, in order to tether a functional group for ligand attachment on the surface of microparticles, a poly(ethylene glycol)-maleimide (PEG-MIHA) linker was attached to the terminal carboxyl group on PGA. Microparticles

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were fabricated using the emulsification method and collagen attached as a ligand. Diameter of particles was ranging from 0.3 to 75 μm with mean diameter of 6 μm. The MPs from 40% C18 -PGA-PEG-MIHA had a higher zeta potential than 40% C18 -PGA. Aggregate formation was evaluated with mouse embryonic stem (mES) cells and neonatal calvarial osteoblast cells. Microparticles made from 40% C18-PGA and 40% C18-PGA-PEG-MIHA had no effect on aggregate formation in mES cells but supported osteoblast cell aggregation. After 14 days, mES and osteoblast cell aggregates with 40% C18 substituted PGA-PEG-MIHA MPs with collagen had the highest cell metabolic activity ($n = 3$, $p < 0.05$). It suggests that 40% C18 substituted PGA-PEG-MIHA MPs with a collagen ligand could act as matrices to support cell proliferation in a 3D structure.

61.P20
Well-ordered calcium phosphate/alginate biphasic scaffolds containing designed hollow fibers for bone tissue engineering and regeneration

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In this work, we present novel calcium phosphate (CaP)/alginate scaffolds with well-organised structures for bone tissue engineering and regeneration. These scaffolds were fabricated by 3D bioplotting using a three channel scaffold printer. The CaP pastes were prepared by mixing CaP powder with biocompatible oils and additives; alginate pastes were prepared by dissolving alginate with 6% polyvinyl alcohol solution. CaP/alginate biphasic scaffolds were fabricated by dosing CaP and alginate pastes layer by layer alternately utilising computer aided design (CAD) and computer aided manufacturing (CAM). Furthermore, alginate hollow fibers were introduced in these scaffolds for mimicking the vasculature. Scaffolds were characterised by light microscopy, SEM and XRD. The mechanical properties were investigated in dry and wet state. Human mesenchymal stem cells (hMSC) were seeded and cultured. Bovine serum albumin (BSA) was introduced in the scaffolds and the release behavior was studied. Results showed that the prepared biphasic scaffolds owned regular structure and controlled pore size and interconnection. Hollow fibers with completely open lumen were successfully introduced. The compressive strength of biphasic scaffolds were significantly increased and toughness were improved. hMSC attached and grew well on the scaffolds. Proteins can easily be incorporated in the materials due to the physiological preparation conditions and BSA released much faster from alginate than from CaP.

61.P21
Integrated bioprocess composed of cells, porous PLGA microspheres and a HARV bioreactor to develop three dimensional tissue engineered construct

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To date, extensive researches on the development of 3D bone and cartilage-like tissue construct have been done for the clinical purpose of restoring bone and cartilage defects. In this research, an integrated bioprocess to generate *in vitro* 3D tissue engineered bone and cartilage constructs was developed using adipose-derived stem cell (ADSC), primary chondrocyte, porous microsphere and a HARV bioreactor. First, porous PLGA microsphere with above 500 μm of diameter was fabricated, which was designed to experience less effect of sheer stress during rotation in a horizontally rotating microgravity bioreactor via previous simulation test. Cells (ADSC or chondrocyte) were seeded uniformly on poly (L-ornithine)-coated microspheres, and the cell-microsphere constructs were cultured under osteogenic or chondrogenic culture condition within a HARV bioreactor, which microspheres and dynamic culture system support efficient 3D cell and tissue growth by facilitating nutrient mass-transport into 3D constructs. Finally, 3D bone- or cartilage- like tissue formation was characterized in molecular and gene level by immunocytochemical analysis, real time-qPCR analysis, and other analysis.

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61.P22
Biological evaluation of a successful transferred double protein coating from 2D PCL coatings to 3D scaffolds.

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Introduction: Scaffolds are being produced from biodegradable polymers in order to replace defects in the human body. The poor biological performance of some of these materials translates into a need for surface modification. In this work we report on the transfer of surface modification, involving immobilization of gelatin and fibronectin, from 2D PCL films to bioplotted 3D PCL scaffolds and the biological evaluation of both substrates.

Materials and methods: Scanning Electron Microscopy (SEM), X-ray photoelectron spectroscopy (XPS) and confocal fluorescence microscopy (CFM) has been used to evaluate the scaffolds. Cell viability and adhesion of HFF (human foreskin fibroblast) cells was evaluated on modified 2D PCL films and 3D PCL scaffolds with an actin cytoskeleton-focal adhesion staining, MTT and calcein staining.

Results: SEM, XPS and CFM confirmed a homogeneous protein-coating on both 2D and 3D substrates. The biological evaluation showed that the double protein coated PCL substrates were superior compared to the other surface modifications, independent of the material dimension.

Conclusion: We have fabricated scaffolds with an interconnected structure and standardized pores applying the 3D Bioscaffolder®. The scaffolds were successfully modified with a double protein coating using a multistep surface modification protocol. From the biological evaluation, it could be derived that fibronectin remains biologically active as it promotes initial adhesion and proliferation.

61.P23:
Melt electrospinning writing fibrous scaffolds for regenerative medicine

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Electrospinning is a dynamic process that involves electrostatically drawing a liquid to create fibrous structures. Increasingly used within regenerative medicine to fabricate scaffolds, electrospinning faces challenges in controlling fibre deposition due to excessive electric instabilities, contributed by volatile solvents. However electrified jets of non-conductive polymer melts are more stable and the deposition path is very predictable. Combining melt electrospinning with automated stages, this new approach to direct writing produces tissue engineering scaffolds with a high level of accuracy and complexity. Melt electrospinning writing in a direct writing mode produced complex microporous fibrous scaffolds with filaments between 5 and 20 μ, with minimum stage speeds required for different conditions. Reducing the flow rate decreased the fibre diameter but increased the minimum stage transla-

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tion speed required to generate a straight fibre. Importantly, melt electrospinning allows the accurate stacking of fibres upon each other, even when using complex shapes such as cursive writing. Melt electrospinning in a direct writing mode produces ordered and intricate biomedical devices and cell invasive scaffolds for regenerative medicine. Electrospinning writing benefits from minimal electrostatic instabilities associated with non-conductive polymer melts and consequently produces ordered and reproducible structures for regenerative medicine.

61.P25: Biofunctionalization of rapid prototyping PLA scaffolds for tissue engineering

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Rapid prototyping (RP) allows the development of precise 3D scaffolds for tissue regeneration. Polylactic acid (PLA) is known for the fabrication of degradable scaffolds; yet, it lacks bioactivity. Adding elastin-like polymers (ELPs) could improve this drawback. Thus, this work aims to develop and characterize ELP-functionalized PLA scaffolds.PLA scaffolds fabricated by a robotic nozzle-deposition system were functionalized with ELP containing the RGD sequence. Both, physisorbed and covalently bonded (NaOH + EDC/NHS activation + immersion in ELP solution) coatings were achieved. Characterization by CBQCA and Micro BCA assays was done. Adhesion, proliferation and morphology of rMSCs were evaluated by LDH activity and immunofluorescence. 3D structures with pores of 165 ± 5 μm, struts of 70 ± 5 μm, 70% of porosity and a compressive modulus of 30 ± 5 MPa were obtained. Confocal images showed an homogeneous surface coating. The micro BCA assay showed a higher ELP density in the covalently functionalized surfaces than on the physisorbed ones. Biological studies showed a positive cell response in the functionalized scaffolds. This work shows the possibility to successfully bioactivate the surface of biodegradable 3D PLA scaffolds with various biomolecules. Scaffolds were homogeneously coated with a covalently bonded ELP showing a positive cell response. RP and biofunctionalization can be tuned to tailor promising degradable, mechanically stable, bioactive and biocompatible scaffolds for TE.

61.P26 Integration of mesenchymal stem cells into biodegradable nanofibers scaffolds by electrospinning and electrospraying methods

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Electrospinning (ES) is an attractive method used in bioengineering to produce scaffolds. Normally, the scaffolds are produced by ES and subsequently seeded with cells. However, uniform cell colonization remains an unsolved problem in 3D scaffolds. This work investigated a direct method to integrate cells into nanofibrous scaffolds by combining ES of fibers and electrospraying (EP) of cells. Mesenchymal stem cells (MSCs) from deciduous teeth in culture media were electrosprayed into PLGA nanofibrous scaffolds. The impact on cell viability and proliferation was determined. The cells were evaluated by scanning electron microscopy (SEM) and confocal microscopy (CM). The assessment with trypan blue revealed no significant loss in viability of MSCs and the MTT analysis showed the proliferation of cells remained into the scaffolds. The cell number increased significantly in culture. SEM images of cells illustrated high cell densities integrated between the fibers. CM images showed that cells were adhered to nanofibers. To assess possible DNA damage, cells were evaluated by comet assay and no genetic damage to the electrosprayed cells appeared until after a period of 15 min. Karyotyping of electrosprayed cells is being performed to evaluate chromosomal abnormalities. The results suggest that ES and EP association is an interesting technique to form 3D cell integrated scaffolds, making it a viable alternative for use in tissue engineering.

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61.P27 Cartilage tissue engineering using a hybrid of polymeric framework and cartilage-derived extracellular matrix with mesenchymal stromal cells from human inferior turbinate tissue

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Cells, biomaterial scaffolds and biological signals are indispensable in regeneration of diseased or traumatized tissues through tissue engineering. Here, maintaining of structural and biomechanical properties of target tissue is important for retaining biological activity. Furthermore, conserving the biological signals is very crucial to repair of the tissue. Herein, we present a hybrid of polycaprolactone framework and decellularized cartilage extracellular matrix (dECM) for cartilage regeneration. This structure is fabricated by customized multi-head tissue/organ building system (MtoBS), which is one of the solid freeform fabrication technologies. The mesenchymal stromal cells from human inferior turbinate tissue, a new promising cell source, are encapsulated into the dECM of hybrid structure as 'cell-laden hybrid structure'. The potential of cell-laden hybrid structure, serves as a biomimetic structure, was investigated by culturing mesenchymal stromal cells *in vitro*. The structure revealed great cell viability and biological activity. Mechanical properties were also improved than the widely used sponge type dECM scaffold. Moreover, the cartilage-derived ECM environment promoted chondrogenic characteristics of MSCs due to the composition and structure of the ECM. This study provides an appropriate basis for fabrication of hybrid type structure that is composed of dECM and synthetic polymer using SFF technology for tissue engineering.

61.P28 Gradients for cell guidance – functionalization by UV-laser-irradiation

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The development of medical devices and implants necessitate cell growth promoting surfaces. Especially neuronal grafts need surfaces which provide guiding structures of growth promoting factors or cell specific binding molecules. Previous studies have shown that UV-laser-irradiation can be used for functionalization of polymers. Several experiments have been performed with polymethylmetacrylate. By irradiation the surface gets locally modified due to oxidation. Hence carboxylic groups are induced and offer binding sites for amine functionalization. In state of the art technology the use of masks is

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common to generate functional patterns for cell guidance. For every guiding structure new mask have to be produced. The current study shows that by UV-laser-irradiation of poly-ϵ-caprolactone (PCL) functional groups can be achieved on the polymer surface. Due to the high spatial and temporal resolution of the laser irradiation the induced energy can be controlled. This offers the possibility to write freeform structures with controlled functionalization density on the polymer surface. A special case of this freeform functionalization is the production of gradients of anchor groups. These anchor groups can be functionalized with amines and growth factors which can be used as cell guiding structures.

61.P29
Fluorescent cell-based reference standards for quality assessment of regenerative medicine products

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Characterisation of cells for identity, purity, biological activity and viability is a regulatory requirement in both Europe and the US. Many of these characteristics can be measured using fluorescence-based analysis, for example, measurement of biomarker expression using spectroscopy, flow cytometry or imaging to confirm cell identity or assess biological activity. Whilst relatively simple methods exist to measure these parameters during standard cell manufacture, they become increasingly challenging to measure in the 3D systems often used in final products. These challenges can be partially addressed using reference standards, such as fluorescent micro-beads, to assess measurement sensitivity, variability and bias. However, these physical materials are not representative of the living cells used in regenerative medicine products, limiting their value in relation to biological samples. We therefore developed and characterised a prototype cellular fluorescent reference standard consisting of two cell lines stably transfected with GFP or RFP. The intercellular fluorescent variability was quantified using flow cytometry and a novel confocal image analysis system and shown to be stable over > 5 passages. Using these well characterised constitutively fluorescent cells we demonstrated how they can be used to calibrate fluorescence-based measurements in complex 3D environments providing a tool to facilitate product development, characterisation and quality assessment prior to release.

61.P30
What you see is what you print: Single Cell Transfer

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Many structures in living organisms show a high complexity. One example is the stem cell niche, where different cell types interact in a highly defined manner. The artificial realization of such structures, need a building technology, which enables a highly defined and reproducible positioning of the cell types of interest. With state of the art printing technology it is not feasible to transfer one single cell in more than 50% of the produced droplets. Laser Induced Forward Transfer (LIFT) however enables us to achieve a 100% single cell transfer rate. During LIFT a material is transferred from a transfer slide onto a receiver slide. The transfer slide consists of three layers: a support layer, an absorber layer, which is locally evaporated during laser irradiation and a transfer layer, which is transferred by the evaporation onto the receiver layer. Cells are contained within this transfer layer and can be selectively transferred via LIFT. Defined cell patterns (3T3, K562) were generated on a gelatin gel receiver slide using a semiautomatic five-axis LIFT system. Cell viability was demonstrated by life-dead assays. Different process parameters like the distance between transfer slide and receiver slide or the laser power have a great impact on the printing result. In future work we will focus on the transfer of different cell types to show the capability of this technology for in vitro stem cell niche generation.

61.P31
Micropatterned co-culture of rat hepatocytes with 3T3 cells using microstencil technique

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Primary hepatocytes have been used for various applications such as liver tissue engineering, and pharmacological and fundamental cell biology studies. For the success of such applications, hepatocytes have to express liver-specific functions at a high level and maintain these functions over a long term. In this study, we investigated the morphological and functional behaviors of the micropatterned co-culture of rat hepatocytes with 3T3 cells.A micropatterned chip was prepared using polydimethylsiloxane microstencil such that the chip contained 724 hepatocyte islands, each 500 μm in diameter, in a triangular arrangement with 800 μm pitch, in which hepatocytes were co-cultured with 3T3 cells. The hepatocytes in micropatterned co-culture exhibited hepatocellular morphology for several weeks of culture by supporting the heterotypic interface between the hepatocytes and 3T3 cells. The albumin secretion activity of hepatocytes was highest in the micropatterned co-culture but decreased in the random co-culture, micropatterned mono-culture (hepatocytes only), and random mono-culture in that order. Furthermore, earlier formation of co-culture promoted higher functional activity of hepatocytes as compared to later formation, and hepatocyte functions were induced with an increasing the density of inoculated 3T3 cells. These findings will provide information useful for designing co-culture conditions.

61.P32
Cell, scaffold and growth factor patterning via 3D printing

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Traditionally, scaffolds for tissue engineering have homogenous, bulk properties which do not vary with location. However, for repair of tissues which have vital-to-function architecture, or for simultaneous repair of multiple tissues, more complex therapies are necessary. The work undertaken here aims to demonstrate that 3D printing is capable of producing scaffolds for these more complex therapies, with mechanical and chemical material properties, cell populations and growth factor signals which are independently patterned in three dimensions. Results so far show that scaffolds can be produced containing both alginate hydrogel and PLGA-based microparticulate scaffold phases, which are suitable for cartilage and bone engineering respectively, raising the prospect of bespoke osteochondral defect repair. Pre-osteoblasts and/or chondrocytes can be incorporated into scaffold materials prior to printing, and show high post-print viabilities of around 80%. Initial studies also show that multiple cell types and multiple scaffold materials can be patterned simultaneously and independently. Ongoing work is focused on further optimising deposition of the microparticulate scaffold material, to ensure adequate mechanical strength for application in bone. In addition, an evaluation is underway of the extent to which patterned growth factor signals are translated into patterned cell behaviour.

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61.P33
Cell LEGO

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New methodology using ssDNA-PEG-lipid as a cell adhesive will be presented to display cells in certain area on a glass plate and to regenerate 3D tissue using cells as building blocks (cell lego). When a ssDNA-PEG-lipid solution is added to a cell suspension, the hydrophobic alkyl chains of the ssDNA-PEG-lipid spontaneously form hydrophobic interactions with the lipid bilayer of the cell membrane. The ssDNA which is presented on the cell surface can be used as an adhesive to immobilize cells on various surfaces. Various patterns can be drawn on glass plates using cells along the patterned aaDNA. Solutions of ssDNA-SHs were printed by an inkjet printer onto a glass plate covered with a gold thin layer. ssDNA-SHs were immobilized onto the surface. Cells with ssDNA'-PEG-lipid, in which ssDNA' was complementary to the ssDNA sequence of the ssDNA-SHs on the surface, were applied to the surface to induce cell attachment through the ssDNA–ssDNA' hybridization. The ssDNA-PEG-lipid was also utilized to immobilize cells on a cell aggregate. ssDNA was introduced onto the surface of HEK293 cells with an ssDNA-PEG-lipid, and ssDNA' was introduced onto the surface of cell aggregates with an ssDNA'-PEG-lipid. Then, the ssDNA'-cell aggregate were mixed with the ssDNA-HEK293 cells. The HEK293 cells were immobilized on the cell aggregate surface through DNA hybridization.

61.P34
Behaviour of human adipose stem cells in microstructured scaffolds

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In vivo, cells are supported by a 3D extracellular matrix (ECM). However, cells are currently cultured *in vitro* on 2D surfaces. To mimic the ECM and the growth supporting environment of the *in vivo* -conditions, well-defined microstructured scaffolds were designed in MATLAB and fabricated from Ormocomp® by a rapid prototyping method of two-photon polymerization (2PP). The scaffolds had a repeating layered structure and a model-based porosity of 96.7%. Human ASCs were seeded on duplicate samples containing two $240 \times 240 \times 120$ μm scaffolds. Cell viability and attachment was evaluated by Live/Dead-staining, and the growth and morphology by bright field, SEM and confocal imaging at each time point (4 h, 2 days and 6 days). In addition, the cell migration into scaffolds was studied using continuous cell imaging. The majority of ASCs were viable at each time point and they seemed to proliferate and migrate inside the scaffolds during the 6 days culture period. At 4 h, the scaffolds were almost empty, at 2 days ASCs grew upwards mainly along the outer scaffold wall, and at 6 days ASCs grew in the middle of the scaffolds in many layers. In addition, SEM images showed that cells formed extensions not only along the scaffold wall but also through the pores inside the scaffolds. Our results on cell viability, attachment growth and morphology propose that well-defined 3D scaffolds fabricated by 2PP are a favorable environment for culturing ASCs.

61.P35
Quantifying spatial variations in cell density in collagen scaffolds: a combined theoretical-experimental approach

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A key requirement of any scaffold is its ability to promote cell function – the material must support cells within a 3D environment that mimics the architecture, biomechanical and biochemical environment of *in vivo* tissue. One option is to seed cells in a collagen gel – plastic compression increases the collagen density (and hence mechanical integrity) to representative *in vivo* values. Such an approach has been developed at UCL, and has been shown to sustain oxygen concentrations at physiological values throughout a scaffold. The current study investigates cell fate spatially in such a construct, using a combination of mathematical modelling and experimental data. Models are presented that track the evolution of the cell population in response to the oxygen concentration field, and also account for cell-cell and cell-scaffold interactions. This is achieved using a continuum framework that explicitly accounts for changes in the volume fraction of cells, fluid and collagen throughout the scaffold. The models are simplified by exploiting the geometry of the scaffold to yield analytical expressions for the cell density as a function of position and time. The model outputs are validated against data on cell viability and proliferation obtained using Live/Dead and Alamar blue assaying at 3 spatial positions in a construct. In this way, a quantitative picture of cell fate (including death and proliferation), and its dependence on the underlying source of oxygen, is developed

61.P36
Ischemia is a prime but not the only cause of hMSC death in tissue engineered constructs *in vivo*

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Local tissue ischemia is a prime cause responsible for the massive cell death in tissue engineered (TE) constructs observed post-implantation. In order to assess the impact of ischemia on the death of implanted hMSCs, which have great potential for repairing damaged tissues, we hereby investigated the *in vivo* temporal and spatial fate of human Luc-GFP-labeled MSCs within fibrin gel/coral scaffolds subcutaneously implanted in nude mice. *In vivo* bioluminescence imaging monitoring and histological analyses of the constructs tested confirmed the irremediable death of hMSCs over 30 days post-implantation. The kinetics of expression of three hypoxic/ischemic markers (HIF-1?, LDH-A and BNIP3) was also monitored. Our results provided evidence that hMSC located within the core of implanted constructs died faster and predominantly as well as strongly expressed the aforementioned ischemic markers. In contrast, cells located in the outer regions of TE constructs were reperfused by neovascularization and were still viable (as evidenced by their *ex-vivo* proliferative potential) at day 15 post-implantation. These results support the explanation that in the central part of the constructs tested, death of hMSC cells was due to ischemia, whereas in the periphery of these constructs, cell death was due to another mechanism that needs to be elucidated.

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61.P37
Bioprinted amniotic fluid-derived stem cells accelerate wound healing in skin

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Amniotic fluid-derived stem (AFS) cells possess similar properties as mesenchymal stem cells (MSCs), such as multipotency, immunomodulation and lack of immunogenicity. MSCs have shown therapeutic potential in treatment of acute and chronic wounds. Given that AFS cells can be obtained less invasively and show greater proliferative capacity in culture, we investigated whether they could also augment wound healing. Full thickness skin wounds were surgically created on the dorsal region of mice and treated by bioprinting a fibrin-collagen gel containing AFS cells, MSCs, or no cells over the wound. At day 0, 7 and 14, AFS and MSC-driven wound closure and re-epithelialization was significantly greater than that of gel-only treatments. Histology revealed increased neovascularization and robust epidermal layers in the AFS and MSC-treated skin compared to gel-only treatments. GFP-labeled AFS cells and MSCs showed that cells were transient, and did not permanently integrate. Staining for von Willebrand factor showed that printed cells did not co-localize with new vessels, despite the neovascular activity. These observations suggest that increased wound closure rates and angiogenesis may be due to delivery of trophic factors, not direct cell-cell interactions. AFS cells perform as well as MSCs in wound closure, and seem to induce greater neovascularization. Therefore, bioprinting of AFS cells might address the clinical need for more effective treatments for large-scale wounds and burns.

61.P38
Bioprinting a hybrid scaffold containing human placenta derived extracellular matrix

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Introduction: Recently, a number of synthetic biomaterials have been bioprinted to three dimensional (3D) scaffolds for tissue engineering applications. However, synthetic scaffolds alone lack biological cues to guide tissue regeneration. Therefore, the objective of this study was to incorporate a human placenta derived extracellular matrix (ECM) (Bhatia MB, Wounds 20, 29, 2008) into a bioprinted synthetic scaffold to create a hybrid scaffold. Methods: To produce the hybrid scaffolds, polycaprolactone (PCL) was first fabricated as a 3D scaffold with a bioprinter (EnvisionTEC). Human placental ECM (Press BH, et al. Poster, TERMIS NA, 2011) was then loaded on PCL scaffolds and dehydrated. In a separate method, ECM slurries and PCL were alternatively printed into each layer of scaffolds with custom designed internal patterns.
Results: The results demonstrated that PCL scaffolds of controlled fiber diameter and pore size could be fabricated using the bioprinter. Both dehydration and bioprinting methods successfully incorporated placental ECM on PCL scaffolds. Additionally, the incorporation of ECM enhanced the ability of scaffolds to support attachment and proliferation of Placenta Derived Adherent Cells (PDAC®), as examined by Calcein AM staining and MTS assay.
Conclusion: These results suggest bioprinting is a promising approach to fabricate hybrid scaffolds that incorporate both natural placental ECM and synthetic components for tissue engineering applications.

61.P39:
3D bioprinting for everybody - application of low-cost 3D printers in tissue engineering

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Introduction: Biological constructs can be manufactured layer-by-layer through 3D bioprinting, in which a cell-loaded hydrogel is deposited to form a predefined structure. For this, materials need to be developed with specific parameters. Especially for viscous hydrogels - which might be indispensable for scaffold stability - many bioprinters experience problems such as material clogging in the tip. To solve such a challenge, open-source printers with modifiable software and hardware are valuable. The purpose of this project was to adapt a commercially-available low-cost 3D printer for bioprinting cell-hydrogel constructs.
Methods: The syringe-based Fab@Home printer was modified with a custom-made temperature-controlled print-head to influence hydrogel rheology. An alginate-hydroxyapatite hydrogel with encapsulated hMSCs was printed, cross-linked with $CaCl_2$, and cultured for 3 days.
Results: Stable 3D printed hydrogel constructs were achieved by modified printer control parameters and usage of the new print-head. Cells survived the printing process and remained viable after 3 days in culture.
Conclusions: A commercially-available 3D printer has been successfully modified for biological usage. Resolution is usually the most limiting factor of low-cost printers; however, the viscosity of our material is here more limiting.
Acknowledgments: We acknowledge Marco Hitz for technical support and funding from the RMS Foundation, Bettlach, Switzerland.

61.P40
A new approach for 3D tissue & organ fabrication inspired from orthopedic surgery

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Fabrication of transplantable 3D tissues or organs *in vitro* is one of the major goals in regenerative medicine. Several scaffold-free systems have been developed to avoid potential side effects caused by scaffold mainly used to build three-dimensional tissue constructs. However, they seemed to be still unable to produce fine structures without contamination from exogenous biochemical materials. Inspired from bone fracture treatments in orthopaedic surgery, we have established a simple method to fabricate 3D scaffold-free cell constructs. This method uses spheroids and temporal fixators which enable placement of various types of three-dimensional cells into desired xyz positions without need of hydrogels or biochemical reactive materials. We also developed a robotic system for scaffold-free cell construction. The prototype can handle two different types of cells and fabricate 10 mm 3 scaffold-free cell constructs. We consider that the simplicity and scalability of this unique system will facilitate its clinical introduction. Near future, with combination of the robotic technology and the bio technology, we may be able to build living organs for autologous transplantation. And this multi-cell construct may be a useful research tool for drug development.

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61.P41
Optimized 3D design and bioprinting of a blood vessel based on autophagy analyses

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In tissues/organs, a complex network of vessels of arteries and veins branching out into capillaries provide nutrients-oxygen and simultaneously remove by-products. Pathologies leading to vascularization abnormalities cause several life threatening diseases. Tissue engineered vessels using scaffolds is one of recent attempt to reconstruct the damaged or diseased vessels. However, the fabricated scaffolds could cause immunogenic reactions and complications during their degradation after implantation. Scaffold-free 3D organ printing is a new and powerful approach in tissue engineering. In this research, 3D-functional tissues were printed using cell aggregates as building blocks (bioink). Here, we developed a novel computer-aided design and 3D printing technique to optimize bioprinting of scaffold-free vascular constructs. Computer-generated scripts directly from 3D computer-aided designs were used to control the bioprinter. Bioink made of fibroblasts and endothelial cells were printed using bio-inert, thermo-responsive hydrogels as support. We optimized 3D path planning and bioprinting techniques essential for engineered 3D tissues and functional units. Furthermore, the effect of 3D printing with various path planning options on stress and death responses of the cells in the bioink and in the printed constructs were analyzed. Consequences of genetic manipulation of these responses were studied to improve post-implantation survival and adaptation.

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62. Tissue Engineered Nutrition

62.01
Framing *in-vitro* meat as art

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Engineered food, and in particular lab grown food, poses a cultural challenge; the evocative language that is used to tell the story of *in vitro* meat indicates the need to develop a more nuanced cultural articulation of the subject. Considering that it is 100 years since A. Carrel started his infamous chicken embryo tissue culture experiment that led to the first envision of *in vitro* meat; most people still seem to find the idea of cells growing outside of a body odd. As part of an ongoing research project into the use of tissue technologies as a medium for artistic expression, the authors grew *in vitro* meat (out of pre-natal sheep skeletal cells) in the year 2000, as part of their research fellowship at the Tissue Engineering and Organ Fabrication Laboratory, Harvard Medical School. Three years later the authors and six other volunteers consumed *in vitro* meat (made of xenopus muscle cells) as part of an art installation/performance titled 'Disembodied Cuisine' in France. The paper will explore new discourses and new ethics/epistemologies that surround issues of partial life and the contestable future scenarios they offer. The authors are now conducting an Interdisciplinary project which concerns with tissue engineered muscle actuators in custom made bioreactors as cultural expression. Tissue engineered muscle actuators has the possibility to grow 'better' *in vitro* meat but can be also used as a case study to examine shifting perceptions of what is alive and 'natural'.

62.02
Skeletal myoblast differentiation on starch microspheres for the development of cultured meat

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The cultivation of skeletal muscle cells *ex vivo* for food purposes has the potential to provide a cultured meat product having significantly reduced environmental impact than conventional meat production. We are exploring the possibility to expand and differentiate muscle progenitor cells on starch microspheres in suspension culture. Starch is an attractive alternative to animal-derived or biopolymer scaffold materials, because it is edible and abundantly available, but needs to be crosslinked and surface functionalized in order to support cell attachment and growth. In this study, we have investigated the influence of surface charge of crosslinked starch microspheres on cell attachment and growth, as well as differentiation of C2C12 myocytes to muscle fibers in this system. The results show that positively charged microspheres, but not negative or neutral microspheres, supported fibroblast and C2C12 cell attachment and growth. Furthermore, C2C12 cells differentiated to myofibers that stained positive for myosin heavy chain under static conditions. Total protein content of cultured cell/starch biomass was within the range measured for hamburger, sausage and steak, while myoglobin levels were a factor of 10 lower, however detectable. These promising results encourage us to proceed in developing this culture system for cultured meat applications further and explore its suitability for up-scaled suspension culture.

62.03
Cultured meat by self-assembly

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Meat production technologies have a number of associated problems, which with increasing demand for meat might become critical. We have developed a new approach to edible meat production that is based on tissue engineering by self-assembly and uses bioprinting. Multicellular units are delivered into a biocompatible support structure according to a design template (compatible with the shape of the desired structure) by a bioprinter. Biological assemblies form post-printing, through morphogenetic processes akin to those in early embryonic development (e.g., cell sorting, tissue fusion). The resulting construct is transferred to a bioreactor for maintenance and maturation to make it suitable for use. We have applied the technology to build three-dimensional tissue and organ structures for purposes of regenerative medicine. Here we describe its adaptation to building meat products for consumption. The technology has several advantages in comparison with earlier attempts to engineer meat *in vitro*. The multicellular units can be reproducibly prepared with mixtures of cells of different type, in particular endothelial cells for vascularization allowing for control in composition that enables the engineering of healthy products of great variety. Printing ensures consistent shape, while post-printing structure formation and maturation facilitates conditioning. Using this approach we have fabricated thin sheets of edible meat which can be folded into aesthetically appealing products.

62.04
Engineering aligned muscle tissue with the incorporation of a vasculature

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Skeletal muscle tissue engineering is a focus of our group with different applications in mind, including cultured meat. During the last years we optimized the engineering of muscle tissues using a hydrogel based system. Importantly, constraining the tissue between two anchoring points led to alignment of the muscle fibers. We further investigated whether we could incorporate a vasculature in the tissues by co-culturing the myoblasts with endothelial cells (ECs). We determined that merely constraining the tissue caused the muscle cells to align and at the same time produce vascular endothelial growth factor. This induced the ECs to organize into vessel like structures in the tissues. We are currently exploring the application of polymer scaffolds made by low temperature electrospinning. We compared the stiffer polymer poly(lactic acid) (PLA) and more elastic co-polymer poly(l-lactide/caprolactone) (PLCL, 70–30 molar ratio). The scaffolds were cut in strips, anchored at both ends and seeded with myoblasts and ECs. The tissues were cultured for 2 weeks, without additional growth factors. The cells remained viable and gene expression analysis confirmed differentiation into myofibers. Strikingly, in contrast to the PLA constructs, in which the myofibers had a random distribution, the myofibers aligned in the direction of constraint in the PLCL scaffold. Although there are indications that the ECs organize into vessel like structures, more studies are needed to confirm this.

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62.05
Enabling technologies for scalable production of *in vitro* meat

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Advancements in traditional agricultural methods may be insufficient to address the global challenge of meat production necessitated by increasing consumer demand and the public health burden associated with animal meat consumption. The industrial production of *in vitro* meat by scalable tissue engineering methods is a proposed solution. We describe a production model involving five principal bioprocess components: (i) isolation and propagation of myoblasts from farmed animals; (ii) serum-free cell culture media formulation; (iii) development of porous spheres from temperature-sensitive edible hydrogels (iv) scalable bioreactor architecture; and (v) mechanical conditioning cycle optimization for maturation of skeletal muscle myofibers. We discuss the potential application of this production model toward the design of neutraceutical foods with preventative or therapeutic benefits in reducing food-borne illness, transmissible infectious disease and metabolic disease.

62.06
Tissue engineering of skeletal muscle and fat for meat production

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Introduction: Medical developments such as stem cell isolation and tissue engineering might be used to grow meat for consumption. It is the aim of this study to produce a proof of concept hamburger that is ready for consumption.
Materials and Methods: Bovine satellite cells (SC, CD56 +) and adipose tissue derived stem cells (ATDCs, pre-plating selection) were extracted from 1 cc of fresh meat. Proliferation in prescribed media was followed by prescribed differentiation protocols. SCs were seeded in a collagen/Matrigel gel and cultured in six wells plates in donut shaped rings. ATDCs were differentiated in a PGL/fibrin scaffold.
Results: SC of bovine origin were efficiently retrieved from fresh skeletal muscle. They could be expanded to ± 30 doublings and sufficient numbers to populate 100 bioartificial muscles (BAMs) while ± 1000 BAMs are required for a hamburger. The average protein content was 78% (w/w). Differentiation of pre-adipocytes led to 80% of the cells turning into fat cells by oil-red-O staining. Matured adipocytes remained captured in the scaffold, forming bioartificial adipose tissue (BAAT). Through additional food preparation techniques, the BAMs/BAATs mixture will form the hamburger.
Conclusion: It is feasible to tissue engineer a combination of muscle and fat from bovine stem cells, thus creating a cultured hamburger. This proof of concept is the first step in optimization and scaling of the procedure towards eventual production of cultured meat.

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63. Extracorporeal Shock Wave Therapy - a New Direction in Regenerative Medicine?

63.01
Keynote: Extracorporeal shockwave therapy (ESWT) – from bench to bedside

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ESWT is used successfully for more than 30 years to disintegrate concrements in the urinary tract. During routine follow-up Gerhard Haupt, a German urologist, observed iliac bone thickening at sites of shockwave passage 6 months after lithotripsy of ureter stones. This was the first time that an interaction of shockwaves with biological tissue was described. Already in 1991 Valchanov published his first experiences treating non-healing fractures by means of ESWT. The first hypothesis of the working mechanism was very mechanical: due to micro lesions created in the exposed tissue reparation processes are induced. However, in 2002 Maier found the most potent osteogenetic effect at energy levels which did not cause any damage. Meanwhile focus is attracted on the biological ESWT effects. Back to 'bench' it could be proven that shockwaves induce production of multiple growth factors by mechanotransduction. Additionally, angiogenesis could be demonstrated in different trials in response to ESWT. Even an effect on migration and differentiation of stem cells was published. Due to the long experience in urology using higher energy levels without observing significant side effects ESWT could develop quickly in new medical fields ('bedside'). In the last years ESWT is successfully used in the treatment of bone healing disturbances, chronic tendinopathies, chronic wounds and burn injuries. Also in the treatment of ischemic heart muscle tissue ESWT could show benefits for the patients.

63.02
Extracorporeal shock waves – a novel method of mechanostimulation in tissue regeneration

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Introduction: Despite clinical success rates from 60% to 90% of extracorporeal shock wave therapy in treatment of acute and chronic wounds, biological influences of shock waves on tissues and bones still remain to be elucidated in detail. Especially *in vitro* approaches may be essential to identify underlying mechanisms of shock wave triggered wound healing.
Materials and Methods: All *in vitro* experiments were carried out with cells subjected to shockwaves using an *in vitro* shockwave trial water bath and the shockwave device dermagold100 (MTS Germany). Readout methods were mainly FACS analysis and ELISA of released cytokines.
Results: Application of the unfocussed shock wave device at medium energies resulted in transient membrane permeabilisation of treated cells observable by uptake and release of polar molecules. Moreover, the mitochondrial membrane potential was restored immediately after treatment without significant reduction of cellviability. In contrast, release of ATP and cytokines was clearly dependent on shock wave energy and the number of pulses applied.
Conclusion: Due to our findings the active process of mechanostimulation rather than transient membrane permeabilisation may be responsible for favorable effects of extracorporeal shock wave therapy in treatment of acute and chronic wounds. This may open a new way for application of shock waves in tissue engineering approaches. Financial support from FFG (# 818412) and City of Vienna is gratefully acknowledged.

63.03
Unfocused extracorporeal shock waves induce anabolic responses in osteoporotic bone

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It was previously shown that non-osteoporotic rats treated with unfocused extracorporeal shock waves (UESW) had higher cortical and cancellous bone volumes and improved mechanical properties. In the current study we examined the effects of UESW in osteoporotic rats. Female Wistar rats received an ovariectomy (OVX). Two weeks after OVX one group received saline (n = 9) and another group received alendronate (n = 9). At 0 weeks 1000 UESW were applied to one hind leg, the other was not treated and served as control. *In vivo* microCT-scanning was used to analyze bone changes. In saline treated rats UESW resulted in higher cancellous bone volume at 2 weeks, but not at 4 and 10 weeks. UESW resulted in higher cortical volume at 2, 4 and 10 weeks with respectively 3.2%, 5.5%, 5.5% more than the untreated control side. In rats receiving Alendronate UESW resulted in higher cancellous bone volume at 2, 4 and 10 weeks. UESW resulted in higher cortical volume at 2, 4 and 10 weeks with respectively 7%, 10.5%, and 12% more than the untreated control side. Large areas of direct bone formation were observed at the cortex and around de novo bone niches in the marrow of UESW treated legs. Intramedullary soft tissue damage, but no periosteal or bone micro damage were observed. In conclusion UESW drastically increase cancellous and cortical bone volume. This study shows promising results for the use of UESW in the treatment of osteoporosis.

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63.04
Myocardial regeneration by low energy shock wave treatment

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Recently we found that epicardial shock wave treatment improves left ventricular ejection fraction (LVEF) in rodent myocardial infarction. The underlying mechanism remains largely unknown. We therefore aimed to clarify mechanisms on cellular and sub-cellular levels. Efficacy of epicardial shock wave therapy was tested in different *in vitro* and *in vivo* models. LVEF markedly improved in shock wave treated pig ischemic hearts 6 weeks after treatment. Quantitative histology revealed significant angiogenesis. Analysis of VEGF1 + 2 receptor phosporylation in human coronary artery endothelial cells showed evidence for direct receptor activation after shock wave treatment. Recruitment of circulating endothelial (progenitor) cells was found in a model of chronic hind limb ischemia in rats. In a subsequent *in vitro* experiment we observed significant migration of endothelial cells after SWT. Inflammation and enhanced proliferation in endothelial cells was modulated via regulation of cytokines IL-6, IL-10 and cyclophilin A and B by stimulation of toll-like receptor 3. Epicardial shock wave treatment induces angiogenesis and vasculogenesis in ischemic myocardium. VEGF receptors are stimulated directly, chemoattractants for recruitment of endothelial cells are released and inflammation is modulated. No adverse effects were found in a large animal model closely resembling human hearts. SWT therefore is deemed suitable for clinical application in ischemic heart disease.

63.05
Extracorporal shock wave therapy (ESWT) in ischemic challenged tissue

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Extracorporeal shock wave therapy (ESWT) has been shown to accelerate tissue repair in acute and chronic wounds, but the mechanism remains incompletely understood. Recently, we showed dose dependent beneficial effects of ESWT on ischemia challenged tissue. Here we wanted to evaluate the influence of ESWT on angiogenesis at different treatment times. Shock waves at 0.1 mJ/mm^2 and 5 impulses/s (total 300 impulses) were applied to the epigastric flap ischemic region at various times pre-, immediately and 24 h post-ischemic insult. The angiogeneic response was evaluated by immunohistochemical means (von Willebrand Factor; smooth muscle actin) as well as *in vivo* vascular endothelial growth factor receptor 2 (VEGF-R2) expression. Flap perfusion as an indirect indicator of functional neovessels was assessed by laser Doppler imaging.Flap perfusion, microvessel number, and survival (through reduced flap contraction and necrosis) were significantly enhanced in the treated groups compared to controls, irrespective of timing of shock wave treatment (pre- versus post-ischemia). VEGF-R2 expression was dynamically up-regulated in response to ESWT. Shock waves induce strong angiogeneic response in ischemic tissue irrespective of treatment time and enhance tissue perfusion thus leading to improved skin flap survival.

63.06
Extracorporeal shockwave treatment induced extracellular ATP release – a potential mechanism to activate wound healing

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Introduction: Extracorporeal shockwave treatment (ESWT), a mode of mechanical stress, has been shown to induce release of ATP from Jurkat T cells *in vitro*. In high concentrations extracellular ATP activates complex signaling responses, which finally increase cell function. In primary human monocytes extracellular ATP has been reported to stimulate VEGF release. Aim of this study was to assess if ESWT triggers ATP release in Jurkat T cells and primary human monocytes leading to alterations in cell function.
Methods: Human Jurkat T cells and blood-derived monocytes were subjected to shockwaves using an in vitro shockwave trial waterbath and the shockwave device Dermagold100 (MTS Germany). Cells were treated with different energy levels and different numbers of pulses. Extracellular ATP and VEGF release was measured in the supernatants of ESWT treated cells.
Results: ATP release after ESWT increases in a pulse- and energy dependent manner. ATP release significantly increases already at a low number of pulses/energies compared to untreated controls. Furthermore first results of ATP induced VEGF release in 2D and 3D culture systems will be presented.
Conclusion: ESWT may promote VEGF release via extracellular ATP, dependent on energy and number of pulses. ATP release and feedback through certain purinergic receptors could be one of the underlying principles of the beneficial effects of ESWT. Financial support from FFG (#818412) and City of Vienna is gratefully acknowledged.

63.07:
Shockwaves induce stem cell differentiation through ATP release and P2X7 receptor activation

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Extracellular ATP signaling is as a key regulator of immune cell function. We reported that shockwave treatment (SWT) modulate T cell function through ATP. Because SWT is clinically used to improve bone healing, we investigated if ATP signaling could contribute to bone formation. Human mesenchymal stem cells (hMSCs) were obtained from healthy volunteers to study whether SWT induces ATP release. SWT caused a dose-dependent release of ATP, reaching in extracellular ATP concentrations of ~7,000 nM. SWT or addition of equal ATP concentrations induced p38MAPK activation, c-fos and c-jun transcription, and mineralization of hMSCs as evidenced by increased alkaline

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phosphatase activity, osteocalcin production, and calcium node formation. SWT and ATP treatment increased the transcription of P2×7-type ATP receptors. Removal of extracellular ATP by addition of apyrase, inhibition of P2-type ATP receptors with KN-62 and PPADS, silencing of P2×7 receptors with siRNA, or blocking p38MAPK signaling with SB203580 abolished these effects of SWT on hMSCs. This indicates that SWT induces cellular ATP release at concentrations that cause autocrine P2×7 receptor activation and downstream signaling responses leading to osteogenic differentiation of hMSCs *in vitro*. We conclude that SWT and other approaches that cause P2×7 receptor activation may have clinical utility in improving bone healing in patients with non- or slow-healing fractures. Supported by grants from the NSF China to TY, XZ, and DZ.

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64. Biofabrication - Platforms for 3D High Throughput Screening and Cell Culture

64.01
Keynote: Bioprinting 3D in-vitro model for tissue science and engineering

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Bioprinting living cells for constructing 3D *in vitro* models represents a new frontier in tissue science and engineering. This presentation will review the salient advance of bioprinting *in vitro* model and its application in broad 3D biology, disease study, drug testing and cell/tissue-on-a-chip. Enabling engineering processes for cell and biologics printing, patterning and assembling will be reviewed. Examples of applying *in vitro* 3D biological models to tissue science and engineering, drug metabolism testing and tumor model study will be presented, along with the update report of newly formed Biofabrication Thematic Group in TERMIS.

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64.02
High throughput stem cell differentiation

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Stem cells are a self-renewing source of differentiated cell types for use in many biopharmaceutical applications. However, high efficiency, cost effective directed differentiation is extremely technically challenging. We describe a high throughput combinatorial technology, Combicult™, that allows tens of thousands of differentiation protocols to be tested simultaneously, greatly accelerating optimal protocol discovery. Combicult™ combines miniaturisation of cell culture on microcarriers, a pooling/splitting protocol and a unique tagging system to allow multiplexing of experiments. Bespoke software utilises criteria such as hierarchical clustering and probability analysis to select optimal protocols for further validation. We present the results of several successful high throughput screens. Novel, serum-free protocols for the generation of cells types relevant for cell therapy and drug development applications (e.g., neurons and hepatocytes) have been discovered. Furthermore, we demonstrate that Combicult™ can be used to discover protocols that are applicable across many cell lines. This is particularly relevant to the study of hES and iPS cells which exhibit significant variability between lines. Combicult™ can greatly accelerate stem cell protocol discovery, efficiently enabling: (i) elimination of variable and expensive components; (ii) improved cell yields; (iii) investigation of stem cell biology and differentiation signaling pathways and (iv) cell line selection.

64.03
In vivo screening of cells in three dimensions enables the discovery of a novel cartilage treatment

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We propose screening *in vivo* as a new strategy to tackle ethical concerns of animal experiments via the first animal-implantable three-dimensional (3D) screening device containing wells made of thermoplastic polymers. When human mesenchymal stem cells (hMSCs) were seeded in the wells, 3D aggregates formed, making wells an ideal carrier for assessment of cells *in vitro* and *in vivo*. We harnessed well volume and culture time to optimize the culture of cells for screening of 36 cartilage-producing conditions involving different numbers of hMSCs and primary chondrocytes (PCs), both as one cell type or in co-culture in one mouse. The results showed positive hits for tissue regeneration including a coculture with 80 : 20 (hMSCs : PCs), which yielded the highest quality of regenerated cartilage according to Bern scores. Upscaling of high hMSCs to PCs ratios in scaffolds with interconnected pores confirmed the higher efficiency of 90 : 10 and 80 : 20 ratios with respect to glucosaminoglycans (GAG/DNA) and GAG/%PCs. Subsequent introduction of mononuclear cells (MNCs) to scaffolds, to replace the hMSCs, provided further preclinical evidence supporting a new cartilage regeneration technology, where 90 : 10 (MNCs : PCs) ratios achieved the regeneration of hyaline cartilage in a one-step surgery. The *in vivo* screening of cells and compounds opens an entirely new range of experimental possibilities to increase the pace of biomedical discovery while reducing the extensive use of experimental animals.

64.04
Melt electrospinning in a direct writing mode

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Fibrous tissue engineering scaffolds are an increasingly important part of regenerative medicine, with recent interest generated in the solution electrospinning of nanofibers. However it is difficult to control the arrangement of solution electrospun fibres due to the chaotic nature of the electrospinning jet. The electrified jet of non-conductive polymer melt, however, is more predictable in its path. Lines of melt electrospun fibres can therefore be written on a moving stage, and the process considered a new additive manufacturing technique. Such melt electrospinning writing can be performed with translating flat or with rotating cylindrical collectors. Cell-invasive scaffolds were fabricated from polycaprolactone and were flat or cylindrical, with filaments between 2 and 25 μm. For any given condition, there was a minimum translation or rotating speed required to generate a straight fibre. Importantly, the process allowed controlled spacing between fibres at different orientations which can be used to control the pore size, geometry and interconnectivity throughout the thickness of the scaffolds. Electrospinning writing can therefore make microporous scaffolds with centimetre lengths, millilitre volumes and micron level accuracy, operating in an

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automated and continuous manner to reproducibly fabricate scaffolds. Electrospinning writing is emerging as a distinct technique to produce ordered and intricate biomedical devices and cell invasive scaffolds for TE constructs.

64.05
Fabrication and assembling of hydrogel fibers into 3D bunchy constructs for engineering cardiac tissue

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Modular approach which assembling micro-tissues into the desirable constructs has attracted great attention of researchers. In this study, hydrogel fibers as novel modules were fabricated and assembled into aligned 3D bunchy constructs using in engineering cardiac tissue. In brief, sodium alginate and fibrin were mixed together as the host material. Hydrogel fibers were fabricated through the self-developed 'extrusion-in-winding' process in which alginate-fibrin mixture with cardiocytes was continuously extruded in rotating $CaCl_2$ solution and turned into fibers collected with a receptor. After immersed in endothelial cell suspension till a layer of cells was built on the surface, the fibers were assembled into aligned bunchy construct. When the parameters were optimized, the slender fibers with a diameter of 150~250 μm were successfully built. Cardiomyocytes were encapsulated in the fibers and over 90% were survived during the extrusion process. And endothelial cells formed a layer on the surface of fibers. In further study, the special-designed cyclic stretching training will be performed on the fabricated constructs to improve cell alignment. Endothelial cells on adjacent fibers were proposed to connect with each other during culture, generating the micro-channels with an endothelial layer among fibers in the constructs. This study provided a novel technique for the fabrication of aligned tissue and presented a great potential for the vascularization of engineered tissue.

64.06
Development of a manufacturing system for cell sheet production

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Autologous transplantation of multilayer myoblast sheet is emerging as a new technique for curing myocardial infarction. This method canovercome the disadvantages such as less take ratio of transplanted cells through the direct injection of myoblast suspension. The sheet of myoblasts also has ability to source the cytokines which improves heart function due to paracrine system including the facilitation of angiogenesis and the attraction of progenitors on affected part. Many researches have been tackled concerning cell source exploring, cell culture, sheet assembling, and *in vivo* animal tests. In manufacturing, the comparison of management between cell processing facility (CPF) and cell aseptic processing system (CAPS) based on the isolator system revealed that CAPS leads to reductions of the running cost as well as operational laboriousness in the small production. Then, we, recently, conducted the design of manufacturing facility to develop the automation system for sheet manufacturing with flexible modular platform (fMP). The isolator system for the sheet assembly made automated formation of multilayered sheets and their incubation. The machinery operations were successfully performed. And this system can realize some procedures by having flexible connections with various modules required for the culture operations under sterile conditions, suggesting the broad versatility for the production in other types of multilayered sheets.

64.P01
Semi high throughput mechanobiological screening of MSCs and ECs for vascularized bone tissue engineering

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Introduction: Vascularization is a bottleneck in tissue engineering. We have pioneered the inclusion of endothelial cells to form a vascular network before implantation. We hypothesize that mechanical stimuli can further optimize this strategy. However, limited quantitative data on the effect of these stimuli on cells is available. Here we show a semi high throughput screening system to study the effect of surface strains, fluid shear stress, and a combination of the former two on the cells that are used in pre-vascularized bone.
Methods: For the application of surface strains to cells, a PDMS membrane stretches over a NOA-81 pillar when negative pressure is applied to the membrane. In a separate setup, fluid flows over the same membrane at varying velocities. Finally the two setups will be combined to apply both mechanical stimuli simultaneously.
Results: A working first stage device has been designed. ANSYS modeling has shown that by varying pressure or the surface of the membrane where the pressure is applied, different strain distributions can be achieved. Fluid dynamics modeling has shown that using a varying width fluid flow channel, different shear domains can be achieved. Using these variables, multiple conditions can be tested for on a single chip.
Conclusion: The design for a device to expose cells to a variety of mechanical signals in a systematic, quantifiable way has been made. A working first stage prototype is currently being validated.

64.P02
A bioinformatics approach to assay development: selection of a cell type to study the interaction between cells and bone graft substitutes

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The biological response, handling and mechanical properties of currently available bone graft substitute materials and implant coatings require further optimization. This may be realized via systematic and high throughput screening (HTS) of biomaterial-induced cellular responses. However, advances are hampered due to a lack of adequate *in vitro* testing models and methods. To this end, we will establish an in vitro HTS system by correlating biomaterial-induced bone formation of 24 known materials *in vivo* with *in vitro* gene expression profiles of cells cultured on these materials. Hence, markers predictive for *in vivo* osteoinductive biomaterial performance will be identified and used in a HTS system to evaluate a broad range of materials with inherently different properties. Initially, two pilot DNA microarray studies were performed to determine the most appropriate time point and cell type for this HTS based on gene expression profiles. We compared six different human cell lines, which demonstrated that the osteosarcoma derived cell line MG63 responded most robustly, reflected in its gen-

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ome wide expression profile, to three well characterized ceramic materials (hydroxyapatite, biphasic calcium phosphate and tricalcium phosphate). By defining these variables, the basis is set to identify a set of markers predictive for *in vivo* material-induced bone formation, which is the first step towards the development of a HTS system to screen *in vitro* the osteoinductive potential of materials.

64.P03
Effect of the surface density of nanosegments immobilized on culture dishes on *ex vivo* expansion

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Hematopoietic stem and progenitor cells (HSPCs) are multipotent cells that have the specific capacity to self-renew and differentiate into all type of mature blood cells. Intravenous infusion of HSPCs has been commonly performed to treat patients suffering from hematological disorders and malignant diseases after radiation and/or chemotherapy. Umbilical cord blood (UCB) is an attractive source of hematopoietic stem and progenitor cells (HSPCs) for HSPC transplantation. However, the low number of HSPCs obtainable from a single donor of UCB limits direct transplantation of UCB to the treatment of pediatric patients. In this study, we investigated the *ex vivo* expansion of HSPCs cultured on biomaterials grafted with several nanosegments, i.e., polyamine, fibronectin, RGDS, and CS1 (EILDVPST), at several surface densities. No direct correlation was found between fold expansion of HSPCs and physical parameters of the culture dishes, i.e., surface roughness and water contact angle of the culture dishes. However, a small amount of grafted amino groups, less than 0.8 residual μmol/cm^2, on the dishes was effective for the *ex vivo* expansion of HSPCs. A high amount of grafted amino groups hindered the *ex vivo* expansion of HSPCs on the dishes. HSPCs cultured on dishes with a high concentration of CS1 (2.40 residual μmol/cm^2) showed greater expansion of HSPCs and more pluripotent colony-forming units (i.e., colony-forming unit–granulocyte, erythroid, macrophage, and megakaryocyte(CFU-GEMM)

64.P04
Combinatorial Cell Culture (Combicult™) of non-adherent cells using encapsulation

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Combicult™ is a high throughput technology which allows tens of thousands of combinations of media compositions to be tested simultaneously in small volumes. We have used this technology to discover effective protocols for the differentiation of stem cells into many terminally differentiated cells including neurons, chondrocytes, hepatic-like cells and others. Our original bead based version of the technology is best suited to cells which adhere to a substrate. In order to extend the use of the technology to non-adherent cells, we have adapted an encapsulation technique which effectively traps the cells inside spheres and allows them to be used in the same manner as cells attached to beads. We will show how we have successfully used this technology in combinatorial screens to discover new protocols for the differentiation and maintenance of non-adherent cells, such as those from various hematopoietic lineages.

64.P05
Gelatin as scaffold for tissue engineering

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The term Cell Sheet Engineering refers to the procedure for which sheet of cells produced on a substrate are used to promote in situ regenerative processes. This method has been implemented by Okano et al. who demonstrated that by using a thermoresponsive polymer substrate, such as poly-N-isopropylacrylamide (PNIPAAm), the formed cell sheet can be easily detached from the substrate just by decreasing the temperature of a few degrees. In this work we have investigated the use of a gelatin hydrogel as substrate for promoting the formation of a cell sheet for cell sheet engineering. The procedure exploits the fact that the used gelatin melts at 37 °C, allowing the detachment of the cells cultured below that temperature when gelatin melts. The solution of gelatin (1 : 6 in culture medium) was cast into a culture plate and aged at room temperature, 100% relative humidity for 24 h and then at 29 °C, 100% RH for 48 h. After aging, cell culture (MRC5) at 31 °C on the gel surface started. In 24 h cells attached to the surface starting to spread, forming a uniform web of cells in 48 h and a confluent layer within one week. This cell sheet was easily transferable, as a print, to any other surface (materials, tissue or organ) kept at 37 °C. In our tests, we transferred it to the bottom of a polystyrene Petri dish and verified that cells were viable and active. We also checked the production of Laminin, Fibronectin and Focal Adhesion Kinase with Confocal Laser Microscopy.

64.P06
Thermo-responsive hydrogel for the generation of implantable cardiac progenitor cell (CPC) sheets for scaffold-less tissue engineering

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Introduction: Conventional tissue engineering (TE) strategies based on biodegradable scaffolds loaded with cells showed drawbacks mainly related to scaffold degradation. To overcome these limitations, cell sheets made by cardiac progenitor cells (CPCs) were generated by the use of a thermo-responsive methylcellulose hydrogel (MC-gel) which allows to harvest cell layers simply by lowering the temperature.
Methods:
Isolation and characterization of CPCs: CPCs, isolated from human atrial biopsies by cell migration and then enriched for the expression of the stemness marker c-kit by means of immuno-magnetic selection, were characterized for their immunophenotype and multipotency.Generation of cardiac progenitor cell sheets: hCPCs were seeded on MC-gel, cultured until confluence and then detached by lowering the temperature to 10 °C for 20 min. The harvested cell sheets were evaluated for the expression of Connexin 43.
Results and Conclusion: hCPCs expressed stemness/mesenchymal markers at different levels (c-kit 90%, CD90 80%, CD105 85%, CD44 100%, abcg2 1%), few cells were positive for early cardiac markers (GATA-4 and MEF2C) and all were negative for cardiac sarcomeric proteins. Intact cell sheets were recovered with functional cell to cell connections (mature Connexin 43 at cell membrane). CPCs-derived cell sheets could find application in heart TE. Moreover, MC-gel can be mould with micropatterns of defined topography aimed at direct cell spatial organization.

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64.P07
Fabrication of micro-tubular tissue structures using microfluidic gel formation system

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Bottom-up tissue fabrication methods using microtissue units as building blocks are powerful tools to reconstruct complex tissues. Here, we developed a method to construct vascular-like micro-tubular tissue structures. Biocompatible alginate micro-gels that show sol-gel changes were used to form microtube structure. Alginate microgel fibers used in this study were fabricated with a coaxial laminar flow micro-fluidic device. We used sodium alginate aqueous solution as an inner flow and $FeCl_3$ aqueous solution as a sheath flow to form Fe-alginate gel fibers. The continuous flow of sodium alginate solution is cross-linked by the diffusion of Fe^{3+} ions from the outer $FeCl_3$ aqueous solution. This micro-fluidic device allowed to generate Fe-alginate gel fibers with a uniform diameter from 100–300 μm by varying the flow rate ratio. On the formed Fe-alginate gel fibers, endothelial cells easily attached, spread and proliferated around the gel fibers as shown in the cell behavior on the tissue culture polystyrene dishes. After cells reach confluency on the gel fiber, gels are dissociated by addition of alginate lyase, resulting in successful formation of micro-scale tubular tissue structures. We believe that our approach would facilitate vascularized tissue formation and be useful for fabricating complex tissue structures by transporting nutrients and metabolic substances through the micro-tubular structures within the reconstructed 3D tissues.

64.P08
Dictation of cell orientation throughout three dimensional hydrogels

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Introduction: In nature, feedback-loops between cells and their ECM act as a guide to develop diverse tissue types. Cell cytoskeletal morphology is closely related to ECM deposition/organisation. In this study, we devise a novel technique that applies portable aligned nanofiber meshes to hydrogel via a layer-by-layer method, creating a highly organized cell population throughout 3D hydrogel, which explores a feasible technique toward constructing tissues with highly organized architecture to mimic the native cell niche.
Materials and Methods: 2% Poly-L, D-lactic acid was dissolved in chloroform and dimethylformamide and electrospun. A novel composite collector produced highly aligned and free standing, thin sheets of nanofibers. These were combined with collagen type I hydrogels to produce a 3D layered architecture. Primary bovine nucleus pulposus (NP) cells were cultured within the construct and their organisation was monitored by confocal microscopy.
Results and Discussion: Highly aligned nanofiber meshes (Ä 500 nm/fibre) were produced. 3D constructs were constructed by applying nanofibre sheets in a layer-by-layer manner, each layer ± 60°. After cell seeding to aligned nanofibers, the whole construct was stablized by collagen hydrogel. Cells orientated in response to the local nanofiber orientation and this has been demonstrated through the thickness of the construct. The low density of nanofibers enabled cell infiltration within the hydrogel.

64.P09
Encapsulation of mesenchymal stem cells (MSCs) with sodium cellulose sulfate (SCS) and poly-diallyl-dimethyl-ammonium chloride (pDADMAC) using vibration technology

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Peripheral vascular diseases represent a major global health problem. Stem cell such as mesenchymal stem cells (MSCs) may promote vascular regeneration. However, current limitations of stem cell therapy include the poor rate of engraftment, limited cell survival after transplantation, and high cost of cell manufacture. The use of biomaterials to encapsulate cells, overcomes these problems, and is likely to improve the therapeutic outcome. Our aim was to develop a microcapsule of Sodium Cellulose Sulfate and poly-diallyl-dimethyl-ammonium chloride (pDADMAC) to encapsulate MSCs. This strategy would enhance the retention of the cells in the transplanted area. In addition, it would protect cells from the host immune system. Encapsulation of MSCs was achieved using vibration technology. The control of several parameters allows the production of microcapsules with desired sizes (200–1000 μm diameter). Morphology and ultrastructure of microcapsules was determined by light microscopy and scanning electron microscopy. The determination of the molecular weight cut-off (MWCO) of the microcapsules suggests that paracrine factors secreted from the encapsulated MSCs will cross the capsule membrane. Further, encapsulated MSCs were viable up to 2 months under normoxia and hypoxia *in vitro* culture conditions. These results suggest that encapsulation of MSCs can be achieved, and it might be a potential option for cell transplantation and delivery of therapeutic products to ischemic tissue.

64.P10
Unification of cell cycle with cytocompatible and reversible-forming polymer hydrogels

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We prepared cytocompatible polymer hydrogels as a matrix with reversible property for encapsulation of functional cells without reduction of their activities. A water-soluble phospholipid polymer, poly(2-methacryloyloxyethyl phosphorylchoine-co-*n*-butyl methacrylate-co-*p*-vinyl phenylboronic acid) (PMBV) can reversibly bind to poly(vinyl alcohol) (PVA) under a biological condition. The PMBV/PVA hydrogel can reversibly encapsulate cells. The functions of cells, including ES cells, in the PMBV/PVA hydrogels were investigated. Storage modulus of the hydrogel could be changed by the PMBV ratio. Cells in the hydrogel were still active after 7 days storage in the room temperature. The proliferation rate of the cells depended on the storage modulus of the polymer/cell complex. The cells proliferated and formed spheroids when the storage modulus of the complex was around 700 Pa. It is indicated that there is a suitable storage modulus for cell proliferation. When the cells immobilized in hydrogel, the storage modulus increased. The cell remodeled its environment in the hydrogel. When the cells did not proliferate, ca. 80% of the cells became G1/G0 phase in 12 h, and it became more than 90% after 2 days. Moreover, the PMBV/PVA hydrogel sphere could be prepared in a microfluidics to encapsulate a single cell in one hydrogel sphere. We will discuss the cell function control by encapsulation with the PMBV/PVA hydrogel to provide good cell resources with uniform cell cycle.

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64.P11
Bone and peripheral nervous system interactions within bone tissue engineering perspective

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The neuro-osteogenic network is a topic of interest and research in skeletal biology. To unravel the cross-talk between bone cells and the peripheral nervous system, namely nerve fibers within the bone microenvironment, we established a novel coculture system for osteoblasts (OB) and dorsal root ganglia (DRGs) based on microfluidic devices. The microfluidic devices are mainly used in neuroscience research since it allows the segregation of somal from axonal part of the neurons. As first step, we adapted these devices to allow the coculture of DRGs with non-neuronal cells, namely OB. To improve the readout of the axonal outgrowth measurements, we performed an adjustment in the microfluidic chamber to align the DRGs in equal distance relatively to the microgrooves. Subsequently, to expand the appliance of the system to studies within the tissue engineering (TE) perspective, we merged the microfluidic system with 3D culture. The extracellular matrix of the bone microenvironment was simulated using different materials for the OB culture compartment and different coatings for the DRGs compartment. In such way, we were able to assess the neurites outgrowth within different materials used for TE strategies. In the scope of bone regeneration, this system proved to be an advantageous protocol for studying the cellular and molecular interactions between peripheral nervous system and bone cells and emerges as potential new tool to be explored within TE perspectives.

64.P12
Versatile biomaterial scaffold platform for critically-sized tissue constructs

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Introduction: We present a versatile silk-based 3D scaffold platform that incorporates tunable oxygen and nutrient delivery conduits. The high level of control over the physical and biological properties of this platform allows for the fabrication of 'tailor-made' scaffolds for a range of tissue engineering purposes, with particular emphasis on scaffold pre-vascularization, integration, full regeneration and engineering of tissue interfaces.
Methods: Silk only scaffolds and blends with silk fibers or tropoelastin were frozen in an isotropic or anisotropic system in the presence of linear wire arrays. Hollow channels formed by linear wire arrays were functionalized with collagen and laminin. Cell infiltration, compartmentalization and channel endothelialization were investigated.
Results: Scaffolds with randomly distributed or aligned pores of different sizes were fabricated. Morphological, mechanical and degradation properties were controlled through silk processing and blending with silk fibers and tropoelastin. Hollow channels with diverse morphologies and cell-interactive properties served as nutrient and oxygen delivery conduits and allowed scaffold pre-vascularization with endothelial cells. Scaffold supported infiltration of mesenchymal and myoblast cells.
Conclusions: We have developed a unique scaffold platform with highly tunable features for 'tailor-made' tissue engineering.

64.P13:
Engineering cell sheets with electrochemical reactions on membrane culture substrate

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Introduction: Engineering cellular building blocks such as cell sheets without scaffold is a promising approach for tissue engineering applications. Here, we report that cell sheets can be electrochemically detached and stacked to fabricate a multi-layered thick cell sheet.
Methods: The oligopeptide, CCRRGDWLC, was designed to contain an arginine-glycine-aspartate (RGD) domain in the center and cysteine at both ends. Because of a thiol group of cysteine, the oligopeptide was chemically adsorbed onto a gold layer on a membrane substrate via a gold-thiolate bond. After cells were grown to form a cell layer. The gold-thiolate bonds were reductively cleaved by applying a negative potential, and the cell sheet was detached along with the desorption of the peptide.
Results: In a quantitative analysis of the electrochemical detachment of single cells, ~90% of adhering cells were detached from the membrane substrate within 5 min. In addition, because of the oxygen supply through the membrane, cells grew and formed a 50 μm-thick cell sheet at 14 days of culture, which was significantly thicker than that in a conventional culture dish. This approach was further applicable to stack them to fabricate ~200 μm-thick multilayered sheets.
Conclusion: Thick cell sheets were prepared with an electrochemical manner. This cell sheet engineering could be a promising approach for engineering transplantable thick cell sheets.

64.P14
Anisotropic cell sheet for three-dimensional design of cell orientation

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Some parts of native tissues have well-organized cell/ECM orientations, and the anisotropy produces important functional consequences. Therefore, an appropriate anisotropy needs to be designed for reconstructing complex tissue. To date, though a variety of microfabricated surfaces have been developed, cell orientation can be controlled two-dimensionally on these surfaces. However, in the regenerative medicine field, tissue is required to be constructed three-dimensionally. Tissue-like cellular monolayer 'cell sheet' has been developed for establishing a new class of tissue reconstruction technology. Thermoresponsive cell culture substrates allow a cell monolayer to be harvested as a cell sheet with associated ECM by reducing culture temperature (e.g., 20 °C). For example, oral mucosal epithelial cell sheets have been already applied to human clinical studies for corneal reconstruction. In this study, micropatterned thermoresponsive surfaces achieved to organize cell orientation and allowed the aligned cells to be manipulated as an anisotropic cell sheet using a gelatin-coated plunger. Because transferred cell sheets were able to maintain their cell orientation, the anisotropic cell sheets were successfully layered with maintaining individual orientations, indicating that cell orientations were designed three-dimensionally. Based on this new technology, complex tissue that requires control of its anisotropy is potentially reconstructed for the future regenerative medicine.

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64.P15
Thermoresponsive block copolymer-coated surfaces for optimizing cell sheet fabrication

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Thermoresponsive surfaces were prepared via a spin coating method with poly(*N*-isopropylacrylamide)-b-poly(*n*-butyl methacrylate) (PIPAAm-b-PBMA) with various block lengths on tissue culture polystyrenes. Grafted PIPAAm amount and thickness of coated polymer layer were estimated by ATR/FT-IR and ellipsometry, respectively. Amounts of PIPAAm chains ranged from 0.7 to 1.8 $\mu g/cm^2$. Thickness of polymer layers were ranged from 7 to 23 nm. For investigating temperature-dependent cellular behavior, bovine carotid artery endothelial cells (BAECs) were seeded on the various polymer-coated surfaces, followed by incubation at 37 °C. After the 3 days culture of BAECs, the cell adhering culture surfaces were incubated at 20 °C, and then the cells were observed microscopically and/or visually for various time periods. Thermally induced adhesion and detachment profiles of BAECs were significantly affected by polymer layer thickness, grafted PIPAAm amount, and PIPAAm chain length. By adjusting the properties of polymer coated surfaces using various PIPAAm-b-PBMA block copolymers, BAECs effectively adhered and proliferated on the polymer coated surfaces at 37 °C, and then adherent cells spontaneously peeled off from the surfaces by reducing temperature below PIPAAm's LCST. In addition, block polymer coated surfaces allowed the confluently cultured cells to be harvested as the cell sheets with their basal extracellular matrix proteins by low-temperature treatment.

64.P16
Oxygen-permeable honeycomb microwell sheet-based formation and size-dependent functional evaluation of 3D pancreatic beta-cell and hepatocyte aggregates

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Reorganized 3D spherical aggregates are very good tissue models because they maintain organ-specific functions better than 2D monolayer cultures. However, we need to carefully consider the aggregate size because they are not vascularized and thus various metabolites should be transported by diffusion as opposed to *in vivo* situations. To address this issue, first we proposed size-controlled formation of pancreatic beta-cell and primary rat hepatocyte aggregates with direct oxygenation using oxygen-permeable polydimethylsiloxane (PDMS) honeycomb microwell sheet. Oxygen supply from the bottom enabled rapid aggregate formation even if the inoculum density was eight times higher than conventional monolayer culture. Second, we investigated size-dependency of organ-specific functions. Properly-organized but smaller insulinoma aggregates showed rapid insulin release against glucose stimulation as well as monolayers did but the released amount was higher than that in monolayers. Organization of hepatocyte was also effective for better and higher albumin secretion and cytochrome P450 activity. However, resorufin (a hepatic metabolite of ethoxyresorufin) was accumulated preferentially within hepatocyte aggregates as opposed to the case in monolayers. As such, efficient and size-controlled formation of organ cell-derived aggregates using PDMS microwells would be a powerful tool in optimizing aggregate sizes for analyzing the metabolic profiles as well as their rates.

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65. Biomechanics and Modelling of Engineered Musculoskeletal Tissues (in coop. ESB-Biomechanics)

65.01
Keynote: Biomechanics of meniscal replacement

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Due to the high mechanical demands to tissue engineering products for musculoskeletal tissues the mechanical properties of biomaterials play an important role in designing scaffolds used for this purpose. Taking the example of meniscus tissue engineering it can be shown that the design of scaffolds for partial or total meniscus replacement is an extreme challenge. The natural meniscus is a complex and highly anisotropic structure with a variety of different components like collagen, proteoglycans, water, and different cell types secreting this extracellular matrix. Furthermore, the surface of the meniscus contains special proteins that help to minimize friction. The collagenous structure with circumferential fibres in the periphery together with the meniscotibial attachments make the meniscus a highly effective structure in reducing articular contact pressure. Hence, when designing a replacement implant, all these specific characteristics have to be considered. Current concepts for meniscal replacement e.g. use porous polymers like polyurethane or silk fibroin, which allow cell ingrowth. However, it yet has to be shown whether such concepts are highly effective in preventing articular cartilage from premature degeneration. Vascularization of the interface between implant and scaffold is another important topic in the field of meniscus tissue engineering. Here, some experimental concepts have been previously introduced but not yet entered clinical practice.

65.02
Keynote: Multiscale mechanics and mechano-biology for bone and bone tissue engineering

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Modern computational engineering science allows for reliable design of the most breathtaking high-rise buildings, but it has hardly entered the fracture risk assessment of biological structures like bones-tissue engineering solutions aiming at their regeneration. Is it only an engineering scientists' dream to decipher mathematically the origins and the evolution of the astonishingly varying mechanical properties of hierarchical biological materials? Not quite: In recent years, we have succeeded, through novel, experimentally validated micromechanical theories, in predicting tissue-specific elasticity and inelasticity of bone and scaffold materials, from the "universal" mechanical properties of the nanoscaled elementary components (hydroxyapatite, collagen, water), their tissue-specific dosages, and the "universal" organizational patterns they build up. Moreover, we extend cell population models of contemporary systems biology, towards biomineralization kinetics, in order to quantify evolutions of bone mass and composition in living organisms. When using these evolutions as input for the aforementioned micromechanics models, the latter will predict the mechanical implications of biological processes. This will open unprecedented avenues in tissue engineering-supported bone disease therapies, including patient-specific bone fracture risk assessment relying on micromechanics-based Finite Element analyses.

65.03
Simulation of the effect of mechanics on cell viability during degeneration of the intervertebral disc

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The link between cell death within the intervertebral disc (IVD) and degeneration needs to be fully understood. Cell viability in a transport IVD model coupled with IVD mechanics has been implemented to study how deformation affects both transport of metabolites and cell death, depending on tissue properties. A finite element model of the L4-L5 IVD based on poromechanics and diffusion of glucose, oxygen and lactate metabolically coupled (Malandrino et al. 2011 PLoS Comp Biol) included an exponential decay of cells over time below a critical glucose concentration. Degeneration was simulated by decreasing IVD height, subtissues fluid contents and initial cell densities and by increasing solid-phase stiffness. Daily compressive loads (standing, resting) were applied. The boundary supply of metabolites was reduced to induce critical glucose. Solutions obtained with degenerated and healthy properties were compared. In the healthy IVD, deformation couplings increased glucose so that cells stopped dying up to 12 h earlier when mechanics was considered. In the degenerated IVD the mechanical effect was negligible. Deformability of healthy IVDs seems thus beneficial for cell viability while an accelerated cell death may occur with degeneration due to the loss of compliance. This novel mechanobiological study highlights the restoration of both cellular and mechanical factors and has a potential impact for regenerative treatments. DISC-REGENERATION (NMP3-LA-2008-213904) is acknowledged.

65.04
Non-label morphology-based detection of accumulated irregularity in cultured cells

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Change in cellular morphology is still the only non-label practical method to control and monitor cells during the in vitro expansion culture for regenerative medicine. To widely distribute the advances in regenerative medicine, technological development to quantitatively automate these manually maintained processes is in urgent need. However, to define the precise 'quality loss' or 'bad quality' for the process automation is nearly impossible. This is because there are numerous markers and various definition theories that cannot be selected to define 'the bad quality'. Even with single event, such as tumorigenesis risk, there are many types of risks. Therefore, in our work, we have focused to detect the 'irregularity' accumulates in the culture process of cells, such as abnormal growth of morphologically irregular cell population. By combining the fully automatic phase contrast imaging system, BioStation CT, and the bioinfomatic modeling technique, we succeeded in detecting artificial irregularity triggered by siRNA to knockdown p53. Although there are many successful reports on siRNA screening approaches using fluorescent images, we found that non-label morphology-based modeling is effective to detect the p53 knockdown event in early stage of cell culture.

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65.05
Fluid and mass transport modelling to drive the design of cell-packed hollow fibre bioreactors

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A significant challenge in bioreactor development is tailoring both the design and operation to the requirements of the cell type under consideration. This requires the provision of operating equations that enable an end-user to design the bioreactor geometry and set its operating conditions to obtain the desired cell culture environment. Here we define these operating equations based on mathematical models of fluid flow and nutrient delivery to a cell population seeded in a hollow fibre bioreactor (HFB). HFBs use a controlled fluid flow coupled with diffusion to augment mass transport. Fluid flow is described by the Navier-Stokes or Darcy equations, whereas mass transport requires advection-diffusion-reaction equations, where the reaction term captures uptake of nutrients by the cell population. These equations are reduced by exploiting the small aspect ratio of a fibre, and small reduced Reynolds number for the flow. Analytical solutions enable operating equations to be derived that describe the dependence of the minimum oxygen concentration on the bioreactor dimensions and operation (flow rates, pressures, seeded cell density).We parameterize the models using experimental data for two specific cell types (cardiomyocytes and chondrocytes), and present operating data for each of these examples, as well as an experimental validation. E.g., opening a port to enable radial flow through the HFB allows a 333% increase in the depth of the cellular region for cardiomyoctes.

65.P01
Development of bone scaffolds with controlled permeability and stiffness

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In the last years there has been a significant improvement in Bone Tissue Engineering with the development of new bone substitutes. When designing the scaffold for the substitute, one of the most determinant factors is its microstructure since it influences not only the mechanical properties but also the ability for cells to penetrate the scaffold and for nutrients, oxygen and waste products to diffuse through the scaffold. Effective permeability has been used as a characterization of scaffold mass transport properties, as it measures quantitatively the ability of a porous medium to conduct fluid flow and it depends on the combination of porosity, interconnectivity, pores size, orientation and tortuosity. In this work it was developed a computational tool to optimize scaffolds' microstructures with controlled permeability and elasticity, using homogenization techniques. Using this computational model it was possible to obtain different geometries depending on the deformation field applied and the importance given to each property which, in future applications, can represent different clinical situations. Some of the designed scaffolds were built using rapid prototyping techniques and tested in vivo in order to study their performance in terms of bone regeneration and in order to validate the proposed computational method. The results show this computational approach may be extremely useful in scaffold design for Bone Tissue Engineering.

65.P02
Toward organ printing: design characteristics, virtual modeling and physical prototyping branched segments of kidney arterial vascular tree

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Organ printing is defined as the layer by layer additive biofabrication of 3D tissue and organ constructs using tissue spheroids as building blocks. The design of the vascular tree is a critically important step in practical implementation of organ printing technology. Bioprinting a vascular tree requires detailed knowledge of the morphometrical, morphological and biomechanical characteristics of the sequentially branched segments of the natural vascular tree as well as insight on post-printing tissue compaction and remodeling. We surveyed these characteristics of the initial segments of the natural kidney arterial vascular tree of the porcine kidney. Computer simulation was used to model compaction of tissue engineered tubular vascular segments with different wall thickness virtually biofabricated from closely packed and fused uniformly sized vascular tissue spheroids. The number of concentric layers of tissue spheroids required to bioprint tubular vascular segments with desirable wall thickness and diameter was theoretically estimated. Our results demonstrate that vascular segment compaction correlates well with reported experimental data. Finally, physical prototyping of linear and branched tubular constructs using physical analogs of tissue spheroids was performed. Thus, virtual and physical prototyping provides important insights into the design parameters and demonstrate the principal feasibility of bioprinting a branched vascular tree using vascular tissue spheroids.

65.P03
3D tissue engineering scaffolds and computational design

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Tissue engineering utilizes porous biomaterial scaffolds to deliver biological factors that accelerate tissue healing. These two functions require scaffolds be designed for mechanical loading and mass transport. The purpose of this paper was to apply both ad hoc and topology optimization homogenization based design approaches to create scaffold architectures, and to determine how these architectures compare to theoretical bounds on effective stiffness. Open cell scaffold architectures demonstrated a wide range of permeability, but were all below isotropic effective stiffness bounds. Wavy fiber architectures provide a means to approach the lower bounds. Using image-based techniques, designed architectures may be incorporated in anatomic shapes. In this research paper we will discuss about our newly developed software that is compatible to use in electrospinning machine to achieve 3D scaffolds with any polymers regardless of its shape, size, physical and chemical properties.

65.P04
Quantitative deconvolution and tracking of differentiating stem cells

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Quantitative image cytometry is possible only if the relative intensity of deconvolved images is preserved. For this reason, relative intensities

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present in fluorescent images are compared before and after processing via deconvolution. We have investigated deconvolution algorithms/softwares performance with respect to retention of relative intensities. Although deconvolved images show improved contrast quality, in all but one method, relative intensities before and after deconvolution are not retained. Following deconvolution, migration and differentiation behavior of cells are tracked. A potential source for cardiac repair is induced pluripotent stem cells (iPSCs). Here we study the formation of cardiac cells from the time-lapse movies of iPSCs by segmentation and tracking techniques. We used image cytometry tools to track nuclei expressing H2B-mcherry, and GFP (for cardiac lineage) in differentiating iPSCs. At first, we performed time lapse microscopy for long-term imaging of live iPSCs at various stages of differentiation. Following image acquisition, we deconvolved the images of fluorescently labeled iPSCs and then applied segmentation and tracking to locate the spatial and temporal positions of iPSCs nuclei as the cells migrate and differentiate.

65.P05
Why do tendon repairs fail? A computational approach

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Severed tendons require close reapproximation to heal, and early active mobilisation of the digit improves clinical outcome. Tendons are repaired using suture, however, techniques with sufficient strength to withstand early active mobilisation present greatest resistance to tendon glide. The most appropriate suture method is currently unknown. A finite element (FE) modelis being developed to simulate active mobilisation of a tendon repaired with four common suture methods; locking Kessler, Savage, Tsuge and locking Cruciate. This will allow observations of stress concentrations, compression and the gap between tendon ends, which would cause re-rupture, ischemia and poor healing respectively. The model requires full characterisation of tendon material properties. Literature will be used to describe tendon in the direction parallel to its long axis. However, tendon is anisotropic and its tensile behaviour tangential to its long axis is not well documented. We have performed transverse tensile testing of ex vivo porcine cadaver fore-trotter flexor digitorum profundus (FDP) tendon, which will complete the FE material characterisation. Ex vivo tensile testingof suture repaired porcine cadaver fore-trotter FDP tendonswill validate the FE model. The validated model will identify the suture technique which provides the most favourable conditions for healing. This FE method can be used in developing novel tendon repairs which will improve tissue healing and clinical outcome.

65.P06
Deciphering the role of matrix metalloproteinase and extracellular matrix changes in the development of in-stent restenosis using a multiscale mechanobiological model

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Stent induced arterial injury due to surgical interventions initiates a complex cascade of inflammatory events within arterial wall which ultimately lead into the development of intimal hyperplasia and arterial stenosis. Computational modelling can be used as a means to model the biological response of arteries to different stent designs using mechanobiological models whereby the mechanical environment may be used to dictate the growth and remodelling of vascular cells. Changes occurring within the arterial wall due to stent induced mechanical injury, specifically changes within the extracellular matrix have been postulated to be a major cause of activation of vascular smooth muscle cells and the subsequent development of in-stent restenosis. In this study a mechanistic multiscale mechanobiological model of in-stent restenosis using finite element models and agent based modelling is developed which allows quantitative evaluation of the collagen matrix turnover following stent induced arterial injury and the subsequent development of in-stent restenosis. Specifically, the model is applied to study the influence of stent material properties and geometrical parameters such as the deployment diameter and stent strut thickness on the level of in-stent restenosis.

65.P07
Morphology-based prediction of differentiation potential of mesenchymal stem cells

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Morphology of cells had long been used as an important indicator of cell quality. However, such experience-based morphological importance has not been well quantitatively examined in the aspect of automation for industrialization. For the cell therapy, such non-invasive technique to evaluate and assess the quality of cells is strongly required for its industrialization with the growth of stem cell research and its achievements. Human bone marrow-derived stem cells (hBMSCs) had been widely studied and applied to clinical cell therapies with their multipotency. However, it is also known that such stem cells require highly skilled cell culture to carefully maintain their undifferentiated status, and to differentiate into the objective type of cells. In our research, we introduced bioinformatic machine learning strategy to build a prediction model, which links 'the cell morphology information' and 'the experimentally determined differentiation results' of hBMSCs.By modeling information from the time-lapse phase contrast images of more than 2,000 images of the continuously passaged hBMSC (over 8 passages), the experimentally defined staining results of differentiations could be predicted successfully. Also from the microarray analysis, we further investigated the gene expression patterns that relate to the morphological prediction performance.

65.P08
Influence of mechanics on microcarrier cell expansion: a computational study

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New tissue engineering strategies try to accurately control cell fate in the earliest stages of in vitro tissue culture. Expanding cells on porous microcarriers and combining these carriers to form aggregates allows for threedimensional cell growth while preventing necrosis due to mass transport limitations. The influence of the introduction of these microbeads on the mechanical microenvironment is still poorly understood. However, it has been shown that the mechanical conditions of cells can have a large influence on cell fate and survival. We developed a computational software framework for individual cell-based models of three-dimensional cell cultures. The model simulates the proliferation of ATDC5 progenitor cells on microcarriers. The mechanics of the cells are modeled by the Johnson-Kendall-Roberts potential and the equations of motion of the cells are calculated for a friction-dominated system. The cell cycle is broken up into two stages: increase of cell volume and cytokinesis. The computational model is used to study the

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sensitivity of the microenvironmental parameters to process design variables for a theoretical system with the physical properties of in vitro microcarrier cell cultures. Simulations reveal that both mean compressive stress and heterogeneity in mechanical stress levels are strongly dependent on the adhesive properties of the substrate.

65.P09
Consistent quasi-static and ultrasonic elasticity determination of PLLA-based rapid-prototyped tissue engineering scaffolds

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This study is concerned with reliable and physically sound elasticity determination of rapid-prototyped tissue engineering scaffolds made of poly-l-lactide (PLLA), with and without small portions of tricalcium phosphate (TCP) inclusions. At the level of overall scaffolds, i.e. that of several millimeters, multiple uniaxial loading-unloading (quasi-static) tests were performed, giving access to the scaffolds' Young's moduli, through stress-strain characteristics during unloading. Additionally, acoustic tests with 0.05 MHz frequency delivered an independent access to elastic properties, in terms of the normal components of the scaffolds' stiffness tensors. The latter strongly correlate, in a linear fashion, with the Young's moduli from the unloading tests, revealing porosity- independence of Poisson's ratio. The magnitude of the latter is in full agreement with literature data on polymers. Both of these facts underline that both ultrasound tests and quasi-static unloading tests reliably provide the elastic properties of tissue engineering scaffolds.

65.P10
Level set based design optimization framework for active scaffolds

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Introduction: Level-set based approach unlike standard topology optimization can overcome computational cost and local minima problems in computational scaffold synthesis. An effective and versatile computational design tool based on the level set method is developed for active porous scaffolds that can deliver biological agents on demand by applying external electromagnetic (EM) fields.
Methods: FEA based optimization framework based on the level-set method is built on commercial software COMSOL to allow for automated updates of the scaffold material satisfying EM actuated release and strength requirements. Frechet derivative is used with adjoint sensitivity on the MATLAB scripting tool. A level set function interpolated into a fixed initial domain of the scaffold material is evolved by using the Hamilton-Jacobi equation to determine the optimal shape.
Results: Results validate sensitivity calculations of EM and mechanical analysis models. Despite convergence challenges, by dynamically adjusting the artificial damping term, well defined active scaffold topologies for desired strength and release performance resulted in well defined microstructural porous layouts with interconnectivity.
Conclusions: Numerical results show that level set based topology optimization method is powerful and flexible in handling complex shape changes for active scaffold performance improvements promoting its use in applications with various metrics from chemistry, fluid flow, and nano-optics.

65.P11
Experimental and computational model of a microfluidic platform for micromass generation and culture

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A number of works have studied microscale systems for 3D cell culture and tissue modeling in high-throughput regime. In microfluidics, geometrical features and shear stresses have been shown to modulate cell docking and cellular aggregate formation. We hereby present a device developed through an integrated computational/experimental approach for controlling micromass generation by non-geometrical parameters. A PDMS microfluidic device with a conical chamber was developed to generate human articular chondrocyte micromasses and CFD simulations were used to predict flow and velocity profiles. Cells were seeded under several conditions, by tuning cell concentrations; flow rates (FR) and seeding times. Micromasses, generated without the traditional centrifugation step, were cultured at different FR for 14 days and evaluated both biochemically and histologically. Time, flow and cell concentration were confirmed to control the number of seeded cells within the chamber. The difference between experimental and simulated cell docking was less than 10%. The micromass shape resulted dependent on the applied FR, and spherical shape was only achieved at the highest FR tested (500 μl/min). Preliminary results in a 4×4 microfluidic array platform showed the feasibility of high-throughput micromass generation. Non geometrical parameters can control cell seeding and micromasses geometry, hence adding a powerful tool for the investigation of microscale tissue models.

65.P12
Micro CT-based multiscale elasticity of double-porous (pre-cracked) hydroxyapatite granules for regenerative medicine

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Hundred micrometers-sized porous hydroxyapatite globules have proved as a successful tissue engineering strategy for bone defects in vivo, as was shown in studies on human mandibles. These granules need to provide enough porous space for bone ingrowth, while maintaining sufficient mechanical competence (stiffness and strength) in this highly load-bearing organ. This double challenge motivates us to scrutinize more deeply the micro- and nanomechanical characteristics of such globules, as to identify possible optimization routes. Therefore, we imaged such a (pre-cracked) granule in a microCT scanner, transformed the attenuation coefficients into voxel-specific nanoporosities, from which we determined, via polycrystal micromechanics, voxel-specific (heterogeneous) elastic properties. The importance of the latter and of the presence of one to several hundred micrometers-sized cracks for realistically estimating the load-carrying behavior of the globule under a typical two-point compressive loading (as in a "splitting" test) is shown through results of large-scale Finite Element analyses, in comparison to analytical results for a sphere loaded at its poles: Use of

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homogeneous instead of heterogeneous elastic properties would overestimate the structure's stiffness by 5% (when employing a micromechanics-based process as to attain homogeneous properties)—the cracks, in comparison, weaken the structure by one to two orders of magnitudes.

65.P13
An advanced cone-and-plate reactor for the in vitro-application of shear stress on adherent cells

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Blood contacting engineered tissues need endothelial cell lining. Endothelial cells (ECs) are permanently exposed to the blood flow and the resulting shear stress, its magnitude varying with the EC site in the blood stream. Along with other mechanical stimuli like vessel wall stretching or hydrostatic blood pressure, this shear stress modulates the endothelial cell function, morphology and gene expression. Here, we describe our improved cone-and-plate reactor that applies up to 10 dyn/cm^2 uniform wall shear stress on a defined, ring-shaped region on a culture dish on different cell lines. At the same time, a hydrostatic pressure of up to 195 mmHg can be applied by increasing the atmospheric pressure in the incubator box. Gas composition can be controlled additionally, used for maintaining CO2-homeostasis or inducing hypoxic conditions. For better comparability, six cone-and-plate systems can be used at the same time at different rotational velocities. The effects on cell morphology, cytoskeleton and cell alignment can be monitored during application using a laser scanning microscope. Flow conditions have been studied and a sufficient area of uniform wall shear stress of 1 to 10 dyne/cm^2 could be shown. To exceed 10 dyne/cm^2, we suggest an increase in medium viscosity.

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66. Animal Models for Preclinicals and Clinical Translation

66.01
Keynote: Animal models for translational research

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Translational research focuses on the development of scientific and medical discoveries to applications in the clinic. This development is usually facilitated by validation in vivo using appropriate animal models. The use of animal models is the only method to demonstrate proof of concept, feasibility, applicability, safety and efficacy of the technology being developed. Although the animal models may not recapitulate all the responses prompted by the human body, they remain as an essential medium that provides forecast of possible responses in patients. Therefore, proper selection and use of animal models that mimic physiologic and pathologic conditions in humans are necessary. In this session, an overview of animal models used in translational research will be discussed. In addition, considerations associated with the selection of animal models and design of in vivo studies will be discussed.

66.02
A "sensate" surface replacement scaffold can monitor loading during cartilage regeneration

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Introduction: Currently available techniques for cartilage regeneration were developed for focal injuries. Our aim was to develop a 'sensate' medial condyle surface replacement scaffold to anchor autologous adult stem cells (ASC's) and facilitate cartilage regeneration while monitoring in vivo joint loading to investigate healing and rehabilitation strategies.
Methods: Scaffolds were prepared with Quickslice and a Stratsys FDM, out of polybutylene terephthalate. Strain gauges were attached to bone interfacing surfaces and wired to a transmitter to measure in vivo joint forces. Scaffolds were seeded with autologous ASC's 48 h prior to implantation and placed into young dogs weighing 25–30 kg. Measurements were collected and activities monitored for 6 months. Joints were harvested to determine tissue quality and quantity. Tissue growth was tested using mechanical indentation and mechanical properties were compared to controls for statistical significance. Condyles were prepared for histomorphometry and analysis was carried out using Image J.
Results: Axial loads were up to 2x BW and shear forces up to 20% of BW. Tissue properties on scaffolds were similar to control cartilage. Histology showed extensive bone growth and tissue coverage with 15% demonstrating joint cartilage structure.
Discussion: The surface replacement accurately measured loads and is a useful tool to develop rehabilitation techniques. Histology indicated good tissue formation. SUPPORTED BY NSFCMMI 08.

66.03
Pre-treatment by mesenchymal stromal cells in rat kidney transplantation induces adverse immune responses

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Mesenchymal stromal cells (MSC) have shown immunomodulatory potential including partial tolerance induction by pre-treatment of donor-specific cells in a rat heart transplantation model. Therefore, we investigated an analogous MSC pre-treatment in a rat kidney transplantation model with a strong MHC mismatch.While transplanting DA to LEWIS, 2 million responder- or donor-specific MSC were given i.v. 4 days prior to transplantation under mycophenolate mofetil immunosuppression for 7 days. Grafted kidneys and responder spleens were harvested at postoperative day 8–9 and examined by quantitative PCR and immunohistology. Sera were screened for alloantibodies by flow cytometry. The application of donor- and responder-specific MSC resulted in elevated humoral immune responses verified by enhanced intragraft B cell infiltration and complement (C4d) deposits compared to PBS treated controls. Moreover, both MSC treated groups displayed moderate signs of inflammation and rejection with slightly higher intragraft mRNA expression levels for TNFα and ICAM. Donor-specific MSC showed significantly higher levels of IgG alloantibodies relative to the PBS- or responder-MSC-treated controls. A higher mRNA expression level for MHC class II and elevated B cell proportions in these spleens corroborated the results.MSC administration before kidney transplantation triggers immune responses or even alloantigen pre-sensitization, thereby worsening the general graft outcome.

66.04
Treatment of congenital birth defects in sheep using tissue engineering: 10 years single institute experience

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Introduction: Different sheep models have been used to improve the treatment of congenital birth defects using tissue engineering techniques. Several congenital defects were prepared in sheep models and reconstructed using collagen-based biomaterials in the pre- and postnatal period. According to the 3R's (replacement, refinement and reduction), adaptations to the protocols were made in previous protocols in order to re-use (reduction) and improve the animal welfare (refinement).
Materials and methods: Pregnant sheep underwent fetal surgery around 79 days' gestation. A neural tube defect, gastroschisis, bladder exstrophy or skin defect was created surgically in the fetal lamb and

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reconstructed using different collagen-based materials in both the fetal and neonatal period.
Results: In total 172 surgical procedures have been performed in 163 pregnant sheep for 12 different study protocols. The average success rate for these experiments was 65%. During the pregnancy period the overall fetal survival rate was 74%. There were five maternal deaths due to complications, which were mainly caused by abdominal wound dehiscence.
Conclusion: The sheep model is a well suited large animal model to perform fetal interventions (e.g. to create congenital defects) and to study the use of collagenous scaffolds for the treatment of these severe defects. With the refinements of our protocols, the number of animals could be reduced and the welfare of the laboratory animals was enhanced.

66.05
In-vivo osteoinductivity assays for bone grafting materials

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This presentation provides a review of in-vivo osteoinductivity models for experimental as well as commercially available bone grafting products. Dr. Marshall Urist first coined the term 'osteoinduction' in 1965 when he discovered that demineralized bone matrix (DBM) generated bone in the muscle pouches of various animal species. Since that time, a variety of in-vivo models have been proposed to validate a material's osteoinductivity by verifying its ability to grow bone where it would otherwise not grow (e.g. a heterotopic or ectopic location). In-vivo osteoinductivity models published in scientific literature, regulatory data, and commercial data were surveyed. The majority of in-vivo osteoinductivity data has been obtained from DBM products; but assays have also been carried out on biologics (e.g. products containing growth factors and/or cells) as well as synthetic biomaterials. Ectopic implantation into an athymic rodent model has become widely accepted as a validation of osteoinductivity, however other animals models have been proposed to verify this attribute. This presentation will provide an overview of animal models, implantation sites, time points, and analyses that have been used to assess the osteoinductivity of experimental and commercially available bone grafting materials.

66.P01
Cell sheet-based fabrication of tumor-bearing model mice

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Tumor-bearing mice were prepared by transplanting cancer cell sheets, compared with the injection of cell suspension. Luciferase gene-coded mouse lung squamous cancer cells were cultured with thermoresponsive culture dishes. By reducing temperature to 20°C, the confluent cells were harvested as intact cell sheets. Then, cell sheets were transplanted on the subcutaneous of BALB/c nu/nu mice. Cell suspension obtained by trypsin digestion was subcutaneously injected on the back of mice as the control. Tumor growth was estimated from tumor volume and luciferin emission measured by in vivo imaging system. Though the formation of small tumor tissues were observed on the backs of mice transplanted as the cell suspension, luciferin emissions were scarcely observed from these tissues except for one mouse. From histological studies, most tumor tissues were determined as granulation tissues, not cancer. For the cell sheet transplanted mice, tumors were emerged on their backs from day 5. The tumor volumes and luciferin intensities increased gradually for all the mice transplanted as cell sheets. Obtained tumor tissues were positively stained by a marker for squamous cancer cells. Cancer-bearing mice can be fabricated by cell sheet transplantation, because cell sheets possess their basal extracellular matrix proteins and thus connect with host tissues effectively. Thus, cancer cells were supplied with sufficient oxygen and nutrition from host tissues to develop tumor tissues.

66.P02
Anticoagulation therapy in a sheep model for vascularized tissue engineering

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The sheep is a widely used large animal model in the field of bone and esp. vascularized tissue engineering as well as in cardiovascular surgery. While sufficient anticoagulation is essential to prevent thromboembolic events in (micro)vascular animal studies, no standardized scheme for sheep has been developed so far. To establish a reliable anticoagulation regime we investigated the effect both of three antiplatelet drugs through aggregometry and of enoxaparin-natrium through antifactor Xa activity over 7 days (n = 5; 1: acetylsalicylic acid [ASS] 500 mg p.o./500 mg i.v.; 2: clopidogrel 75–350 mg p.o.; 3: ticagrelor 90–360 mg p.o., 4: enoxaparin-natrium 1–5 mg/kg; all semidaily). Besides in vitro testing the antiplatelet effect of ASS, analysis of clopidogrel metabolism was performed.ASS failed to inhibit sheep platelet aggregation in vivo and in vitro. While clopidogrel was only effective in 2/5 sheep, clopidogrel and one of its metabolite were identified in every plasma sample. Ticagrelor inhibited platelet aggregation in 1/5 sheep. Therapeutic antifactor Xa levels were achieved with enoxaparin-natrium, but lamb required higher dosages than adult sheep. Our results indicate that high dosages of clopidogrel and ticagrelor can inhibit platelet aggregation merely in several sheep despite sufficient resorption of clopidogrel. Efficient anticoagulation can be ensured using age-dependent doses of enoxaparin-natrium, thus making the sheep model more reliable in vascular TE studies.

66.P03
Establishment of a new ischemic excision model

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To test therapeutic schemes improving blood perfusion in a preclinical setting, we developed a delayed wound healing model caused by insufficient perfusion of the wound area. To induce ischemia, an abdominal flap (8 × 8 cm) was harvested. After ligation of an unilateral caudal epigastric bundle the flap was sutured back and wounds (ø1.5 cm) were created in the ischemic and non-ischemic side of the flap. Wounds were analysed by a planimetric software (Lucia G1) and expressed as a percentage of the total postoperative wound surface area. Laser Doppler imaging was performed to confirm ischemia in the corresponding area. Histological and immunohistochemical staining were performed to follow new vessel formation. Animals were divided into three groups (n = 6–8) according to the treatment of the excisions: Group VEGF (100 ng human VEGF-165 in 1 ml fibrin sealant), group fibrin (1 ml fibrin sealant) and group sham (controls). Laser Doppler imaging revealed enhanced perfusion over 7 days in all groups, with an insufficient perfusion in the ischemic area. After 7 days all wound areas decreased compared to day 0. Wound healing in vital areas occurred

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faster than in ischemic areas, with up to 50% smaller wounds (sham group). VEGF treated wound closure was slower than in fibrin or sham group. Histology and immunohistochemistry need to be analyzed to follow new vessel formation and reveal differences in wound contraction and reepithelialization.Supported by EC-1011014483 Angioscaff (FP7).

66.P04
Reproducible animal model for the study of anal sphincter dysfunction for regenerative medicine

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Purpose: Although internal sphincter resection (ISR) can avoid permanent colostomy in patients with lower rectal cancer, it causes anal sphincter dysfunction, resulting in a life-long, debilitating disorder due to incontinence of stool. Regenerative medicine should be able to improve this condition, however, no good animal experimental model has yet been established that accurately shows the low internal anal sphincter pressure. This study tried to establish and evaluate the novel animal model with anal sphincter dysfunction.
Methods: Twenty male Sprague-Dawley rats were allocated into sham operation, and ISR groups. The ISR group underwent removal of a left semicircle of both internal and external anal sphincter. Both groups were evaluated for their resting pressure at before surgery and on postoperative days (POD) 1, 7, 14, and 28. Anal pressures were recorded and statistically analyzed.
Results: The sham operation group recovered baseline anal pressure on POD7. The ISR group showed a significant decrease in anal pressure on POD1 ($P < .005$), and low pressure continued until POD28 ($P < .001$). The defect of the anal sphincter muscle was confirmed histologically in the ISR group.
Conclusion: The immediate recovery of anal pressure is one of the problems in conventional sphincter dysfunction animal models. However, the novel model has continuous anal sphincter dysfunction for one month and may contribute to further study of the efficacy of therapies involving regenerative medicine.

66.P05
The biology of the ovine functional spinal unit

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There is a need for the development of in vitro models that incorporate natural tissue for the pre-clinical testing of novel therapies within the functional spinal unit (FSU). The sheep spine is a close biomechanical match to human spine and was selected for model development. The aim of this study was to characterise intervertebral discs (IVDs) and facet joints from the cervical, thoracic and lumbar regions of sheep spines aged 1, 3–4, 5–6 and 8–10 years in order to determine the age of the animal that would provide healthy and degenerate tissues. Whole FSUs were scanned using micro computed tomography. The morphology of the IVDs and facet tissue was recorded using photography and histology. Grading systems were used to determine the extent of gross morphological degeneration in the facets and histological degeneration in the IVD and facets. Differences were found between all age groups. Cartilage defects on the surface of the facets increased with age which was supported by cartilage thinning, trabeculae thickening and calcification. The IVDs became thinner and fibrous with age and displayed features of lamellar splitting, clefting and cell necrosis. The IVD and facets only began to show signs of degeneration at 3–4 years of age. Therefore, FSUs from sheep up to 3–4 years of age could be used in healthy models and FSUs from sheep 6–10 years of age in degenerated models for the in vitro assessment of early interventions in the spine.

66.P06
In vivo evaluation of muscular regeneration treated with human mesenchymal stem cells (HMSCs) from the umbilical cord matrix and thrombin in a rat anterior tibial myectomy model

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Introduction: Stem cells may prove to be a new source of cells for cell therapy in functional and morphologic regeneration of muscle, by replacing the degenerated cells, by producing growth factors or by modulating the inflammatory response. We tested in vivo the application of HMSCs isolated from the Wharton's jelly associated to a biocompatible vehicle derived from human thrombin to induce muscle regeneration in a rat anterior tibial myectomy model.
Methods: HMSC from Wharton's jelly umbilical cord matrix were cultured and the karyotype of these cells was evaluated at metaphase before in vivo application. Sasco Sprague rats were used and after a standardized 5 mm diameter myectomy lesion of the tibial anterior muscle, the defect was filled with different preparations: a cellular suspension containing 1×106 HMSCs and thrombin (MSCTb Group); conditioned media combined with thrombin (ConditionedTb Group); a ControlTb and a Control Group with and without the addition of thrombin to the lesion. At day 15 the muscles were collected and immunohistochemistry markers were used as well as myotube counting for evaluation of the inflammatory and regeneration status.
Results and conclusion: HMSCs associated to thrombin presented promising results in terms of morphologic and functional recovery of the rat anterior tibial muscle after myectomy. Further testing will be performed with different times, in order to assess detailed information about distinct stages of muscular regeneration.

66.P07
Multimodal adhesion prevention in a rabbit adhesiolysis reformation adhesion model

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This study compares effectiveness of a liquid instillate (4% icodextrin, ICO) and a polyethylene glycol polymer barrier (PEG), alone and combined, to prevent adhesions in a rabbit adhesiolysis reformation model using adhesion area, prevalence, and severity. On day 0, 30 cm^2 of the cecum was abraded and a 15 cm^2 partial myectomy of the transverses abdominis was performed. On day 14, adhesions were lysed, and treated based on random group assignments. On day 28, adhesion contact area was measured and severity was scored by a single observer blinded to the treatment group. Adhesions were totaled and analyzed as surgical site and nonsurgical site adhesions. ICO was used as a diffuse adhesion barrier administered initially as an intraoperative irrigant then as a postoperative instillate, 50 ml. PEG was used as a localized adhesion barrier and sprayed onto tissues, 0.1 ml/cm^2. ICO combined with PEG statistically reduced adhesion area significantly at surgical and nonsurgical sites relative to controls, and reduced prevalence and severity of adhesions the most. PEG alone statistically reduced adhesion area significantly at nonsurgical sites relative to controls, and reduced prevalence and severity of adhesions the greatest at the surgical site. The synergistic effect of a diffuse liquid, 4% icodextrin, and

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solid adherent barrier, PEG, is an innovative combination that warrants further clinical investigation for multimodal adhesion prevention.

66.P08
Surgical porcine myocardial infarction model through permanent left circumflex artery occlusion

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Purpose: To develop and validate a surgical porcine myocardial infarction model.
Methods: All pigs underwent a thoracotomy and the left circumflex artery (LCx) was ligated ~3 cm from its origin from the left coronary artery. Model Development: Pigs were euthanized on day 2 ($n = 7$), 14 ($n = 3$) and 28 ($n = 3$). Troponin I was measured on days 2 and 7, and histology on days 14 and 28. Model Validation: rhVGEF ($n = 5$) or buffer ($n = 5$) was injected into the myocardium in the area supplied by the LCx 10 min post-occlusion. Echocardiography and histology were performed on day 28.
Results: Model Development: Troponin I increased to 4.8 ng/ml on day 2 returning to baseline by day 7. Infarct size was 10%, 7% and 8% of the total left ventricle area on days 2, 14 and 28. Model Validation: Although infarct size was similar in buffer and rhVEGF groups (8.7% vs 8.9%), absolute fractional shortening decreased 9.3% (23.5%Δ) in the buffer group whereas rhVGEF only decreased 3.0% (10.4%Δ) compared to baseline. Absolute ejection fraction decreased 15.5% (22.8%Δ) in the buffer group whereas rhVGEF only decreased 9.0% (17.5%Δ) compared to baseline. These functional changes correlated with a greater total number of blood vessels in the affected area in the rhVGEF group (9.6 MM) vs. buffer (6.3 MM).
Conclusion: The surgical porcine myocardial infarction model described herein detected functional and histological improvements associated with rhVGEF treatment and may have utility in evaluating other therapeutics.

66.P09
Tissue engineering approach for vertebral arch regeneration in a fetal sheep myelomeningocele model. Preliminary results

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Introduction: Myelomeningocele (MMC) is a congenital malformation characterized by a neural tube closure defect and secondary traumatic and chemical injury of the exposed elements. Amniotic fluid (AF) has emerged as a valuable source of fetal stem cells. These cells have shown potential applicability in fetal tissue engineering strategies, a promising alternative to surgical reconstruction during the gestational period.
Materials and methods: Ovine fetuses ($n = 9$) underwent two surgery procedures: Creation of MMC lesion at day 75 of gestation and, at day 95, spinal defect coverage. During surgical creation of MMC, AF was collected to isolate and ex vivo expand ovine AF mesenchymal stromal cells (AF-oMSCs). The animals were assigned to three different groups according to the treatment tested: (1) AF-oMSCs imbibed in a platelet-poor plama (PPP) gel, (2) AF-oMSCs assembled with Demineralized Bone Matrix (DBM) and PPP and (3) PPP scaffold mixed with DBM. At full term of gestation (day 150), the lambs were delivered by cesarean section, euthanized and histologically examined.
Results: The application of AF-oMSCs in a PPP scaffold did not appear to stimulate bone regeneration. When DBM was added within the mixture, de novo bone formation was observed at the covered area. The application of DBM with no presence of AF-oMSC resulted in re-absorption of the DBM pieces.
Conclusion: Combination of AF-oMSCs and DBM-based scaffold could be a promising approach for prenatal MMC repair.

66.P10
Comparative studies on esophageal glands in animal models for regenerative medicine applications

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Background: This study aimed to investigate different animal models with regard to EG and to identify tissues appropriate for esophagus tissue engineering.
Methods: Esophagus was obtained from ovine, avian, bovine, murine and porcine sources and investigated with regards to the presence of EG. Histological specimens were stained using HE to locate EG in the upper, middle and lower portions of each esophagus. If EG were identified, the tissue was further investigated using PAS and AB to determine the presence of neutral or acidic glands. AB in 3 pH levels (0.2, 1.0 and 2.5) was then used to detect the presence of sulphated mucous.
Results: The esophagus sourced from murine, bovine and ovine sources was devoid of EG. In avian esophagus, acidic and sulphated mucous producing EG were identified throughout the esophagus with larger EG located cranially. On the other hand, in porcine esophagus predominantly neutral and sulphated mucous producing EG with isolated pockets of acidic and sulphated mucous producing EG were identified in the cranial and middle areas of the esophagus. Distance of EG to lumen ranged from 127–340 μm (avian) to 916–983 μm (porcine). EG consisted of 35% (avian) to 45% (porcine) area of the submucosa. EG had an area of 125 000 qμm (avian) to 580 000qμm (porcine).
Conclusion: The results of these investigations demonstrate that porcine esophagus is optimal for EG investigation in terms of scaffold development for tissue engineering.

66.P11
Autologous and heterologous adipose derived stem cells seeded on oligo(polyethylene glycol) fumarate hydrogel scaffold are effective in the regeneration of critical osteochondral defects in minipig model

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Adipose-derived stem cells (ASCs), with their wide availability and multidiffferentiative potential, may represent an efficient tool for bone

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and cartilage regeneration. In this study ASCs were isolated from seven adult minipigs, expanded in culture and used in association with a oligo(polyethylene glycol) fumarate hydrogel scaffold (OPF) for the regeneration of critical osteochondral defects. Four defects (diameter 9 mm; depth 8 mm) were made on the peripheral part of the trochlea in each animal and treated with OPF scaffold which was pre-seeded with autologous or heterologous ASCs or used as cell-free. Empty defects were considered as negative control. Each isolated ASCs population was also tested in vitro. Six months after implantation, all samples showed quite satisfactory filling of the lesions, with the exception of one animal, whose joint appeared to be infected at the moment of harvesting. Good osteointegration was also observed by MRI evaluation. As revealed by histological and immunohistological analyses, OPF+ASCs constructs were able to promote a complete regeneration of the subchondral bone and a cartilage-like tissue. No differences were observed between autologous and heterologous constructs, thus supporting the possibility of an allogeneic use of this cell source. These results demonstrated that ASCs-OPF constructs has the potential for being considered a treatment option for the regeneration of osteochondral defects.

66.P12
Recellularization of a natural intestinal matrix using chicken chorioallantoic membrane as an in vivo bioreactor

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Aim: The chicken embryo chorioallantoic membrane (CAM) has been widely used as an in vivo model to investigate its proangiogenic action on acellular matrix. In this study we evaluated cellular migration into an acellular intestinal scaffold.
Methods: After ethical approval, fertilized GFP+ chick eggs (n = 39) were incubated at 37°C at constant humidity. After 8 days of incubation, 1 mm diameter acellular matrices from rat small intestine were placed on the CAM between branches of the blood vessels. Grafts were examined daily and until 10 days following placement. Immuno- and histochemical analyses using DAPI, GFP, Cytokeratin-8 (Cyt8), smooth muscle actin (SMA) and haematoxylin and eosin (H&E) were performed. Slides were then visualized using confocal microscopy.
Results: H&E analysis demonstrated cellular migration from the CAM into the graft, beginning at day 5 reaching its maximum at day 10, with cells moving from the periphery of the scaffold adjacent to the CAM towards the centre. Immunopositivity for DAPI and GFP confirmed viable cells originating from the CAM. However, no cellular differentiation was evident, since Cyt8 and SMA were negative.
Conclusion: The present study is, to our knowledge, the first demonstration that the CAM system can be used for testing cellular migration and proliferation in a rat intestinal matrix. Also, GFP+ CAM represents an efficient model for studying chemotaxis and could be the first step for an in vivo bioreactor.

66.P13
Clinical modeling for lateral mandibular body reconstruction: initial results from a pig mandible model

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In combat casualties, the majority of the facial fractures are to the mandible (36%). The frequency and complexity of these injuries requires novel approaches to improve the outcome for mandibular battle wounds. The objective of this study is to validate a large animal critical size defect (CSD) model for assessing biomaterials prior to clinical trials. Bilateral non-healing size defects were created in each of the mandibular bodies of five mature mini pigs. All defects were plated, and each animal had one defect treated with autograft, and the other left untreated (negative control). Evaluation with computed tomography (CT) imaging will be completed at 8 and 16 weeks; with post harvest μ-CT and histological assessment at 16 weeks. This design allows evaluation of post operative complications, jaw stability, and the surgical site's healing potential with and without the clinical 'gold standard', autologous bone graft. Initial results indicate that the plated defects are stable, and that animals tolerate the surgery well. Untreated defects at 8 weeks do not show significant signs of healing suggesting that this may be a CSD. The lack of a validated CSD model in large animals has hampered progress in pre-clinical studies of biomaterials. Further assessment using standard histology and uCT will be performed and reported to quantify healing prior to assessment of candidate biomaterials.

66.P14
Transplantation of human fibroblast cell sheets for colorectal endoscopic submucosal dissection in a xenogeneic model; an animal feasibility study

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Background: Colorectal endoscopic submucosal dissection (ESD) is a novel technique for en-bloc mucosal resection of colorectal carcinomas. However colorectal ESD is sometimes complicated by colon perforations. In this study, we tried to perform transplantation of human fibroblast cell sheetsina xenogeneic model to prevent perforation.
Method: Human fibroblastcells were isolated from the skin of volunteer donors and were amplified for 2 weeks by explant culture on temperature-responsive culture dish. A few weeks before transplantation, swinesunderwent sigmoid-stomy for bowel preparation. ESD was performed at the swine rectum. Immediately after ESD, cultured human fibroblastcell sheets were transplanted. Then, the swine was sacrificed one hour after transplantation for histological analysis.
Results: Rectal ESD and cell sheet transplantation were safety performed on three swines. Fibroblast cell sheets were histologically conformed on the ulcerated area after ESD. Fibroblast cell sheets can be attached to the ulcerated site in short-term.
Conclusion: Transplantation of the fibroblast cell sheets isfeasible in a xenogeneic model. In the near future, we plan to conduct experiments for engraftments of fibroblast cell sheets in a swine autologous model.

66.P15
Studying bone colonisation by breast cancer cells using a novel in vivo model

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The skeleton is a preferred site of breast cancer (BC) metastasis. To date, treatment options for patients with bone metastasic disease are at best palliative and the disease is still incurable. To identify key mechanisms of BC osteotropism and allow the development of new therapeutics, more suitable animal models that allow to study human BC-bone interactions are needed. Ectopic bone models based on tissue engineer-

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ing have previously been shown to serve as homing sites for BC cells. In the presented study we set out to establish a novel humanised ectopic bone model for BC bone colonisation. To that aim electrospun polycaprolactone scaffolds were seeded with primary human osteoblasts and implanted together with bone morphogenic protein-7 subcutaneously into immunodeficient mice. X-ray imaging revealed significant bone formation over the next 12 weeks. Then, luciferase expressing human BC cell lines of different bone-metastatic potential, MDA-MB-231 and MDA-MB-231BO cells, were implanted in proximity to the scaffold. Non-tumorigenic MCF10A cells served as controls. Tumour growth was monitored by bioluminescent imaging. After 5 weeks, specimens were analysed by micro-computed tomography, histology and immunohistochemistry. We conclude that the presented in vivo model is highly suitable to study BC-bone interactions, and expect to gain in our future work new insights into the mechanisms of human BC bone colonisation in vivo.

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67. Orthopaedic Area (in coop. EORS)

67.01
Keynote: From imaging technologies to smart biomaterials and tissue engineering: novel concepts for bone regeneration

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In recent years numerous strides have been made in bone reconstruction. Especially in the craniofacial skeleton where complex anatomical structures and functional characteristics represent a challenging situation for the reconstructive surgeon a paragdigm shift has occurred. Efforts are no longer limited to non-biodegradable prosthesis merely acting as space fillers in osseos defects. Instead integrative implants are developed, which actively regenerate bone tissue leading to improved functional and aesthetic postoperative results. This lecture will give and overview about the state of the art in biomaterials for bone regeneration from a clinical and biomaterials perspective followed by the authors own experience with a concept to tissue engineer patient specific bone grafts. The author has developed an integrative concept combining medical imaging, design and fabrication of bioactive biomaterials, cell transplantation and reconstructive surgery. This technology successfully was applied to regenerated complex shaped skeletal defects. In this lecture long term results of several animal studies in rabbits, pigs and dogs are presented as well as international clinical studies.

67.02
Keynote: Convergence of delayed allogenic cell injection in a scaffold-based bone engineering therapy concept

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In orthopaedic and trauma surgery, extensive bone loss is associated with major technical and biological problems. Bone grafts as the gold standard treatment possess osteoconductive and osteoinductive properties, however, they face significant disadvantages. These include limited access and availability, donor site morbidity and haemorrhage, increased risk of infection, and insufficient transplant integration with following graft devitalisation, subsequent resorption and decreased mechanical stability. As a result, recent research focuses on the development of alternative therapeutic concepts. The concept of tissue engineering has emerged as a promising new approach to facilitate bone regeneration. However, despite initial success, traditional cell-based strategies are still infrequently translated into routine clinical practice although the basic and preclinical research literature clearly indicates the feasibility of osteogenic cell application for the reconstruction and repair of bone. Areas that need to be addressed include consensus as to the most suitable cell type, cell amount and state of differentiation, suitable carriers for cell and growth factor delivery, and cell survival upon transplantation. The aim of this present study was to assess the regenerative potential of biodegradable composite scaffolds in conjunction with delayed allogenic cell sheet injection compared to autologous cancellous bone graft (ABG), which is the current clinical gold standard for humans.

67.03
Reamer Irrigator Aspirate (RIA) putty for engineering craniofacial bone grafts

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The low incidence of mesenchymal stem cells (MSCs) in bone marrow aspirates has inspired searches for alternative cell sources. Recently, a method for reaming the bone marrow to obtain solid reamer-irrigator-aspirator (RIA) putty has been developed to provide significantly higher levels of MSCs. In this study, we investigated the characteristics of RIA from the femurs of adult pigs histologically and found that various tissue fragments are evident, creating highly complex microenvironments within the putty. A heterogenous cell population was extracted by digesting the putty. They exhibited different morphologies but gradually adopted a more fibroblastic morphology with sub-culture. The cells demonstrated the potential to form colony forming units-fibroblasts and differentiated along osteogenic, chondrogenic, adipogenic, and vasculogenic lineages. We prepared cylindrical scaffolds using decellularized bovine trabecular bone. A central channel was created and the walls of the trabecular region were coated with collagen. We implanted the putty into channels and cultured in osteogenic medium up to 4 weeks. Cells within the channel remained viable and proliferated. Cells in collagen-coated scaffolds also migrated into the trabecular region to form more uniform tissues. Micro-CT analysis and immunohistochemistry were used to assess de novo bone formation. Our results demonstrate that RIA is a clinically-relevant, rich source of MSCs that can be used to generate bone grafts.

67.04
Cell therapy without exogenous stem cells: insights from human experiments using serum-augmented bone grafts

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Platelet-rich-plasma (PRP) is used in many areas of orthopedic surgery with the promise of stimulating the anabolic processes. We used PRP mixed with autologous bone grafts in the therapy of bone ischemia. Patients with femoral head necrosis were either treated with decompression drilling as a control (n = 13) or with autologous PRP mixed with bone chips (n = 19). After 6 years the PRP group had significantly lower failure rate (21% vs 67%) indicated by prosthesis implantation. The general opinion is that PRP is a source of growth factors, however, it contains high levels of human serum albumin, which is known to have an anabolic effect in ex vivo stem cell culture. We hypothesized that using only serum albumin can have a significant effect on stem cells in bone grafting. First we showed in preclinical studies that allografts can be augmented with albumin which results in better remodeling. In a first-in-human study we implanted albumin-coated allografts in revision arthroplasty surgery (n = 10). At 1 year follow-up histology and SPECT-CT analysis showed intense remodeling at the graft-host interface. We conclude that PRP supplementation or even just serum albumin may be enough to support BMSCs and improve the success of bone grafts, allowing a simplified and effective way of cell therapy. Supported by TÉT-SIN-CELLTHER, TÁMOP-4.2.1/

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67.05
Functionalized biphasic silk scaffold for anterior cruciate ligament regeneration and bone tunnel healing in the porcine model

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The use of tendon autografts is the current gold standard for ACL reconstruction. However, problems such as donor site morbidity and integration site or enthesis dysfunction persist. We propose the use of a functionalized biphasic silk scaffold for complete ligament and bone regeneration. In a preclinical porcine trial, Bombyx mori silk knitted/sponge hybrid scaffolds were fabricated with functionalized ends for in-situ osteogeneic differentiation of loaded MSCs within the bone tunnels. The scaffold ends were functionalized by incorporating nano-hydroxyapatite, with (Exp) and without (Ctrl) BMP-2 loaded. These two groups were each seeded with porcine MSCs prior to implantation in 14 Yorkshire pigs. After 24 weeks, the knee joints were collected and scanned using micro-CT prior to histological preparations and mechanical tests. Gross observation of excised knee joints showed no signs of osteoarthritis and that the ligament portion was regenerated. Bone tunnel narrowing with presence of new mineralized tissues was observed in Exp. Histological observation further substantiated new bone and enthesis formation in Exp, with dense collagen I rich fibers developed in the ligament region. Better integration was also observed mechanically in Exp compared to Ctrl. From this in vivo study in a large animal model, it was conclusive that the BMP-2 eluting biphasic silk scaffold can serve as an advanced TE treatment modality for ACL reconstruction that avoids current treatment limitations.

67.P01
The prevention of orthopaedic related bacterial infection by novel surface design

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Osteomyelitis from bacterial attachment and proliferation on surfaces of biomedical devices and implants are significant issue in implant surgery. Prevention of biofilm formation by preventing the initial bacterial attachment can be a promising strategy to reduce osteomyelitis. Different kinds of approaches are suggested such as grafting of antibacterial polymer and peptides, coating of inorganic or metal ions and loading of antibiotics. However, there are disadvantages such as weak coating surfaces, cytotoxicity effect, complicated fabrication methods and possible development of antibiotic resistance. Therefore, we proposed to covalently coat small molecule X which provides excellent antibacterial effect while maintains biocompatibility. Our results show that the titanium surface coated with small molecule X (Ti-X sample) has at least 80% reduction against S. aureus on both short and long term culturing and is biocompatible with the osteoblast. As this novel surface coating can be easily fabricated, cheap and permanent, could be a potential solution for preventing osteomyelitis in orthopaedic surgery.

67.P02
Effect of IL-1β on the proteome of chondrocytes derived from human osteoarthritic cartilage - a pharmacoproteomics approach for drug screening

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Inflammation plays a pivotal role in cartilage destruction in osteoarthritis. IL-1β affects the balance of biosynthesis and degradation of extracellular matrix (ECM) constituents by chondrocytes which hallmarks osteoarthritis and induces the production of the pain mediator prostaglandin E2 via cyclooxygenase. Today's treatment options are restricted to symptome-modifying drugs. However, analgesics or anti-inflammatory drugs partially show just limited efficacy with respect to pain relief or cause undesirable side effects. Hence, the identification of disease-modifying and efficient symptome-modifying drugs is a main challenge in osteoarthritis research. Pharmacoproteomics is a promising approach for drug screening. In order to establish a reference system for monitoring substance effects, we performed proteome profiling of human osteoarthritic chondrocytes. Alterations of the secretion performance and the metabolism of chondrocytes due to IL-1 β treatment were assessed by 2D-PAGE and shotgun proteomics. We focussed on the determination of interindividual differences as well as those arising due to stimulation by IL-1β. The resulting database was used to assess the effect of different derivatives of hyaluronic acid in order to evaluate the applicability of this approach. Our approach enabled insights into molecular alterations due to cytokine and substance effects at a comprehensive systemic level.

67.P03
Bone ingrowth and biological fixation of selective laser melted porous scaffolds for the reconstruction of severe bone defects

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Custom-made scaffolds are applied clinically nowadays to reconstruct massive bone defects encountered at the time of revision acetabular surgery. CT-based preoperative planning combined with additive manufacturing provides the flexibility to produce orthopaedic implants with a personalized external shape and a controllable internal network of pores. This study quantified the in vivo bone ingrowth and fixation of clinically used titanium scaffolds with different porous networks, surface treatments or calcium phosphate coatings. Six adult goats were implanted with cylindrical Ti6Al4V scaffolds (Ø8 mm × 14 mm, porosity 75%) fabricated by selective laser melting (SLM). Scaffolds were press-fitted into 12 holes drilled in the subchondral bone of both femurs and tibias of each goat. Fluorochrome labels were injected at 3, 6 and 9 weeks. In vivo CT scans and X-rays were taken. Resonance frequency analysis, micro-CT, histology, and pull-out tests were performed postmortem at 6 and 12 weeks. Micro-CT analysis and histomorphometry showed bone infiltration into the scaffold's pores from the bottom and sides. The amount of new bone formation increased slightly between week 6 and 12. Higher pull-out forces were measured for scaffolds with regular internal structures. Shifts in the resonance frequency spectrum indicated that implant stability increased with time. SLM manufactured titanium implants allow bone ingrowth and obtain strong biological fixation in a 3 months goat model.

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67.P04
A 3D artificial meniscus design for meniscus tissue engineering

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Introduction: Meniscus tissue serves in joint lubrication and stability, and shock absorption. The main meniscal problem is the tears and currently there is no perfect treatment. Tissue engineering appears to be a promising solution. In this study, a multilayered 3D meniscus based on collagen type I was prepared and evaluated.
Materials and methods: Three different foams and electrospun nano/microfibrous mats were used to produce a multilayered 3D construct. The top layer consisted of collagen-chondroitin sulfate-hyaluronic acid (Coll-CS-HA) prepared by freezing at −20°C and lyophilization. The middle and bottom layers were made of insoluble collagen type I from bovine Achilles' tendon by lyophilization after freezing at −20°C and −80°C, respectively. All the foams were dehydrothermally (150°C, 48 h) and then chemically (EDC/NHS) crosslinked.
Results and Discussion: Coll-CS-HA presented the lowest mechanical properties, followed by −80°C and −20°C Coll foams. 3D construct had the highest mechanical properties. The compressive modulus of the 3D construct (445 kPa) was twice as high as that of −80°C Coll foam (ca. 240 kPa). Young's modulus (2.97 ± 0.33 MPa) of the 3D construct was significantly higher than −80°C foams. In dry state the 3D construct showed higher compressive and shear properties than the natural tissue but the tensile properties were much lower.
Conclusion: The 3D meniscus substitutes have mechanical properties suitable for partial meniscus replacements.

67.P05
In vitro and in vivo evaluation of bioactive hybrid materials coating for infection resistant implants

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Rhode Island Hospital, USA; Brown University, USA; BioIntraface Inc, USA

A biocompatible coating that resists bacterial biofilm formation on medical devices has recently been developed in our laboratory. The novel coating consisting of titanium oxide/polymer doped with silver was created by metal-organic method. This was tested in vitro against Staphylococcus aureus and osteoblasts (bone forming cells) using rapid screening techniques. A range of compositions were identified which inhibited S. aureus growth, while facilitating osteoblast viability and proliferation. The coating was then applied to intramedullary (IM) nails and evaluated in vivo in a caprine model. In this pilot study, a fracture was created in the tibia of the goat and S. aureus were inoculated directly into the bone canal. The fractures were fixed by either coated (treated) or non-coated conventional IM nails (control) for 5 weeks. Clinical observations as well as microbiology, mechanical, radiology, and histology testing were used to compare the animals. The release of silver and accumulated silver in blood and organs were determined by surface and element analysis. The data showed that the treated goat lost less weight and needed less pain reliever compared to the control. Moreover, the treated goat was able to walk using all four limbs after 5 weeks, while the control was unwilling to bear weight on the fixed leg. In summary, the hybrids are potential antibacterial materials which are being investigated in our on-going full scale animal study.

67.P06
Array-based identification of potential osteoarthritis key genes using mesenchymal stromal cells from osteoarthritic versus healthy donors

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Osteoarthritis (OA) is one of the most frequent degenerative joint disorders. However, the exact aetiology of OA remains unknown. The aim of this study was the identification of pivotal OA relevant genes and pathways in mesenchymal stromal cells (MSCs) using a gene expression array-based approach. Based on RNA microarray data from bone marrow derived MSCs of $n = 13$ patients with idiopathic hip OA and $n = 15$ age-matched healthy donors gene lists were filtered according to significance, fold change, literature search, STRING 9.0, and Pareto analysis. A total of $n = 89$ candidate genes were analyzed using PCR Arrays. Pathway analysis was performed using NetAffx™. Statistics were calculated by Student's t-test, $P < 0.05$. A total of $n = 35$ significantly regulated genes were detected. These include candidate genes, e.g. secreted frizzled-related protein 1 (SFRP1), pleiotrophin (PTN), and growth hormone receptor (GHR) closely linked to bone and cartilage development. Furthermore, extracellular matrix associated genes were significantly regulated. Moreover, pathways of Wnt and Jak-Stat signaling were identified as regulated signal transduction cascades in OA-MSCs. Using an array-based approach we identified potential key genes and signal transduction pathways involved in OA. Furthermore, these data support the hypothesis that MSCs play a central role in the aetiology of OA and will help to facilitate the application of autologous cell-based strategies for musculoskeletal tissue regeneration.

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68. Bench to Bedside Examples

68.01
Keynote: Challenges and hurdles of stem-cell based clinical trials under the European law

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Since 2008 cell-based therapies are considered as ATMPS (Advanced Therapy Medicinal Products) in Europe and are equivalent to drugs. Thus, the process of applying cells as a therapeutic product has not only become extremely complex but also time and money consuming. Major hurdles have to be taken. First, the regulatory barriers for stem cell based-therapy are obviously considerable, and both public and private funding seems to be difficult. Second, large animal studies to show the proof of principle and biodistribution of the cells can present challenges of their own. Third, identifying accurate cell test systems, e.g. for genetic stability is challenging and has to be discussed with the authorities in detail. Fourth, not very much appreciated are issues related to research psychology, career development and the enormous problems in creating a truly translational environment, where clinicians, scientists and regulatory affairs experts come together in a productive fashion. Consequently, comparing MSC-based products with that of other emerging technologies during their histories reveals similar delays in clinical application. In this presentation the author tells his experience gained from the EU FP-7 large scale project ADIPOA, were fat-derived autologous MSCs are injected into the osteoarthritic knee. The hurdles from planning the clinical trial to finally inject the cells into the first patient are discussed. Overcoming these hurdles requires money, persistence, focus and power of endurance.

68.02
Lab to OR - the evolution of fibrin sealants in regenerative medicine and reconstructive surgery

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Reconstructive surgery of diseased or injured organs is a major medical challenge that will keep growing with the expansion and aging of the population. The promise of bioengineered materials to replace or cover defective anatomical areas depends on the ability to position and fixate transplants in a secure and compatible way and to allow it to template and regenerate with the local tissue. Fibrin Sealants (FS) have been utilized in plastic surgery with skin and tissue-engineered substitutes for reconstructive & aesthetic procedures. Particular benefit has been reported in burns and in procedures requiring flaps or grafts in complex grafting sites. FS can improve graft take, provide local haemostasis, reduce infections and stimulate wound healing. These effects are achieved by providing a supportive matrix enhancing cellular motility and consequently allowing an improved migration of keratinocytes, fibroblasts and other repair cells into the wound area. FS were also shown to be a good vehicle to deliver agents like stem cells or growth factors thus further contribute to wound healing acceleration. Here we review the development of a new fibrin sealant - ARTISS from the lab to the OR. The review will cover the in-vitro assessments, the preclinical activities, dose finding studies and the outcomes of the prospective randomized clinical trials performed to achieve the regulatory approvals for plastic & reconstructive indications.

68.03
Versatility of the Dermal Regeneration Template (Integra®) engineered tissue in the reconstruction of different districts of the body

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Introduction: INTEGRA® is a temporary bilaminate membrane system made of a superficial silicone sheet and a deep 3D porous layer of cross-linked bovine tendon collagen and glycosaminoglycan used to formation of a permanent and very well neo-vascularised dermal layer.
Materials: January 2004–2011 84 patients (46F and 38M, 3–82 years, mean age 54) were treated: 38 post-surgical cases (31 post oncological demolition, seven post traumatic), five vascular ulcers, four burns, six retracting scars and one Proteus Syndrome. The INTEGRA® was accurately shaped on the lesion, fixed with the Appose®, dressed with Vaseline and sterile gauzes (T0). New medications occurred at T7, T14, T21. After 4 weeks a split-thickness autograft (0.16 mm) was performed. 11 pz after oncological reconstruction underwent cutaneous biopsy at T30, T90 and T360; every time we evaluated also width/thickness (W/T), elasticity/softness (E/S), aesthetic aspect (A.A) by means of subjective graduated scale from 0 (unsatisfactory) to 3 (optimal).
Results: Histological findings showed neoderma formation with collagen fibers deposition, autologous fibroblast migration, fairly good neoangiogenesis, without nerve terminations and annexial structures. W/T parameter was a grade 3 in 70% of cases, E/S grade 3 in 60%, A/A grade 3 in 50%. No observed retracting scar.
Conclusions: 2/3 of pts obtained satisfactory morfo-functional results. In selected patients INTEGRA® can be considered a valid, optimal cost/benefits ratio and safe technique.

68.04
Adipose stem cells and biomaterials for reconstruction of human bone defects

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The most reliable method of reconstructing large bony defects involves painful harvest of bone. In the future, tissue engineered products will replace harvested flaps. Adipose stem cells combined with osteostimulative biomaterials provide an attractive alternative for bone tissue engineering.Here we describe our patient cases with cranio-maxillofacial bone defects that have been treated with the combination of autologous adipose stem cells and biomaterials, β-tricalcium phosphate and bioactive glass. So far we have treated more than 20 patients. In addition, approximately half of the patients have received biomaterial incubated with BMP-2 to induce osteogenic differentiation. First clinical cases were performed in November 2006 and treatments have succeeded well. Treatments have been done according to good manufacturing guidelines. Sterility, endotoxins and mycoplasma have been routinely tested. In addition, cell surface markers, biocompatibility of biomaterials and osteogenic differentiation in vitro have been analysed. Our surface marker expression data is in accordance with the results reported earlier. Furthermore, our data shows that adipose stem cells have the capacity to undergo osteogenic differentiation. These cases show that the use of autologous adipose stem cells combined with oste-

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ostimulative biomaterial results in generation of new viable bone and can be utilized in fabrication of custom-made bone flaps.

68.05
Small molecule enhancer of BMP activities and inhibitor of osteoclast activity

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Bone morphogenetic proteins (BMPs) are the key cytokines in bone formation and repair. In humans, however, huge amounts of BMP are needed to show effect or to measure up to results normally achieved by the use of autologous bone. Since high BMP dosages could induce side effects and increase the cost of the treatment, several strategies have been employed to reduce the amount of BMP needed for clinical applications: optimization of the BMP release by the delivery system, inhibition of BMP antagonists, enhancing BMP activity by small chemicals or other means. To that end we screened small chemicals for their capacity to enhance BMP activity in pluripotent cells and identified NMP (N-methyl-pyrrolidone) as a novel enhancer for BMP activity. Since NMP is an FDA-approved excipient, a guided bone regeneration (GBR) membrane loaded with NMP was developed as a 3rd generation membrane, where biocompatibility, biodegradability and bioactivity are combined. Beside in vitro and preclinical trials on NMP and other BMP enhancers, we will also report on the outcome of two clinical trials on bone regeneration and bone augmentation using the NMP releasing membrane in the dental field. Lately we also discovered that NMP has a second activity, namely to inhibit osteoclast maturation and osteoclast activity. Therefore, NMP could be a versatile tool in osteoporotic patients as well.

68.06
Stimulation of hair growth in humans by cell-secreted proteins

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We have evaluated a bioengineered human cell-derived formulation, termed Hair Stimulating Complex (HSC), generated by cells grown in hypoxic bioreactor cultures on beads and containing KGF, VEGF, follistatin and noggin on hair growth in male pattern baldness. Follistatin and noggin antagonize activin and BMPs, which maintain the quiescence of hair follicle stem cells. We hypothesized that HSC might increase progenitor and transit amplifying keratinocytes, leading to an increase in the thickness of the hairs and a reversal of the miniaturization process. The initial pilot study was a single site, double-blind, randomized, placebo-controlled trial involving 26 male subjects with androgenetic alopecia. At baseline the treatment site received four intradermal injections of 0.1 cc of HSC and the control site a placebo comprising non-conditioned medium. Parameters of hair growth were determined using the Trichoscan image acquisition and analysis system. HSC was safe and showed a statistically significant increase in hair thickness density at 3 months ($P < 0.03$), primarily attributable to a change in hair thickness. We also saw an increase in hair thickness density at 1 year ($P < 0.03$), attributable to an increased number of hairs. The results support the hypothesis. A Phase I/II 55 patient trial with 8 injections of HSC and control at baseline and a repeat dose at week 6 has completed enrollment. The efficacy results represent a novel regenerative medicine approach in hair restoration.

68.P01
Periodontal regeneration with autologous periodontal ligament-derived cell sheets in clinical settings

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Periodontal regeneration has been challenged with chemical reagents and/or biological approaches, however, there is still no sufficient technique that can regenerate complete periodontium, including alveolar bone, cementum, and well-oriented collagen fibers. We previously showed that autologous transplantation of PDL cell sheets combined with beta-tricalcium phosphate regenerated true periodontal tissue in canine models. Translating to a clinical setting, we have optimized the methods for extraction and cultivation of human PDL cells, and validated both the safeness and efficacy of human PDL cell sheets. Finally, we have started the clinical trial named 'Autologous transplantation of periodontal ligament cell sheets for periodontal reconstruction', after the approval of the Ministry of Health, Labor and Welfare in Japan. Patient's PDL cells are cultured with autologous serum, and cell sheets are fabricated in temperature-responsive dishes with osteoinductive supplements. Three-layered PDL cell sheets are transplanted to the denuded root surface, and beta-tricalcium phosphate granules are filled in the intrabony defects. In this presentation, current status of this clinical trial will be reported.

68.P02
Development of tissue engineered 3D fiber-deposited scaffolds for ossicular chain repair

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Conductive hearing loss is a major problem that impairs the normal function of the middle ear due to ossicular chain (OC) damage. To date, the OC reconstruction is performed using biological grafts or alloplastic biomaterials; however, in about 20% of cases graft resorption or prosthesis extrusion can occur. In this study, we propose new partial ossicular replacement prosthesis (PORP) scaffolds for OC repair. Three-dimensional fiber deposition technique was used to produce PORP-like scaffolds with different sizes and porosity based on poly(ethylene oxide terephtalate)/poly(butylene terephtalate) (PEOT/PBT) copolymers. Gas plasma treatment was subsequently employed to modify surface topography in order to enhance cell adhesion. The produced scaffolds were characterized by means of Scanning Electron Microscopy and Atomic Force Microscopy. PORP scaffolds were cultured in vitro with human mesenchymal stem cells (hMSCs) from three patients. Cell viability was investigated with Resazurin assay and confocal laser scanning microscopy and resulted stable up to 3 weeks. Cellularity, alkaline phosphatase activity and osteocalcin, together with gene expression and histological data along the culture period were performed to assess the osteo-differentiation of hMSCs in PORP scaffolds, indicating that a tissue engineering approach can be a promising alternative to the currently used ossicular prostheses.

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68.P03
Phase I study of double loaded dendritic cells in ovarian cancer: from bench to bedside

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Life Research Technologies, Austria; IMC FH Krems, Austria; Life Research Technologies, Austria; Cell Pro Danube, Austria; Depart. Of Gynecology, General Hospital Korneuburg, Austria

The crucial role of dendritic cell (DC) in inducing immunity has boosted translational research to understand and exploit their unique immune-modulatory capacity for cancer. Immunotherapy targeted against cancer aims at activating the immune system to destroy tumor cells. A major advantage of immunotherapy when compared to radio- and chemo-therapy is its low toxicity. However, considerable challenges remain for improving the efficacy of therapeutic immunizations against cancer. Here we want to present approaches developed to successfully transfer preclinical data and results from healthy donors to the clinic and ovarian cancer patients. Ovarian cancer represents one of the most aggressive gynecologic cancers with unmet medical need. Ovarian cancer patients in remission typically have little tumor mass and a functional immune system, making them good candidates for proof of efficacy for autologous DC therapies. Therefore ovarian cancer after completion of first line therapy was chosen for our clinical trial. Each patient will be vaccinated intradermally with 6*106 double loaded autologous mature dendritic cells loaded with two universal tumor antigens. Treatments of blocks 1 and 2 have been completed without any major side effects. Enrollment of block 3 began in November 2011. Challenges specific to this advanced therapy approach experienced during the phase I study will be reported and possible solutions discussed.

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69. Clinical Aspects of Tissue Engineering

69.01
Keynote: New materials-based strategies for regenerative medicine

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This talk will provide an overview of our recent developments in bio-inspired materials for tissue regeneration and strategies to translate these to the clinic. Engineering of large-volumes of bone in vivo will be presented using relatively simple yet effective biomaterials approaches based on hydrogels or strontium containing bioceramics. Strontium ranelate has found great success as an oral anti-osteoporosis drug with effects on both osteoblasts and osteoclasts. We demonstrate that the incorporation of strontium within biomaterials results in the rapid high quality production of new bone as assessed in terms of bone histology, mechanics and nanoscale size and arrangement of hydroxyapatite crystals. In other applications, we have developed modified poly-ÿ-glutamic acid based materials that can be processed to generate materials with a range of mechanical properties appropriate for ligament and other tissue repair. The remarkable influence of the materials on stem cell behaviour will be presented. This work is funded from several sources including the EPSRC, the ERC, the Wellcome Trust, the Rosetrees Trust and the Technology Strategy Board.

69.02
Retrospective analysis of patients with ulcers of (arterio-)venous, traumatic or decubitus origin treated with an autologous skin substitute

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Introduction: We have developed an autologous full-thickness living skin substitute consisting of reconstructed epidermis on fibroblast-populated human dermis (SS). The aim of this study was to evaluate the safety, efficacy and applicability of the SS together with the transferability of the protocol between different centres for treating chronic, hard-to-heal ulcers in an outpatient (OP) and hospitalized (H) setting. Ulcers of (arterio-)venous, post-traumatic or decubitus origin were treated in multiple studies between 2004–2009.
Methods: Sixty-six ulcers (size: 0.75–150 cm^2; duration: 0.25–32 years) with a minimum follow-up time of 24 week after one SS application were assessed. Wound-bed preparation consisted of VAC-therapy (H) or application of acellular dermis (OP). Ulcers were evaluated weekly. Time to heal, adverse events and recurrence rate one year after complete healing were recorded.
Results: Complete ulcer healing occurred in 41/66 ulcers (62%). Ulcer size was significantly reduced at week 12 compared to week 0 and at week 24 compared to week 12. At 12 weeks, ulcer size was significantly reduced in the H group compared to the OP group. However after 24 weeks this difference was no longer observed. One year after complete closure 81% of the ulcers were still closed. Only one minor adverse event was recorded.
Conclusion: This retrospective analysis shows that SS provides a safe and successful treatment for particularly hard-to-heal chronic ulcers of various origin.

69.03
A global assessment of stem cell engineering

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Stem cells have become increasingly important to the various cell therapy approaches being developed as part of regenerative medicine. This includes both embryonic and the various adult stem cells. Because of the importance of engineering and various engineering approaches to the stem cell field, the U.S. government has funded a study where the purpose is to do a global assessment of stem cell engineering, i.e. the interface of engineering with the stem cell field. This study spans from basic research to the translation of benchtop stem cell research to the patient bedside. In this preliminary report, activities in stem cell engineering in Asia, Europe, and North America will be highlighted. From the gaps will be identified where engineering in the future can make a major contribution.

69.P01
xCELLigence real time cell analyzer single plate system as quality control for human platelet lysate

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As fetal calf serum (FCS) causes the incorporation of xenogenic substances and fears about disease transmission, the experts in the fields of regenerative medicine und cellular therapy are looking for alternatives. Another aspect is the fact, that the content of FCS is not exactly known and also no real quality control parameters regarding the composition are implemented leading to a high batch to batch variability. Human platelet lysate (hPL) is already known to be a potential substitute to FCS in cell culture. In order to have a well defined product with defined criteria, a quality control system needs to be established. The xCELLigence real time cell analyzer single plate system (RTCA SP; Roche, Germany) is usually used for toxicity studies. Our aim was to evaluate, whether the device can be installed as quality control in the production of platelet lysate as well. Several cell lines (HeLa and fibroblasts) and primary as well as immortalized adipose derived stem cells were cultivated to determine the most suitable cell line. Additionally, coating of the wells with fibronectin and serum albumin was performed to look for further optimization. The results obtained from the xCELLigence RTCA SP system were compared to classical methods like trypan blue staining and colorimetric proliferation assays (WST-1 assay) and will be presented.

69.P02
Application of proteomic techniques for the development of human platelet lysate

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Over the last years the Blood Transfusion Centre Linz worked on several projects concerning the field of human cell therapy and tissue engineering. In the course of these studies it became obvious that

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supplementation of cell culture media with foetal calf serum (FCS) will not lead to safe cell products. FCS may contain xenobiotic molecules, viruses and prions, which are suspected to be assimilated from the cultivated cells. A suitable alternative easily available at the Blood Transfusion Centre Linz is human platelet lysate (hPL). It is produced either from expired thrombocyte concentrates or buffy coats and subjected to strict regulatory requirements and guidelines. Therefore hPL is free of infectious contaminants and viruses. In order to provide a high grade media supplement a thoroughly product optimization was carried out. Two different starting materials as well as differing production procedures were evaluated. Beside the analysis of default parameters, e.g. platelet number, total protein concentration and concentration of growth factors, Proteomic tools, e.g. gel electrophoresis and mass spectrometry supported the optimization process. The protein alterations in hPL based on differing materials and procedures were tracked by 2D DIGE and changing protein spots were identified by MALDI TOF MS. Due to clearly visible differences of the achieved 2D gels it was possible to eliminate or further optimize production steps not leading to the desired outcome.

69.P03
Posterior iliac crest outperforms anterior when obtaining fibroblast-colony forming unit from bone marrow

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The number of Mesenchymal stem cells (MSC) isolated is commonly estimated by counting -Colony Forming Unit- Fibroblast (CFU-F). MSC represent a small fraction of the cells contained in the bone marrow (BM), thus MSC are frequently expanded ex vivo to reach clinically relevant numbers. Unfortunately, MSC expansive capacity is limited and after several duplications MSC undergo replicative aging that is associated with a decreased proliferation and progressive loss of differentiation capacity. Therefore, there is need to optimize MSC collection to obtain an high number of cells at harvest to minimize the number of duplications needed to reach clinically relevant numbers. No data exist about the influence of the anatomical site of harvest on the number of MSC collected in a BM. Our goal was to compare the anterior iliac crest (AIC) and the posterior iliac crest (PIC), analyzing bone marrow cellularity, CFU-F, MSC kinetics, and differentiation potential. We collected the same volume of BM from both the AIC and PIC of 22 donors and compared the samples. In the PIC the number of MSC progenitors was 1.6 times higher compared to the AIC as evaluated by CFU-F assay. No differences were found in terms of phenotypic characterization, kinetics, and differentiation potential of MSC derived from the AIC or PIC site. We conclude that the PIC is preferable for harvesting BM because it contains a greater number of MSC precursors that would be relevant for one step clinical applications.

69.P04
Short-term serum-free culture of amniotic fluid-derived stem cells

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The expansion of stem cells depends on the culture conditions and requires medium supplemented with fetal calf serum (FCS) to generate relevant numbers of cells. However, the presence of FCS is a major obstacle for their clinical use. Therefore, we have evaluated the capacity of expansion of amniotic fluid-derived stem cells (AFSCs) in a commercial serum free medium in comparison with a classical medium -MEM containing 15% FBS. AFSCs were expanded in two different culture media. Proliferation rate of the cells was higher in serum free medium than in -MEM containing 15% FBS. Stem cells isolated in the both media were morphologically similar, and they were expressed identical surface antigens and mRNA of stem cell markers. AFSCs in the both media were also able to differentiate into the myogenic cells. These results suggest that serum free medium is an optimal medium for expansion of stem cells. It allows a better cell expansion, preserves cell characteristics, and reduces the culture period. This medium seems suitable for clinical scale expansion of stem cells. [Supported by the grants of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A091224) and Regenerative Medicine R&D Project from Daegu Metropolitan City.]

69.P05
Development of novel serum preparation device

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Introduction: The use of autologous serum in medical treatment has been increasing. The serum for clinical use should be prepared under aseptic condition to avoid infection. Recentry, we have developed a completely closed system for about 10 ml serum preparation that is suitable for the centrifuge widely used in clinics and laboratories. In this study, the ability of the device in terms of serum preparation and the quality of the serum were examined.
Methods: Twenty millilitre of whole blood samples from 13 healthy donors were taken into the device. Fibrinogen concentration and number of platelets in the blood samples were measured during 30 min of agitation. After separation of the serum by centrifugation, the concentration of three kinds of growth factors(GFs) in the serum before and after freezing under −80°C for 1 year were quantified.
Results: It was judged that coagulation of blood was completed after 30 min of agitation process since fibrinogen and platelets were decreased dramatically during the process. The amount of prepared serum was about 40% of collected blood. The concentration of GFs in the serum were the same level of normal human serum. And there was no significant decrease of GFs after one year freezing.
Conclusion: The newly developed device named 'CELLAID-tube type' could be the fast and safety device for preparing serum and has already CE marked. We believe 'CELLAID-tube type' will be useful in therapies using autologous serum.

69.P06
Japanese guidelines on ensuring quality and safety of products derived from engineered human stem cells—after public consultation—

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Research and development on the application of human somatic stem cells, embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) is being conducted around the world. Identifying technical requirements for utilization of the various types of stem cells is critical for their timely and relevant application in patients. For adopting regenerative medicine through manufacturing and marketing authorization of cell/tissue-based products under the Japanese Pharmaceutical

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Affairs Law, the general considerations and technical concerns of the Japanese government are outlined in the Notification No. 1314 of Secretary-General of Pharmaceutical and Food Safety Bureau (PFSB), Ministry of Health, Labour and Welfare (MHLW), dated December 26, 2000. PFSB also published 'Guideline on Ensuring Quality and Safety of Products Derived from Autologous Engineered Human Cells/Tissue' (PFSB/MHLW Notification 0208003), as well as 'Guideline on Ensuring Quality and Safety of Products Derived from Allogenic Engineered Human Cells/Tissue' (PFSB/MHLW Notification 0912006) in 2008. In addition, an MHLW's study group has been developing five guidelines concerning the technical requirements for ensuring the quality and safety of products derived from engineered human autologous/allogenic somatic stem cells, autologous/allogenic iPSCs (and iPSC-like cells), and ESCs. In this presentation, we introduce these new guidelines and points to consider after the public consultation.

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70. Translation in Tissue Engineering and Stem Cell Therapy (in coop. Korea TERMS)

70.01
Keynote: Changing perspectives on translational research

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Recent advances in tissue engineering and regenerative medicine have provided new therapeutic opportunities for repairing damaged tissues and organs. While therapeutic paradigms that utilize the principles of regenerative medicine have shown to be effective, only a limited number of technologies have been successfully translated to patients. This is largely due to various scientific challenges encountered in the tissue building process, and delayed translation by the limited understanding of regulatory processes involved in the development of clinical therapies. To effectively develop and translate regenerative medicine therapies in a timely manner, a new strategy has to be designed and implemented. This session will focus on the approaches that would lead to rapid translation in the academic setting.

70.02
Keynote: Human articular cartilage repair using umbilical cord blood derived allogenic mesenchymal stem cells

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Repair of articular cartilage defect is still a challenging problem. We developed a novel method to repair the articular cartilage defect of human knee joint using the composite of hyaluronate and mesenchymal stem cells isolated from human umbilical cord blood in neonates. We evaluated the safety and efficacy of the composite for the repair of articular cartilage defects of human knee joint. Arthroscopically proven ICRS grade 4 lesions were included in the phase I-II clinical trial. MSC concentration of 0.5×10^7 cells/ml were implanted to the lesion as 0.5 ml/ÿ. There was no significant adverse event of more than grade 3 according to the WHO toxicity criteria. The overall Repair assessment was improved in 67%. The biopsy result showed highly hyaline-like regenerative tissue according to the H&E, Saf-O staining and Col II immunostaining. The promising result of this study warranted further investigation in this strategy of articular cartilage repair. Phase III clinical trial was performed as a multicenter study with a randomized control group of Microfracture. One hundred and eight patients with ICRS grade IV chondral lesions mostly from osteoarthritis were included in this study. The evaluation included clinical scoring, arthroscopic grading of the repair tissue, and histological assessetment. The result of the phase III clinical trial will be presented in this lecture.

70.03
An efficient 3D microfibrous system for human pluripotent stem cell self-renewal and direct differentiation

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Realizing the potential of stem cell-based therapy requires defined culture systems with efficient expansion, differentiation and isolation protocols to generate clinical-grade cells. Current 2D cultures for hPSCs lack scalability, imposing a major limitation on their biomedical applications. We report a 3D microfiber system that efficiently supports hPSC self-renewal and controlled differentiation. The unique feature of this system is the 3D ECM-like environment where cells are embedded, that affords: (1) uniform high cell loading density in cell-laden constructs; (2) quick recovery of encapsulated cells with excellent cell viability; (3) long-term hPSC self-renewal under chemically defined conditions; (4) direct differentiation of the expanded hPSCs into tissue-specific lineage. Four hPSC lines propagated in the scaffold for 10 passages were capable of maintaining self-renewal as shown by the expression of stem cell markers and stable karyotype in vitro and the ability to form derivatives of three germ layers both in vitro and in vivo. The combination of 3D stem cell expansion with direct differentiation led to the development of >90% mature neurons in differentiated hPSCs. When transplanted into kidney capsule in SCID mice, these derived neurons incorporated into kidney surface without tumorigenesis in the transplant recipients. These results bring us one step closer towards realizing large-scale production of stem cell derivatives for clinical and translational applications.

70.04
Electromagnetic fields (EMFs) can promote neuronal differentiation of human bone marrow-derived mesenchymal stem cells

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Introduction: The neuronal differentiation of bone marrow mesenchymal stem cells (BM-MSCs) has great potential for cellular therapies and might be useful in the treatment of diseases such as stroke or spinal cord injury.
Materials and methods: Here, we analyzed the combined effects of EL-FEMF (extremely low frequency electromagnetic fields) and differentiation medium (contatin hydrocortisone, insulin, forskolin, valproic acid, and KCl) on neural induction in human BM-MSCs. To induce neuronal differentication, BM-MSCs were incubated in differentiation medium for 1 day and treated for 7 days with 50 Hz, 100 Hz, and 200 Hz electromagnetic fields subsequently.
Results: The results showed that exposure to neural differentiation medium and EMF decreased the cellular proliferation and enhanced the cellular differentiation. We detected a significant increase of neuronal markers such as NF-L, Map2, and NeuroD1 by western blotting. Cells which were treated with 100 Hz EMF showed higher expression of NeuroD1, NF-L, and Map2 than 50 and 200 Hz. It confirmed by immunocytochemical analysis. And then, we confirmed that mean cal-

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cium current density increased after ELFEMF stimulation at 12 day of differentiation. We also found that ELFEMF increase the CREB phosphorylation at 24 h of differentiation.
Conclusion: Taken together, treatment of ELFEMF can be a good tool for neuronal differentiation and clinical implications.

70.P01
Costa Rican experience in the establishment of tissue engineering, a technological university approach

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Life expectancy is reaching 80 years with an increase in diseases, disorders and accidents.Aware of the importance of Tissue Engineering, the Costa Rica Institute of Technology (ITCR) aimed to develop and facilitate the implementation of bioengineering technologies to improve the life quality of Costa Ricans. We strengthened relationships with the International Atomic Energy Agency -our main financial and technical contributor-, with education and research institutions. We established collaborations with the national health security system and private health care providers. Research of the ITCR initiative included-Production of human skin cells for therapeutic use-Evaluation of matrices made from aquatic animal wastes for cell culture-Technical support to create the first Tissue Bank in Central America in the Children's National Hospital-Viability and proliferation evaluation of cell lines and primary tumor cell cultures under different insults detecting oxidative stress, apoptosis or necrosis. We trained professionals, lead activities to promote peaceful uses of atomic energy and helped design the National Plan for Science, Technology and Innovation 2011–2014 of the Ministry of Science and Technology. We found Tissue engineering can be significantly improved by interdisciplinary groups. Nevertheless, regulations concerning research involving human beings in Costa Rica need to be reviewed in order to guarantee the development of ethical, pertinent, relevant investigations.

70.P02
In search of finding a suitable business model for regenerative medicine – Reasons why cell-based therapies are still not commercially successful

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Regenerative therapies promise to be a cure for many so far incurable diseases. However, despite extensive investments in the last two decades, most cell-based therapies have failed to deliver clinical and financial success. In this paper, we report on our conduct on an economic analysis of cell-based therapies. Investigating a number of developments in the field, we identified different business models: Firstly, an autologous, service-based business model requiring a cell-biopsy, external cell cultivation and a re-implantation of the final product. Secondly, an allogenic, 'off-the-shelf' product that is – similar to a drug – directly brought to the clinic without any customizing. Thirdly, an autologous, point-of-care business model is described where cells are processed within the operating room and directly re-implanted. Benefits and drawbacks of the different business models are highlighted. The financial impact on the healthcare system is shown by comparing costs for conventional and regenerative treatment of exemplary chosen diseases. These insights might help to derive an appropriate commercialization strategy for cell-based products or services. Our top-level view gives insights to the development of the cell-therapy industry and suggestions for successful business model design. We conclude that not only great science is necessary for successful commercialization of cell-based products, but also a suitable business model.

70.P03
Encapsulated ovarian endocrine cells produced sustained levels of steroid hormones in vivo

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Although hormone replacement therapy is able to compensate for the loss of hormone production due to ovarian failure, delivery through pharmacological means results in consistently high serum hormone levels and clinical complications including heart disease and cancer. Controlled release through cell-based therapy is considered an innovative approach to overcome these complications because it provides a more physiologically-relevant system of delivery. Therefore, the purpose of our study was to design a tissue construct of the endocrine ovary using encapsulation techniques and to test the function of the construct in vivo. Granulosa and theca cells were isolated from the ovaries of Fisher 344 rats. Tissue constructs of ovarian endocrine unit resembling the native follicles were fabricated and implanted in omentum pouches created in each of five ovariectomized (ovx) Fisher 344 rats. A control group of five ovx rats were implanted with blank microcapsules. Plasma levels of 17 β-estradiol (E2) and progesterone (P4) were measured in all animals for 4 weeks. The ovarian tissue construct produced sustained levels of E2 and P4, which were significantly higher than the baseline levels measured in the control group during follow up. In summary, using the encapsulation techniques, we have demonstrated for the first time that the endocrine unit of the ovary could be recapitulated ex vivo and the tissue-engineered construct produced sustained levels of E2 and P4 in vivo.

70.P04
Restoration of hair-inducing capacity of cultured human dermal papilla cells by three dimensional spheroid culture

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The neogenesis of the hair follicle through follicular cell implantation for the treatment of hair loss is believed to greatly depend on the ability to reproducibly expand hair-inductive dermal cells in vitro. Two-dimensional (2D) cultured dermal papilla (DP) cells are known to gradually lose hair-inductive capacity during subculture. Recent studies showed that sphere formation enhances hair-inductive activity of cultured murine vibrissal DP cells. These results strongly suggest that hair-inducing capacity of human DP cells can also be restored by three-dimensional (3D) spheroid cultures. This suggestion prompted us to evaluate the hair-inducing capacity of cultured human DP spheres. We observed hair follicle formation when DP spheres from various passages (p3, p4, p6 and p10) of culture are mixed with newborn mouse epidermal cells. In contrast, hair follicles were never observed when 2D cultures from the same population are employed. To demonstrate that the origin of the DP cells in reconstituted hair follicles was of human origin, DP cells were labeled prior to sphere formation with the fluorescent dye, DiI. We observed that 25/32 (78%) of DP were completely labeled. In conclusion, using a hair reconstitution assay, we show that sphere formation increases the ability of cultured human DP cells to induce hair follicles from mouse epidermal cells.

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70.P05
Short term pre-clinical safety of human bone marrow derived mesenchymal stem cells following intramuscular delivery

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Stem cell therapy offers an exciting approach for the treatment of diseases such as critical limb ischaemia (CLI). Although, human bone marrow derived mesenchymal stem cells (hMSCs) have been proposed as a treatment for CLI, pre-clinical safety data is required prior to use in humans. This study was designed to evaluate the short-term safety of intramuscular injections of good manufacturing practice (GMP) grade hMSCs in immunodeficient mice. hMSCs were cultured according to GMP protocol and met the release criteria prior to use in this study. Mice were assigned to receive intramuscular injections of hMSCs or saline and safety assessment was undertaken 7-days later. Our dose extrapolation calculation from humans to mice was 30 000 cells. To illustrate safety we increased this figure of 30 000 to 300 000 as this represents 10 times the maximum dose that we propose to administer in our human study. Animals were monitored for abnormal clinical signs post injection. Blood for biochemistry was collected and macroscopic and microscopic examinations were carried out on all hMSC treated and control mice. No mortality in reaction to the treatment occurred in any of the mice prior to the scheduled termination. There were no treatment related side effects on the biochemical parameters measured. No macroscopic or histopathological findings were evident in any of the saline or hMSC treated hindlimbs or organs after 7-days. The intramuscular administration of GMP grade hMSCs cells was safe.

70.P06
Stimulation of sub-sonic vibration promotes the differentiation of adipose tissue-derived mesenchymal stem cells into neural cells

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Introduction: Adipose tissue-derived stem cells (AT-MSCs) have been proposed as a new source for nervous tissue damage due to their capacity of neural differentiation. Nowadays, Many studies have demonstrated that sub-sonic vibration (SSV) is an effective cell differentiation method. But there have been no studies on the effect of SSV about AT-MSC differentiation into neural-like cells in vitro. Therefore, we examined the effect of SSV on AT-MSCs to investigate the differentiation potential of neural-like cells.
Materials and methods: Human AT-MSCs were stimulated continuously by SSV (1.0 volts) at frequencies of 10, 20, 30, and 40 Hz for 4 days. After stimulation, we performed cell proliferation assay and measured RT-PCR, Western blot and Immunohistological analysis using neural cell type-specific genes and antibodies.
Results: After exposure, we observed morphological changes of AT-MSCs. Cell bodies adopted a neural-like structure, becoming elongated and spindle-shaped to spherical with several branches. Further, SSV induced gene and protein levels of neural markers and they were mainly up-regulated at 30 Hz.
Conclusions: These results demonstrated that SSV affects AT-MSCs differentiation potential and 30 Hz SSV affected neural differentiation on AT-MSCs.

70.P07
Multi-layered hybrid biomaterial for the engineering of colon tissue

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Objective: Surgical augmentation or patching of colon tissue still represents a major medical problem. We have developed a novel biomaterial designed for the bioengineering of large intestine, and evaluated its biocompatibility, tissue formation and integration in a mouse model.
Methods: Hybrid biomaterials consisting of a decellularized basal membrane (BAM) and a wet-bond layer of PLGA fibers directly elcetrospun onto the abluminal side of the BAM were produced. Elliptical patches were populated in vitro with primary smooth muscle cells (SMC) on the PLGA side and/or oral mucosal cells (OMC) on the BAM side. We then implanted these constructs into the native colon of normally colonized black six mice. Unseeded biomaterials served as control. The grafts were retrieved at postoperative day 14 or 28, and tissue formation, integration and biocompatibility were assessed.
Results: Primary SMC survived and proliferated well on the hybrid biomaterial in vitro. Surgical implantation of the biomaterial was successful and served as barrier for colon bacteria. No animal died due to infection. The hybrid biomaterial, whether previously seeded with cells or not, gave support to the regeneration of new colon wall in vivo. Cell seeding of the biomaterial prior to implantation resulted in a reduction of graft shrinkage of 20% (SMC) and 25% (SMC and OMC), respectively, compared to controls.
Conclusion: This novel hybrid scaffold might be useful for colon engineering in the near future.

70.P08
Neural differentiation of human umbilical cord derived mesenchymal stem cells by sub-sonic vibration

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Introduction: Adult stem cells such as umbilical cord-derived mesenchymal stem cells (UC-MSCs) have the potential to differentiate into various types of cells, including neurons. Mechanical signals have great potential to regulate biochemical signal transduction pathways induced by soluble factors for the control of stem cell differentiation. This study attempted to account for the effects of SSV by describing the proliferation and differentiation properties of MSCs.
Materials and methods: hUC-MSCs were isolated from Wharton's jelly, including the smooth muscle layer of the umbilical cord. SSV was applied to these cells continuously for 5 days. We analyzed using RT-PCR, real time-PCR, and western blotting and immunohistochemical staining.
Results: Growth of cells was inhibited by 30 and 40 Hz SSV. Morphological changes were induced by SSV, and cell shape was very neuron-like. In normal expansion media, downregulation of nestin expression was followed by tissue-specific intermediate filament protein expression. SSV significantly increased gene expression of MAP2, NeuroD1, Neurofilament, GFAP, and O_4.
Conclusion: In this study, the morphology of hUC-MSCs was altered to resemble neurons by SSV. The mRNA and protein levels of neuron-specific markers, including MAP2, NF-L, and NeuroD1, increased. And the other neural cell markers like GFAP and O_4 were increased. These results suggest that hUC-MSCs differentiated into neural cells upon SSV nonselectively.

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70.P09
Comparative study of chitosan/fibroin-hydroxyapatite membrane and collagen membrane for guided bone regeneration on rat calvarial defects: micro CT analysis

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Bone healing and regeneration is one of the most important processes in oral and maxillofacial surgery. Guided bone regeneration (GBR) is well-established therapy that promotes the neobone using a barrier membrane. Required properties of the GBR membrane are bioactivity, bioresorption, and cell viability as well as space-maintaining ability. Much attention has been paid to chitosan-based biomedical materials because of their unique properties such as biodegradability, nontoxicity, and biocompatibility. Silk fibroin acts as an enzyme immobilization matrix with good mechanical properties, and has blood compatibility and good dissolved oxygen permeability in the wet state. Hydroxyapatite, a major inorganic component of natural bone, has been used extensively for biomedical implant applications and boneregeneration due to its bioactive and osteoconductive properties. We have made Chitosan/Fibroin-Hydroxyapatite composite membrane (CFB-HAP) to act as a suitable membrane for bone regeneration. The purpose of this study is to compare the new bone formation and healing in rat skull defect using the new CFB-HAP membrane to collagen membrane (Bio-Gide$^{?}$) which widely used at dental office as a barrier membrane of guided bone regeneration by means of microscopic computerized tomography analysis. The results showed no significant differences between CFB-HAP membrane and collagen membrane in bone volume and bone density, so we concluded that it could be used as a GBR membrane.

70.P10
Novel xeno- and serumfree culture conditions of adipose stem cells for clinical applications

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Human adipose tissue is an abundant source of multipotent stem cells. Human adipose stem cells (ASCs) have shown to have therapeutic relevancy in diverse clinical applications. However, expansion of ASCs is often necessary prior to clinical use. Standard in vitro cell expansion techniques utilize animal-derived reagents which are not recommended in clinical cell therapies due to safety issues. By replacing animal-derived components with xeno- and serumfree (XF/SF) reagents, safety and quality of transplanted ASCs can be enhanced. In the study, an animal component free workflow for isolation and expansion of ASCs was developed. Stem cell characteristics such as immunophenotype, self-renewal capacity and differentiation potential were assessed. These cell characteristics were studied in XF/SF conditions and compared with studies performed in human serum or traditionally used fetal bovine serum supplemented medium. The results showed, that XF/SF conditions maintained the features of ASCs by retaining self-renewal capacity, immunophenotype and differentiation potential into bone-, fat- and cartilage-like cells. Furthermore, chondrogenic differentiation was exceptionally strong in XF/SF conditions. The results suggest that ASCs expanded using an animal-free workflow has great potential in clinical cell therapies, especially for cartilage applications. Since clinical cell therapies using ASCs are under way, a strong focus on safety and quality of stem cells is needed.

70.P11
Restored skeletal muscle function from hydrogel and cell injection

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Introduction: For restored skeletal muscle function of urethral sphincter, we investigated cell-based matrix using hybrid hydrogel (gel) and c-kit (+) human amniotic fluid stem cells (hAFSCs).
Materials and Methods: The hAFSCs was isolated amniotic fluid from women who underwent a routine amniocentesis at a gestational age from 15 to 19 weeks. Using 4-weeks-old female ICR mice, the pudendal nerves on both sides were transected and injected with gel plus hAFSCs into urethral sphincter region. The urine leak point (LPP), closing pressure (CP), real time PCR, and histologic studies were measured 1, 2, and 4 weeks post injection.
Results: The LPP and CP of gel/hAFSC were significantly higher than those of the hAFSCS and gel. The early and middle expressed genes had a high initial expression, and then gradually decreased, while, the late expressed gene had a low initial expression, and then increased for gel and gel/cell group. At 1 and 2 weeks, the injected hAFSC were visible around the urethral sphincter in hAFSCs by human nuclei staining. MyoD and α-SM. MyoD was low in the gel and gel/cell. However, α-SM was strongly detected in gel and gel/cell at 1 week, and maintained expression in gel/cell as time passed.
Conclusion: Hydrogel enhanced cell localization and protected against cell loss, and played very significant role in urethral sphincter regeneration.

70.P12
Technologies for live stem cell transportation

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Described is the utility of a device for the transport of living cellular products for both therapeutic and research markets. Current methodology involves cryopreservation of cells, shipping and distributing cold-chain. Post transport manipulation to resuscitate and passage to overcome the lag phase and remove toxic cryo-protectant is a requirement. These interventions could render a potentially valuable therapeutic product unable to reach the intended market due to added facility and operator costs. This product platform utilizes controlled nutrient release and metabolic waste capture maintaining acceptable conditions in an enclosed, low volume culture and delivery vessel. It is an enabling device facilitating the worldwide shipping of live cell products at ambient conditions utilizing novel technologies allowing attachment dependant cells to be minimally manipulated when using this platform for transport. Ion exchange and controlled release of media components are interfaced with enclosed static cultures of bone marrow derived human mesenchymal stem cells (hMSCs) sealed in the proprietary units over a period of days. The metabolic substrates and products, population number, viability and onset of apoptosis are all interrogated as are the capacity for differentiation and the presence of required surface markers. All of which show a window at which live cell transport is a viable and advantageous alternative to the incumbent cryopreservation system.

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70.P13
In vivo evaluation of O_2 releasing suture material for improved wound healing in hypoxic environments

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Introduction: O_2 plays a pivotal role in wound healing. We therefore have developed an O_2-releasing suture material and evaluated its influence on healing of hypoxic colon tissue.
Materials and Methods: A PGA suture material was coated with PLGA containing oxygen-producing calcium peroxide (CPO) nanoparticles. To evaluate the limitations of our approach Lewis rats underwent an ischemia induction of a bowel segment that consequently was transected and reunified using O2-producing (group A), untreated (B) or PLGA-coated (C) sutures. Mechanical, physiological and histomorphological measurements were performed, at postoperative day (pod) 1, 3, or 7, respectively.
Results: Perianastomotic in vivo tissue oxygen saturation was maintained above baseline level in group A at all assessed pod's, and was significantly higher ($P = 0.020$) compared to B at pod 3. Thickness of colonic mucosa, a surrogate parameter for healing, demonstrated to be significantly more pronounced using O_2-producing sutures compared to untreated or PLGA-sutures. Mechanical stability of the anastomosis was also improved in group A compared to B and C at pod 7.
Conclusion: Oxygen-producing sutures promote anastomotic healing even in challenging environments, and may be clinically used under critical wound conditions in near future.

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71. TE for In Vitro Tests - Part II

71.01
In vivo like tissue fabrication to support efficient and cost-effective drug development

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The tissue engineering market is poised for exceptional growth in the next decade. The global market potential for tissue engineering and regenerative medicine products will exceed $118 billion by 2013. Market opportunities for tissue engineered products based on biological factors such as biomimetic scaffolds, cells (e.g., stem cells) and growth factors range from offering new treatments for neurodegenerative disorders, cancer therapy and cardiovascular diseases up to organ transplantation. Further targets will be providing new solutions for any kind of tissue repair and regeneration. Three dimensional tissues are not only used for regenerative medicine but also as models for drug screening, development and testing in the pharma, medtech and cosmetic industries. Establishing new EU legislations like prohibiting testing cosmetics on animals (testing ban) and a marketing ban of cosmetics tested on animals create an urgent demand for new standardized artificial organomimetic skin models for substance testing. Furthermore, concerns regarding potential health risks of nano-materials released into the environment, and the innovation gap in the pharmaceutical industry strongly drives the development of biological relevant (in vivo like) in vitro tissue models.

A powerful TISSUE fabrication technology based on a novel biomanufacturing technique responds to these challenging industrial requirements: Manufacture on demand skin, lung and bone tissue equivalents in a high throughput format (HTS). The presentation focuses on bio-printing, an innovative technology producing cell-containing scaffolds in a layer by layer deposition process creating 3D tissue specific models. This approach allows manufacturing of composite structures out of matrices, cells and bioactive molecules (e.g., growth factors) that more closely mimic living tissue.

71.02
Keynote: Monitoring type I allergic responses within a microfluidic 3D-co-culture system

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Type I allergies affect more than 25% of the world's population and include a wide range of IgE-mediated disorders such as hay fever, asthma, food allergies, anaphylactic shock and atopic dermatitis. The development of new tools for improved allergy diagnosis and for safer, more efficient allergen-specific immunotherapy, are vital to the prevention and treatment of allergy related diseases. Standard in vitro methods that employ 2D cell monocultures that are grown under static conditions in plastic substrates have shown to exhibit low reliability, reproducibility and accuracy. To increase the accuracy of cell-based assays, it is important to consider that in vivo cells cohabitate in three dimensional cell communities that form tissue. Here, microchip technology is likely to provide the next generation of cell analysis tools capable of testing small cell populations in environments of increased physiological relevance. In the presented work, biochips containing embedded contactless dielectric microsensors are combined with microfluidics to create a 3D human artery wall model capable of continuously and non-invasively monitoring dynamic cell population responses.

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72. Cooperative Research - European Projects and Networks

72.01
Keynote: ANGIOSCAFF

J Hubbell
Ecole Polytechnique Fédérale de Lausanne (EPFL), Switzerland

No abstract available.

72.02
Four approaches to improve neuro implants-Nano4Neuro

B Schlosshauer, H Hartmann, M Doser, A Pêgo, E Fattal, M Wiberg and J Kjems
NMI Reutlingen, Germany; ITV, Germany; INEB, Portugal; CNRS, France; IMB, Sweden; iNano, Denmark

Neuronal regeneration depends partly on the continuity of the nerve tissue. In the human peripheral nervous system nerve gaps are bridged by autologous nerve transplants or simple nerve guide tubes. For the spinal cord no implants have yet been approved. To improve implant performances, we employ four tissue engineering strategies. (A) Pharmacological intervention with siRNA is used in order to desensitize regrowing axons with respect to repulsive components of fibrotic and glial scars. (B) The induction of neovascularization is enhanced to foster rapid nutritional support. (C) Biological micro-guidance cues are provided by recruiting Schwann cells as bands of Bungner as evident in vivo. (D) Novel biomaterials are developed either as nerve guide tubes or as injectable hydrogels, providing guidance and substrates for neuronal regeneration.The siRNA silences a central messenger pathway (GTPase RhoA) onto which various repulsive signals converged. Neovascularization is achieved in sponges synthesized from selected collagen fragments. Micro guidance of axons via glia cells was realized by hundreds of polymer filaments inserted into nerve guide tubes or directly into lesioned sciatic nerves. Biomaterials are based on in situ enzymatically crosslinked hydrogels or on resorbable polymers. Grant support: BMBF 01KQ0902R, EuroNanoMedicine 13N11036.

72.03
EuroSTEC, Soft Tissue Engineering for Children

W Feitz, P Geutjes, E Oosterwijk, W Daamen and T Kuppevelt
RUNMC, The Netherlands

EuroSTEC; Soft tissue engineering for children. A review of progress of 5 years of collaborative work of 15 European partners. Through modern tissue engineering approaches we have developed new insights so that they can be used to treat children with structural disorders present at birth in the future, such as spina bifida, urogenital defects, gastroschisis, diaphragmatic hernia and oesophageal atresia. A translational route through in vitro and animal experiments have lead to early clinical trials. Ethical and regulatory issues have been addressed. A variety of cells, including amniotic stem cells, fibroblasts, muscle cells and urothelial/epithelial cells have been cultured in vitro and when possible seeded into biomatrices. Biomatrices prepared with and without cells, have been implanted using different novel animal models for major congenital birth defects, and evaluated for their capacity to regenerate the correct tissues. The project combined European leaders in the field of biomatrices, cell culture, animal models, surgery, and ethical and regulatory issues. Overall developments and management aspects as well as scientific outcome will be presented.

72.04
Keynote: Gene activated matrices for bone and cartilage regeneration in arthritis - GAMBA – a EU-funded project

C Plank, M Alini, M Murphy, O Zelphati, P Borget, G van Osch, C Gentili, G Daculsi, K Zöller and M Anton
Institute of Experimental Oncology and Therapy Research, Technische Universität München, Germany; AO Research Institute Davos, Switzerland;Regenerative Medicine Institute, National University of Ireland Galway, University Road, Ireland;OZ Biosciences, France;Biomatlante SA, Vigneux de Bretagne, France; Department of Orthopaedics and Otorhinolaryngology, Erasmus MC, University Medical Centre Rotterdam, The Netherlands; Department of Oncology, Biology and Genetics, University of Genova, Istituto Nazionale per la Ricerca sul Cancro, Italy; INSERM Université de Nantes Faculté de Chirurgie Dentaire, France; ScienceDialogue Dr. Karin Zöller, Maren Schüpphaus and Sven Siebert GbR, Germany

The GAMBA consortium develops a gene-activated matrix platform for bone and cartilage repair with a focus on osteoarthritis-related tissue damage. The scientific and technological objectives of this project are complemented with an innovative program of public outreach, actively linking patients and society to the evolvement of this project. The GAMBA consortium aims at implementing a concept of spatiotemporal control of regenerative bioactivity on command and demand. This is achieved with gene vectors comprising growth factor genes under the control of promoters which are individually inducible with endogenous biological and exogenous physical or pharmacological stimuli. The gene vectors are co-embedded with mesenchymal stem cells in different modular compartments of a composite biomaterial matrix consisting of an embedding hyaluronan polymer matrix with engineered thermal properties and Micro Macroporous Biphasic Calcium Phosphate (MBCP™) providing mechanical support. Spatiotemporal control of bioactivity and responsiveness to physiological conditions is represented, firstly, in the spatial distribution and release profiles of gene vectors within the composite matrix and, secondly, by controlled differential induction of growth factor gene expression. We report on the concepts, objectives and some preliminary results of the GAMBA project which is funded in 7th Framework Programme of the EU, grant number NMP3-SL-2010-245993.

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73. Education in TERM (in coop. Expertissues)

73.01
Keynote: The European perspective: short courses in the EC FP7 "EXPERTISSUES" Network of Excellence

P Hatton
University of Sheffield, UK

Education is one of the most important subjects in the field of tissue engineering and regenerative medicine. It is important in that the field is rapidly evolving, and sound educational programmes are therefore essential in both developing young talent and providing research groups with the skills and knowledge necessary to make progress. It is all the more important in that it education and training affect the whole community including clinicians, academics, and industry-based scientists and engineers. Given this key place, the EC funded FP6 Network of Excellence "EXPERTISSUES" (now the European Institute of Excellence for Tissue Engineering and Regenerative Medicine) included education and training as a key activity. As a key part of this activity, the Network developed a number of short training courses to explore the opportunities for European and International training, and from our experiences we have identified a number of best practices. This presentation will review these education and training activities, many of which have continued to evolve and are still available as a service to the global TERM community. The author wishes to acknowledge all the EXPERTISSUES partners and the EC for funding under the 6th Framework Programme (NMP3-CT-2004-500283-2).

73.02
Keynote: Industry perspectives on education & training needs

H Hasibeder
CellSeed Inc. & CellSeed Europe, Japan

The tissue engineering and regenerative medicine industry is relatively young compared to the medical device or pharmacetical equivalents, but it is already clear that a specific set of skills and attributes are required for workers in this emering sector. This presentation will provide an industrial perspective on the educational needs, the value of current programmes, and the future trends in the tissue engineering industry from a global perspective.

73.03
Keynote: Challenges in tissue engineering education: South-East Asian perspective

J Goh, E Yim and M Raghunath
National University of Singapore

Tissue Engineering is an inter-disciplinary topic and as such present a number of challenges in the formulation of an educational curriculum. The course teachers must be practitioners and research-active with good clinical connections to provide relevant mentorship. We have found that problem based learning with a healthy emphasis on hands-on practical and student presentations are extremely valuable. In terms of the types of tissues to focus on in the educational curriculum, particularly in the South East Asia region is bone regeneration for reconstruction and skin tissue engineering for burn treatment. These are the unmet needs important to countries where there are high incident of traffic accidents and recovery from 'recent' war. An interesting point is the religious consideration needed when using certain animal tissues and cells as teaching materials in the South East Asia region.

73.04
Keynote: Undergraduate research experiences and career trajectories

E Brey, ME Faurot, AA Meyer and NG Lederman
Illinois Institute of Technology, USA

Government and academic institutions support undergraduate research with an expected outcome of broadening participation in science and engineering careers. The Illinois Institute of Technology is highly supportive of undergraduate research with many projects focused on tissue engineering. The goals are to immerse undergraduates in cutting-edge research in an effort to influence their long-term interests in science and engineering. The programs are also intended to inform undergraduate students' understandings about research design and practice. The program's influence was explored using pre- and post-Likert tests and open-ended survey items coupled with a content test and semi-structured interviews. Data analysis of survey items focused on career and research topics were on average above 4 on a 5-point Likert scale suggesting that the students had high expectations of the program initially and that these expectations were met upon program completion. Additional findings fell into three main categories: understandings about the process of research development and practice, career and graduate school decisions, and role of laboratory personnel. Implications of these findings include a framework that might directly inform the improvement of undergraduate research programs. In addition, undergraduate research programs have the potential to engender undergraduate students' informed conceptions of engineering research design and practice early on in their career trajectories.

73.05
Keynote: Experiences of leading a taught MSc in cell & tissue engineering

N Forsyth
Keele University, UK

The MSc programme is designed to deliver a combination of both didactic and experiential learning. This is achieved through the combination of lecture-based teaching, laboratory-practical and associated reporting and the research dissertation. The development and maintenance of these activities is essential to maximize student learning. The research areas associated with Cell and Tissue Engineering are fast changing with significant changes occurring between academic years and even in some instances academic semesters. The application of didactic learning approaches can present a unique challenge where established theory becomes research-driven postulation at the MSc level. This presentation will discuss these challenges and provide examples which demonstrate the rate of change in a fast-flowing field. In addition we will provide an overview of research project design which enables both student and supervisor to retain engagement and motivation.

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73.06
Keynote: A practical approach for developing an undergraduate tissue engineering laboratory class

M Micou and D Kilkenny
University of California, USA; University of Toronto, Canada

Over the past decade, there has been a tremendous increase in the number of biomedical engineering and bioengineering programs offering lecture courses in tissue engineering, yet very few offer a complementary lab course. The small number of existing lab courses may be attributed, in part, to a lack of widely available instructional materials. To help meet this need, fifteen tissue engineering experiments that are suitable for use in an instructional setting were developed and will be published by CRC Press/Taylor Francis in new textbook entitled A Laboratory Course in Tissue Engineering. The lab exercises are based on both classic tissue engineering experiments and modern techniques and emphasize the importance of engineering analysis, mathematical modeling, and statistical design of experiments. Furthermore, experiments were developed to conform to a schedule commonly used for undergraduate lab courses and most use a shared set of equipment that is commonly found in labs equipped for tissue culture. This presentation will provide an overview of these experiments and suggestions to facilitate the development of a hands-on tissue engineering undergraduate course.

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74. EuroStec

74.P01
Regenerative urology clinical trials: an ethical assessment of road blocks and solutions

A Oerlemans, W Feitz, E van Leeuwen and W Dekkers
Radboud University Nijmegen Medical Centre, The Netherlands

Two clinical applications of tissue engineering have been described within experimental studies for bladder and urethra reconstructions, but extension of the described techniques to the broader urological patient population has not happened so far. In this paper we aim to identify the ethical road blocks in the clinical evaluation of tissue engineered products under the new EMA ATMP regulations for pediatric urological conditions and, ultimately, to recommend strategies to overcome them. The use of human tissue engineered products (HTEPs) to treat children with congenital urogenital defects poses challenges in the clinical testing phase, connected to three features of the application of this treatment in this patient group: (1) those associated with the product, namely the multifaceted complexity of the HTEP; (2) those connected to the procedure, namely the lack of an RCT-tested gold standard to compare the new treatment to and difficulties surrounding standardization of the treatment protocol; and (3) the patient's young age and associated problems concerning possible long term effects and the informed consent process. Due to these problems, a conventional RCT is not the methodology of choice to evaluate this treatment in this patient group. The unpredictability of HTEPs necessitates stringent and long term surveillance and registry to ensure the safety of patients treated with these products.(This paper is part of the EuroSTEC project.)

74.P02
Patterns of moral argumentation in the discussion of tissue engineering

A Oerlemans, M van Hoek, S van der Burg, W Dekkers and W Feitz
Radboud University Nijmegen Medical Centre, The Netherlands

In a debate of any new technology, there are certain arguments and patterns of argumentation that occur repeatedly. In their 2007 paper, Swierstra and Rip identify characteristic tropes and patterns of moral argumentation in the debate of ethics of new and emerging science and technology (or 'NEST-ethics').If Swierstra and Rip are correct, we should be able to identify these patterns in the discourse around around tissue engineering (TE). Taking their NEST-ethics structure as a starting point, we will consider whether all relevant issues are present in the discussion, and argue what aspects we think ought to be a part of a rich and high-quality debate of TE.This paper will attempt to answer the following questions: (1) Can we distinguish the characteristic recurrent tropes and argumentative patterns of NEST-ethics as described by Swierstra and Rip in the discourse surrounding TE? (2) What aspects of argumentation are missing from the discussion of TE? Why do we think these aspects deserve attention? (3) Based on the analysis of the TE debate, what do we feel is lacking from the structure of NEST-ethics as put forth by Swierstra and Rip? (This paper is part of the EuroSTEC project.)

74.P03
Urethra repair using smooth muscle cell seeded collagen-based scaffolds

LF Arenas, L Micol, D Tiemessen, W Daamen, T Kuppevelt, E Oostenwijk, P Frey, P Geutjes and W Feitz
Radboud University Medical Centre, Nijmegen, Urology, The Netherlands; École Federale Polytechnique, Switzerland; Radboud University Medical Centre, Biochemstry, The Netherlands

Introduction: Some clinical conditions involving the urethra may be a challenge for surgical repair due to a limited source of available tissues. Investigators tested variable kind of biomaterials. The objective is to investigate the role of the smooth muscle cell (SMC) for urethra regeneration.
Material and methods: One centimetre Tubular 0.5 wt% collagen-based scaffolds were constructed using subsequent freezing, freeze drying and carbodiimide crosslinking techniques. 32 New Zealand rabbits underwent an open bladder biopsy for SMC harvesting. SMC were isolated, cultured and labelled with EdU. For cellular group, 1.5 106 SMC were seeded. The tubes underwent o/n rotation, subsequent static culture prior to in vivo implantation for correction a circumferential urethral defect. Urethrograms were performed after 1 and 3 months follow up. The penises were sended to histology. (H&E, IHC and EdU).
Results: The group of seeded scaffolds presented 1 stricture after 1-month (25%) and 1 fistula after 3 months (25%). For the acellular group, 3 strictures were found after 1 month (75%) and 1 after 3 months (25%). Histological analysis revealed more muscle cells, vessels and less inflammatory cells compared to the acellular group. All scaffolds showed luminal urothelial revetment. In the 3 month seeded group hair follicles were observed. EdU positive SMC were detected after 3 months.
Conclusion: The SMC was related to improved results in rabbit model. EdU-SMC is detectable after 3 months in vivo.

74.P04
Pilot study on early versus late ovine fetal interventions in esophagus tissue engineering

AK Saxena, H Ainoedhofer, H Hader and ME Höllwarth
Medical University of Graz, Austria

Background: The aim of this pilot study was to compare early versus late ovine fetal interventions with regards to outcomes.
Methods: Fetus from Austrian Mountain Sheep were selected for interventions between the 70–80-day (Group-1, $n = 3$) versus 120–130-day (Group-2, $n = 3$) of gestation. The fetuses were exteriorized and the fetal neck was exposed to obtain full thickness biopsies of 1–3 cm from the esophagus after which an end-end anastomosis was performed. A laparotomy was performed and the omentum was exposed. The esophageal biopsies obtained were processed under sterile conditions to obtain organoid units (OU) which were seeded on collagen scaffolds, tubularized and implanted into the omentum.
Results: Outcomes of the surgical procedures were evaluated on the following criteria: (1) Biopsy size: Cervical esophageal biopsies of 1–3 cm were obtained in both groups, however in Group-1 reanastomosis was not possible in 3 cm and a collagen conduit was used for replacement. (2) In-situ tissue engineering: Marked differences in omental size and consistency were observed, however construct implantations of 4 cm were successful in both groups.(3) Fetal mortality: In Group-1, there

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was cardiac arrest for unknown reason in one ewe after the procedure, with normal term deliveries in rest of the lambs. (4) Quality of engineered esophagus was comparable in both groups.
Conclusions: Fetal interventions are feasible in ovine esophageal tissue engineering.

74.P05
Coverage of an experimental spina bifida defect by gluing a growth factor loaded collagen scaffold improves the neurological outcome

P Geutjes, A Eggink, L Roelofs, J Crevels, I Heschel, L Olde-Damink, D Tiemessen, E Oosterwijk, T van Kuppevelt and W Feitz
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Introduction: Intra-uterine coverage of spina bifida may protect the neural tissue and improve neonatal outcome. The aim of the study is to investigate whether neurological damage can be prevented by covering an experimental spina bifida lesion with a growth factor loaded scaffold.
Materials and methods: In 32 fetal lambs a spina bifida lesion was created surgically at 77–79 days' gestation. In the control group the defect was left uncovered (group 1). In the treated groups, the defects were covered with membrane scaffolds (Matricel GmbH) (group 2), or with growth factor loaded membrane scaffolds (VEGF and FGF-2) (group 3). The scaffolds were fixed using running sutures. In an additional group the growth factor loaded scaffolds were glued using fibrin glue (group 4). Neurological and histological evaluation was performed 1 day or 3 months after delivery.
Results: The survival rate was 53%. Gluing the scaffold on the experimental spina bifida defect showed a significant improved neurological outcome (group 4). Four out of six lambs could walk, drink and censored pain in the legs. In the uncovered group (group 1) all lambs had loss spinal function. In both sutured groups (group 2 and 3) about 50% showed improved neurological outcome.
Conclusion: Covering the experimental spina bifida by gluing the growth factor loaded membrane scaffold improved the neurological outcome. Although to confirm, the scaffolds with VEGF and FGF-2 had a beneficial effect on wound healing.

74.P06
Biodegradable, net-like scaffolds significantly improve the mechanical properties of tissue engineered skin grafts

F Hartmann-Fritsch, T Biedermann, E Braziulis, J Luginbühl, L Pontiggia, S Boettcher-Haberzeth, T Van Kuppevelt, KA Faraj, C Schiestl, M Meuli and E Reichmann
Tissue Biology Research Unit, Department of Surgery, University Children's Hospital Zurich, Switzerland; Department of Surgery, Pediatric Burn Center, Plastic and Reconstructive Surgery, University Children's Hospital Zurich, Switzerland; Department of Matrix Biochemistry, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, The Netherlands

Extensive skin loss, such as skin defects associated with deep burns or other trauma, represents a significant clinical problem. A promising approach to treat large skin defects is the use of tissue engineered full thickness skin analogues with near normal anatomy and function. In addition to their excellent biological properties, such skin substitutes should exhibit optimal structural and mechanical features. This study aimed to test novel dermo-epidermal skin substitutes based on collagen type I hydrogels, physically strengthened by two types of polymeric net-like meshes. One mesh is already in clinical trials, the second one consists of a clinically established material. Both meshes were integrated into collagen type I hydrogels and dermo-epidermal skin substitutes were generated. Skin substitutes were transplanted onto immuno-incompetent rats and analysed three weeks thereafter, employing histology, immunofluorescence and scanning electron microscopy.The substitutes exhibited a well stratified epidermis that had homogeneously developed over the entire surface of the grafts. The epidermis had deposited a functional basement membrane and dermo-epidermal junction, displayed a well defined basal cell layer, several suprabasal strata and a stratum corneum. Additionally, the grafts were well vascularised.These novel dermo-epidermal skin substitutes are very promising skin analogues for treatment of full thickness skin defects.

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75.1. Commercialization of Regenerative Products: The Academe/Industry Partnership

75.1.01
Commercialization of regenerative medicine products: the academia-industry partnership

A Vertès
London Business School, Sloan Fellowship, UK

Partnering trends reveal stem cell technology deployment by big Pharma is fast occurring, but these large firms focus on adopting stem cell-based research and development tools for preclinical and toxicology studies of conventional compounds. Therapeutic stem cell technology adoption is still lagging, with the notable exceptions of the Osiris-Genzyme-Sanofi, Athersys-Pfizer, and Mesoblast-Cephalon-Teva partnerships. Technology and market risks are chief hurdles to large corporations, and big Pharma remain conservative in their adoption of radical innovation requiring clear-cut confidence-in-safety, confidence-in-efficacy, and demonstration through commercialization of the first cell-based therapies. Breakthroughs needed include a clearregulatory path, a higher degree of consolidation between biotechs in this field, and a proven business model that mid-size Pharma might deliver first in their race to compete. Big Pharma-Academia incubators might also play a key role here. Considering their lower technologicalhurdles, deals on mesenchymal stem cells or haematological stem cells are likely to occur first, with a focus on allogeneic stem cell technologies since autologous ones better fit entities with large service components such as hospitals. Clinical demonstration of benefit and understanding mechanisms of action in vivo are the obvious key enablers of the nascent industry, with the first successes, or additional failures, being of critical importance to maintain momentum.

75.1.02
Commercializing regenerative medicine therapeutics and sustaining a successful business

A Arshad
Scientia Advisors, Cambride, USA

Commercializing therapeutics and sustaining a successful business requires a compelling value proposition. To ensure a therapy is available to whomever needs it on a global basis, there are some keyconsiderations when developing a value proposition:- Address unmet needs-Build awareness among early adopters and followers,- Develop a sound business and economic case to key stakeholders including payers,- Proactively cross all the regulatory barriers,- Develop a channel and network of alliances and manufacturing capabilities. Post launch success involves additional approaches in life cycle management, proactive competitive messaging, continued innovation, and customer relationship management. While this is true for anytherapy, the field of regenerative medicine, in particular presents a much greater challenge due to the relative immaturity of the industry and complex regulations. Ahmed will provide an overview of how companies have thus far performed in addressing these challenges. Particular focus will be on the need for organizations to be 'market focused' while building the framework for their overall business approach to develop and successfully commercialize regenerative therapies. Ahmed will also discuss the need for close coordination between academia, industry, and investors to address the shortage of capital that has limited the advancements of potential breakthrough products.

75.1.03
Translating academic concepts to commercial medical products: A complex academic/industrial partnership

A Coury
Genzyme, USA

Many concepts for medical products that significantly improve patient care come from academic faculty. Their ideas can be tested with limited funding often procured from public or private non-profit sources with much freedom for discovery research. Beyond this stage, costs ramp up rapidly and require successive funding. Dozens of requirements for success should be considered before initiating product development. They include: market projections, legal, regulatory and clinical requirements, human and physical resources, exit strategy and other factors. These imperatives generally impose constraints on a full-time academic's leadership capabilities beyond exploratory stages and technology transfer to a start-up or established company is warranted. During product development, several regulation-mandated stages with escalating costs occur. Applying start-up company 'survivalist' principles of lean, urgent operation with leadership by a 'champion' is usually effective at every stage. Adequate funding must be secured through relationship building, advanced pre-clinical, sometimes clinical progress and strategic publications. The academic institution must buy into a development plan that is not excessively onerous to the ultimate investor. Ideally, academic-industrial transitions proceed in consideration of the mutual benefit of all of the stakeholders.

75.1.04
Commercializing T cell immunotherapies

R Knaus
Cell Media, UK

Cell Media's business model is based on the commercialization of T cell immunotherapies for which clear evidence of safety and efficacy has been provided by academic studies.

Over the last 20 years, hundreds of patients have been treated with antigen-specific T cells in numerous investigator sponsored clinical studies for infections and cancer. Despite compelling evidence of safety and efficacy from these trials, most products have not achieved widespread clinical adoption and reimbursement. Cell Medica's lead commercial products include Cytovir CMV for the treatment of cytomegalovirus (CMV) infections in bone marrow transplant recipients and Cytorex EBV for the treatment of Epstein Barr Virus (EBV) associated lymphomas. Both products were clinically developed by academic groups for more than 15 years, without achieving widespread clinical adoption. Academic efforts to establish the clinical adoption of cell therapies have suffered from a variety of factors including: the lack of data from randomized controlled trials proving a hard clinical endpoint and pharmaco-economic data, the complex logistics for procurement of donor material and the timely delivery of the product to the patient, evolving and untested regulatory challenges, inefficient and non GMP-compliant manufacturing processes that are not scalable for commercial manufacturing, the lack of a solid patent protection in light of published results and the lack of financial resources to take cellular therapies from academic trials to widespread commercial application. Cell Medica will present examples how these challenges can be overcome and how a profitable business can be built to make these prod-

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ucts commercially available to address important unmet medical needs.

75.1.05
Successful translation of academic research using combinations of mesenchymal stromal cells with pharmaceutical agents for clinical applications in regenerative medicine

P Ghosh
Mesoblast Ltd, Australia

Mesenchymal stromal cells (MSC) are a source of self-renewing, multipotent undifferentiated cells that may be isolated from bone marrow, adipose tissue, muscle, synovium etc which possess the capacity to differentiate into cells of the mesenchymal lineage such as bone, cartilage, and tendon. This property offers the potential of repairing or regenerating injured or degenerate tissues of the musculoskeletal system. Apart from engraftment at sites of injury followed by differentiation into the required cellular phenotype, MSC also exert paracrine effects on the injured cells thereby supporting their recovery. Over the last several decades a plethora of research publications have reported that transformation of MSC into differentiate mesenchymal cells requires a variety of cues, including a suitable extracellular matrix or inductive scaffold, mechanical stimuli, and exposure to growth factors (GFs) particularly members of the FGF, IGF, PDGF, and TGF-beta super-families. Unfortunately, co-administration of MSC with GFs to achieve localized tissue repair in patients can present significant problems. Notwithstanding the high cost of most recombinant GF, they generally have short half-lives in vivo and may provoke adverse local reactions such as fibrosis or ectopic bone formation. An alternative strategy is to formulate MSC with a pharmaceutical agent that possess the capacity to act on the MSC to promote their differentiation along a specific pathway while concomitantly enhancing their viability and replication. Examples of the success of such an approach are provided by the combination of MSC with Hyaluronan, embryonic Heparan Sulfate or Pentosan Polysulfate, which from their origins in academic laboratories are now under commercial development as novel therapeutics for a variety of applications in regenerative medicine.

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75.2. The Regulatory Imperative International Perspective

75.2.01 Lecture on the US regulatory environment of regenerative medicine

C Witten
FDA's Center for Biologics Evaluation and Research [CBER], USA

Research in the field broadly defined as tissue engineering/regenerative medicine (TE/RM) includes a variety of areas such as cell biology, genetic engineering, biomaterials, and development of animal models of disease. Collaborations across institutions are common, including scientific collaborations across borders. It is a promising time for the regenerative medicine field, and hopefully will lead to development of products for important clinical needs. In the United States, regenerative medicine products, like other medical products, meet the definition of device, drug, biologic, human tissue, or a combination of these definitions. With the exception of a subset of human tissue all medical products are subject to some level of premarket review. The characteristics of the regulatory pathway, premarket review, and other requirements follows as a consequence of the regulatory definition a specific product meets. The United States began to actively work toward a regulatory scheme for the current emerging area (TE/RM) in the late 1980's and early 1990's. Early efforts were primarily focused on developing a product review paradigm for specific products under development. For example, development of novel interactive wound dressings during this time period led FDA to leverage its experience to adapt a regulatory approach to the products. The approvals of Apligraf in 1998, and Dermagraft, in 2001, were the first approvals of cell-scaffold combination products. Although comprehensive regulations for cellular and tissue-based products were not formalized until 2005, the review of these products as devices incorporated consideration of the principles needed to ensure safety from communicable disease transmission from the donor to the recipient of the product as well as appropriate control of product manufacture to insure the consistent production of safe and effective cellular constructs. The approval process for a combination of bone morphogenetic protein (BMP) on a collagen sponge, in a spinal cage for spinal fusion, led to an increasing recognition of the need for cross-disciplinary scientific review teams for TE/RM products, mirroring the multidisciplinary development teams in the scientific/development community. Concurrently, early development in the areas of cellular therapy and gene therapy led to initiation of dialogue between FDA and researchers/product developers in these areas. A critical step in FDA's development of a comprehensive scheme for human cell and tissue based products was the announcement in 1997 of the "Proposed Approach to Regulation of Cellular and Tissue-Based Products". This proposal was an attempt to address the fragmentation of the regulatory approach towards these products (both traditional tissue-based products and innovative cell or tissue-based products. This proposal was implemented with the development of a set of risk-based rules governing registration and listing, donor eligibility, and good tissue practices, finalized in 2005 in FDA regulations collectively referred to as "the tissue rules" (21 CFR Part 1271). Along with the development of the tissue rules, as noted above, the need to integrate reviews and share knowledge across the centers has led to an increase in consultation and dialogue in these areas across the FDA's three medical product centers, the Center of Device and Radiologic Health (CDRH), the Center for Biologics Evaluation and Research (CBER), and the Center for Drug Evaluation and Research (CDER). In addition to an increase in cross-center reviews for specific products these efforts have also resulted in other beneficial outcomes including several cross-center guidance documents, for sponsors in this area. Documents for development of wound healing products,, cellular therapy for cardiac disease, and cartilage repair products are examples. Similarly, planning for FDA Advisory Committee Meetings and other activities involving the TE/RM field commonly draws on expertise from CBER, CDER, and CDRH. For example, FDA held an Advisory Committee meeting in June 2011 on cell and gene therapy product development for retinal diseases. Because of its cross-cutting nature of the topic, the planning committee included staff from across FDA. Similarly CBER and CDRH have established several cross-center training efforts in the TE/RM area. These include several ongoing seminar series for staff scientific training in both general TE/RM topics, as well as in more specific niche areas. In addition the CDRH and CBER have established, under the auspices of the FDA Commissioner's Fellowship Program, a Regenerative Medicine Fellows Training Program. This program allows non-FDA engineers, physicians, and scientists to participate in an intensive two-year fellowship training in the regulatory science and review of regenerative medicine products. The long-term aim is to help develop a cadre of professionals both inside and outside the FDA who are knowledgeable about the developmental and the regulatory process for products in the TE/RM field. Although development by FDA of a scientifically informed, comprehensive and increasingly integrated regulatory framework for TE/RM products is beneficial, FDA is not the only US government agency with the potential to influence the direction of this emerging field. In fact, more than a decade ago recognition of this fact led to the creation of the Multi-Agency Tissue Engineering Science interagency working group (MATES) to provide a platform with which member agencies can interact and exchange information on their activities in the TE field. MATES includes representation across the government from regulatory, research, and funding agencies (for example FDA, National Institutes of Health (NIH), National Institute of Standards and Technology (NIST), National Science Foundation (NSF), and Department of Defense (DOD)). MATES interactions have lead to multiple joint projects over the years such as workshops, targeted funding announcements, and website (www.tissueengineering.gov) The FDA also has engaged in significant outreach to stakeholders in TE/RM. In addition to FDA presentations at scientific meetings. FDA has co-sponsored (or helped organize) a number of symposia and freestanding workshops with various partners. Examples include the 2007 FDA-NIST In Vitro Characterization Workshop, the 2009 NIH-JDRF-FDA Workshop on Next Generation Beta Cell Transplantation: How Good Does a Beta-Cell Have to Be?, and the 2011 FDA-NIH Workshop on Pluripotent Stem Cells in Translation: Early Decisions. The FDA has produced a series of webcasts with basic information regarding the regulatory process (OCTGT WebLearn and CDRH WebLearn). The agency has published guidance documents that provide the agency's official thinking on a number of regenerative medicine related product development topics. The Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) meetings serve to provide FDA with outside expert advice on specific issues in a public setting. Liaison meetings with various broad-based stakeholder groups provide another opportunity for information exchange. Although science is international, medical product regulations in each country differ based on differences in political systems or philosophy. In order to allow progress in emerging fields to progress safely, regulatory agencies are challenged with interpreting their current regulations in such a way as to maintain the balance between safety and innovation. The most important factor in international convergence is a mutual understanding of the underlying scientific questions that are raised in application of the regulations, particularly in novel emerging technology areas. FDA is actively involved in activities to advance international convergence of regulatory efforts. Our principal partner in these efforts since 2007 has been the European Medicine Agency (EMA). FDA/CBER/Office of Cellular, Tissue, and Gene Therapies (OCTGT) and EMA/Advanced Therapy Medicinal

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Products (ATMP) have regular bilateral discussion under FDA-EMA Bilateral Agreement. FDA and EMA have also participated in other international forums such as ICH and the Asia Pacific Economic Cooperation Life Sciences Innovation Forum (APEC/LSIF). There is an increasing international understanding of the various regulatory models which has the potential for reduction of duplicative product development. There also are international efforts of importance in other related areas, such as biovigilance initiatives, efforts to improve clinician recognition of, and global communication about, serious adverse events and reactions related to clinical use of human organs, tissues, and cells (including assisted reproductive therapies), efforts in the areas of xenotransplantation, validation of alternative methods to animal testing, and membership in scientific standards organizations such as ASTM International and ISO. In summary the regulatory environment for (TE/RM) in the United States in 2012 is a rapidly maturing, increasingly coordinated framework of risk-based regulations that reflect the scientific characteristics of the varied types of products that fall under the rubric of TE/RM so that there is a pathway that allows for any specific product to succeed based on its scientific/clinical merits.

75.2.02 Characterisation of cells for ATMPs

A Wilson
CellData Services, UK

Characterisation is a key aspect of development of ATMPs. Understanding of the identity, purity and functionality of the cell population and finished ATMP is critical for several reasons. It is essential to understand how the chosen population of cells behaves throughout the manufacturing process. The use of biological reagents to induce differentiation, processing to remove impurities, and the basic cell expansion process itself will have measurable effects on the cells. Whether a process is intended to maintain a specific phenotype or to induce differentiation, this will need to be demonstrated by data confirming that the desired population is maintained or produced by the process. Combination with scaffold materials, medical devices etc can also induce changes in phenotype or behavior and this, too, needs to be quantified. It is important to assess functionality of the cells at key points in the process, whether by protein expression or demonstration of a particular required function. Specifications for the cells and for the finished product are generally derived from a subset of the characterisation tests applied during development. The characterisation process allows for identification of key tests that can reliably indicate that the process is under control and that the product is acceptable from the quality perspective, and also supports the setting of data-driven limits for the specifications. Regulatory authorities will require characterisation data to assess comparability between different product iterations, for example in respect of changes to the process between different stages of clinical development, and following post-authorisation changes, perhaps scale-up or transfer to a new manufacturing site.

75.2.03 Regulatory clarity to maximize the success of advanced therapies

MP Martinez
TiGenix, Spain & Belgium

Characterisation is a key aspect of development of ATMPs. Understanding of the identity, purity and functionality of the cell population and finished ATMP is critical for several reasons. It is essential to understand how the chosen population of cells behaves throughout the manufacturing process. The use of biological reagents to induce differentiation, processing to remove impurities, and the basic cell expansion process itself will have measurable effects on the cells. Whether a process is intended to maintain a specific phenotype or to induce differentiation, this will need to be demonstrated by data confirming that the desired population is maintained or produced by the process. Combination with scaffold materials, medical devices etc can also induce changes in phenotype or behavior and this, too, needs to be quantified. It is important to assess functionality of the cells at key points in the process, whether by protein expression or demonstration of a particular required function. Specifications for the cells and for the finished product are generally derived from a subset of the characterisation tests applied during development. The characterisation process allows for identification of key tests that can reliably indicate that the process is under control and that the product is acceptable from the quality perspective, and also supports the setting of data-driven limits for the specifications. Regulatory authorities will require characterisation data to assess comparability between different product iterations, for example in respect of changes to the process between different stages of clinical development, and following post-authorisation changes, perhaps scale-up or transfer to a new manufacturing site.

75.2.04 Regulatory experiences learned in the clinical development of MultiStem

RW Mays
Athersys, Inc., USA

Athersys, Inc. is a clinical stage biopharmaceutical company engaged in the discovery and development of therapeutics designed to extend and enhance the quality of human life. The driving technology platform at Athersys is MultiStem®, a patented and proprietary cellular product consisting of a clinical grade preparation of adult (non-embryonic) stem cells obtained from bone marrow. MultiStem appears to provide benefit through several mechanisms, primarily, via the production of therapeutic proteins and other molecules produced in response to inflammation and tissue damage. Athersys believes that MultiStem represents a unique 'off-the-shelf' stem cell product based on its apparent ability to be used without tissue matching or immunosuppression and its capacity for large-scale production. MultiStem has received regulatory allowance from the FDA and multiple countries in the EU for testing in man and is currently in Phase I/II or Phase II clinical trials for treatment of ischemic stroke, inflammatory bowel disease, myocardial infarction and bone marrow transplantation and oncology treatment support. The regulatory experiences working with FDA and EMEA officials during the clinical development of MultiStem will be discussed.applications in regenerative medicine.

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75.3. Making Collaborations Work

75.3.01
"Establishing a multibillion-dollar cell therapy industry requires a simple ABC approach - Academics, Businesspeople & Clinicians working together to integrate basic discovery, clinical translation and commercialization into a seamless process'

C Mason
University College London, UK

Deploying living cell as therapies is a disruptive technology - a step-change in patient care, clinical practice and business models by providing life-changing and even potential cures for a broad spectrum of medical needs. The cell therapy industry (CTI) had global sales of $400M in 2008, and is predicted to grow to $5B in 2014 with even greater growth expected to follow1. However, these revenue forecasts are dependent on the industry overcoming major and distinctive hurdles that are presently causing significant market failure. The emerging CTI, with its living cell-based products and services, has its own unique set of requirements and challenges that are totally different to those of pharma, biotech and the medical device industry. This will require the collaboration of academics, businesspeople, and clinicians plus the robust and patient support of states and governments. However, once resolved, the CTI will become a sustainable multibillion-dollar global industry establishing cell therapy as the fourth and final therapeutic pillar of healthcare2.

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75.3.02
Translational research in spinal cord injury: from molecule to man

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My clinical experience as a neurosurgeon and clinician-scientist has strengthened my understanding of human pathologies and has led me to develop a laboratory with significant expertise in models of spinal cord injury (SCI). This knowledge base has led to numerous research collaborations with industry partners who are looking to access established clinically relevant animal models of SCI and to translate these discoveries into clinical practice. Our laboratory has successfully partnered with industry to investigate a number of bioengineered strategies. Transcription factor mediated strategies to increase VEGF expression and protein therapies to block Fas activation following SCI have both successfully improved neurobehavioural outcomes. Current studies using self-assembling peptides that provide a growth supportive environment in injured tissue have also shown considerable promise. Our laboratory has also used a bioengineered hydrogel containing hyaluronan to reduce inflammation and scarring in addition to deliver drugs following SCI. In addition to pre-clinical research, we recently completed a Phase I/IIa clinical trial in 48 patients with severe cervical and thoracic SCI with Cethrin, a recombinant protein Rho inhibitor - with encouraging results. In summary, a collaborative environment between academia and industry can be hugely beneficial, particularly in SCI where combinatorial approaches are needed. Bioengineered strategies offer attractive solutions to meet this need and will likely be the key to facilitating clinical translation. It is important for universities to leverage these relationships in the pursuit of cures for diseases.

75.3.03
Utilising economies of scope to use the NHS as a cell therapy development and delivery partner

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There are numerous challenges holding back the provision of cell therapies at the manufacturing scale across the UK. Without production space within GMP regulated facilities the research and development protocols cannot be translated into viable new treatments. However there is a solution in the UK that utilises the infrastructure of the NHS to deliver these treatments to the bedside. The difficulties apply to all cell therapies but are most evident at the autologous end of the manufacturing continuum. There are a number of reasons for this with the top two being; (a) single/small batch production limits the economies of scale and (b) the requirement to manufacture close to the patient leads to the costly and challenging validation of multiple sites. This pushes the costs, in many cases, out of economic range for use within a "free-at-point-of-care" health care system. However health services around the world regularly mange the delivery of existing cell based therapies (e.g. blood, tissues, organs and stem cells,), all of which require processing to high regulatory standards in temperature controlled environments and within strict timeframes. Therefore the provision of new cell therapies needs to utilise an organisation that regularly provides cell based treatments under a single quality system and is able to manufacture in multiple locations both safely, ethically and economically. This economies of scale model is evident within the UK this presentation looks at NHS Blood and Transplant as a case study. Here we show that within the NHS there is the infrastructure, skills, capability and capacity to partner national and international companies and academic institutions in the development and provision of new cellular therapies.

75.3.04
Microstructure and chemistry affects the in vitro bioactivity of calcium phosphate bone graft substitutes

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Despite some controversy as to the methods reliability, its relevance to the understanding of mechanisms behind osteointegration, and the actual nature of the calcium-rich precipitate formed on materials Kokubo's Simulated Body Fluid has been used to evaluate the Bioactivity of numerous types of materials ranging from calcium phosphate (CaP) ceramics and Bioglasses through to Chitosan and natural Pearl. The objective of our study was to compare the Bioactivity of CaP of varying chemistry and strut-porosity through determining the rate of formation

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of hydroxycarbonate apatite (HCA) crystals on the material surface and the extent to which these crystals formed after the material was soaked in simulated body fluid for between 3 and 30 days. The propensity for the formation of either HCA or Octacalcium Phosphate (OCP) as a continuous, un-broken layer over the implant surface immediately after implantation into bone is in theory likely to be significant to the processes of osteointegration; the greater the surface area covered by this layer the better the opportunity for interdigitation with host bone. In practice this assumption is still under debate as the in vivo studies in support of the outcomes of the bioactivity test are often contradictory. In our study formation of HCA varied among different materials. Under the conditions of our test the rate of HCA growth was related to the strut-porosity and the absolute amount formed was dependent upon the chemistry of the substrate.

75.3.05
Technological advances in graft manipulation and cellular therapies – removing the bottlenecks

J Campell
Miltenyi Biotec Ltd., UK

Miltenyi Biotec is a world-leading company in magnetic cell selection and cellular therapy, with particular expertise in the selection of stem cells and cells of the immune system for novel clinical applications. While the science underlying regeneration of tissues and the immune system is advancing at great speed, translation of this knowledge into truly realisable treatments, and delivery within cost and regulatory constraints, is increasingly difficult. In this presentation I will focus on the latest status of cellular therapies in both tissue repair and graft manipulation; examine the bottlenecks in current cell processing procedures; and highlight the new technological solutions that can bring cell therapy nearer the bedside, including the new CliniMACS® Prodigy automatic cell selection system.

75.3.06
ApaTech as an example of how 'academia/industry partnerships' can overcome barriers to commercialization of regenerative medicine products

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The chances of new med-tech enterprises thriving in the present economic and regulatory climate are slim. The current estimated failure rate for such venture is in the region of 75% so it is worthwhile for academics and entrepreneurs to reflect back on the stories of the fortunate few so that they can learn how to give themselves the best chance of beating the odds in bringing new ideas to market. The success with which ApaTech were able to drive the commercialisation of intellectual property emerging from Queen Mary College, University of London, can be attributed to three critical success factors: (1) Effectively identifying an unmet need for the emerging technology and developing it to fit the requirements of the end-user; (2) Reducing time-to-market to a minimum through lean product development, rapid manufacturing scale-up, and strategic commercial distribution; (3) Building confidence with the commercial partners by protecting the IP portfolio, delivering regular new product introductions, avoiding stock-outs, and exceeding sales targets. None of these, however, would have made any difference without having the right collection of technical and commercial experts in the team from day one. In my presentation I will provide real-life examples of how the ApaTech team demonstrated these critical success factors and will provide my own view as to where the most significant challenges lie ahead for anyone wanting to follow in ApaTech's footsteps.

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75.4. Translation Research of Advanced Therapies - Next Academia-Industry Partnership

75.4.01
Production of cell therapies from clinical trial to commercial distribution

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Genzyme has extensive experience with the production of cellular therapies and manufactures three for commercial distribution world-wide; Carticel, MACI and Epicel. Carticel (autologous chondrocyte implantation) and MACI (Matrix-induced autologous chondrocyte implantation) are used to treat focal defects of the knee. Epicel is autologous cultured keratinocytes grafts for burn patients. In the past years, we have also been involved in numerous clinical trials using cellular treatments for a wide range of disease states, i.e. cardiac repair, Parkinson's, etc. There are significant challenges to consistent, quality production of cell therapies on a clinical and commercial scale. The operations to allow GMP production for these programs must be well established and require that manufacturers establish quality systems for the design, manufacture, packaging, labeling and storage of products distributed for human use. One of the biggest challenges is the development and validation of quality assays that characterize the cellular product for release. For the chondrocyte products manufactured for cartilage repair, release assays must ensure quality of the cultured chondrocytes. An Identity assay must have the ability to distinguish chondrocytes from other related cell types. Cultures derived from fibrous tissue dissected from the cartilage junction can not be confidently classified as either chondrocytic or synovial, and may contain a mixture of cell types. Therefore, an acceptable lot release identity assay for cultured chondrocytes must not only detect chondrocytes, but must also discriminate against synovial and heterogeneous cell cultures. A Potency assay for chondrocytes must correctly identify cells that have the potential to form hyaline-like cartilage. All of these assays must be rapid to accommodate production and shipping schedules for cell therapies, which typically have a shelf life of a few days.

75.4.02
An infection control solution for catheter exit sites: Translation from mice to device

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Healionics, USA; Universidad Favaloro & McGowan Institute for Regenerative Medicine, USA

Patients requiring long-term catheterization are at high risk to acquire blood stream infections, with many of these infections proceeding extraluminally. We are commercializing STARcuff – a porous exit site sleeve that provides a natural antimicrobial barrier by attracting macrophages, promoting vascularized dermal ingrowth, and forming a stable epidermal seal. Our translation effort stems from extensive in vitro and small animal model university research. We have shortened our commercialization path by shifting to a large-animal-first, full-size-device approach and focusing on scaffold compositions of only established medical grade polymers with no added biological agents. Percutaneous implants comprising 10-cm segments of either silicone or Carbothane hemodialysis catheters were inserted into the dorsum of pigs. Half were modified with STARcuff on the extraluminal surface. The exit sites were bandaged with Tegaderm and disinfected with chlorhexidene scrub during weekly bandage changes. In the first 28 days post-implantation, 39% (11 / 28) of the unmodified control implants required removal due to purulent infection of the exit site, compared to only 4% (1 / 26) of the STARcuff implants. The STARcuff significantly reduced the rate of catheter exit site infection in a porcine model. This technology has promise as an infection-prevention feature for any percutaneous implanted device. The use of unrestricted silicone with no added agents ensures a faster regulatory pathway.

75.4.03
Financing regenerative medicine: Azellon Ltd, an asset centric business model working in public: private partnership

B Horsburgh
Azellon, UK

Azellon is a spin-out company from the University of Bristol founded on the work of Professor Anthony Hollander. The company is developing a stem cell bandage for the treatment of mensical tears for which no effective first-line therapy exits. Azellon is a virtual company that has been financed by private investors and substantial grants from the Wellcome Trust and the UK Technology Strategy Board (TSB). This approach has enabled the company to reach clinical trials and a significant value inflection for a modest equity investment.

75.4.04
Challenges in translational research: what role for SMEs?

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Stemmatters, Portugal; 3B's Research Group/ICVS, Portugal

During the last decade, health biotechnology industry has rapidly expanded, accounting for approximately 3,500 companies and for overall sales of more than $170bn in 2009. Contribution of regenerative medicine products is still reduced due to the lack of successful product launches, which has contributed to limit investment. Still, this area is gradually attracting more funding due to hybridization of health sectors, with an increase of small players and the growing interest of large pharmaceutical and medical device companies that are starting to invest. However, challenges ahead remain. In a regulatory perspective, new products must necessarily demonstrate safety and efficacy, which has large operational implications in terms of resources involved. In a financial perspective, it is critical to assure adequate financing at all stages of development. While these requirements can be surmountable for large companies, for small ones, they are especially challenging. Small companies have to demonstrate solid intellectual property base and successful proof of concept, which combined with previous demands, makes the global challenge highly risky. Nevertheless, the role of small companies is crucial, as they bring innovation to this sector and provide a de-risking opportunity for new entrants. This presentation presents a strategic and operational framework that sets up the fundamental requirements for competiveness of small companies in the field of regenerative medicine.

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75.4.05
Cardiac derived cells for treatment of heart diseases - efforts to translate research into a marketable therapy

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Technology: A unique cardiac derived cell type (CAPcells) was recently identified and fulfills major criteria for cell therapy. Properties include: e.g. improve muscle function in cardiac stress models, engraft in the heart after i.v. injection, anti-inflammatory properties, inhibit apoptosis and fibrotic cell differentiation (Haag et al., 2010, Linthout et al. 2011, Haag et al. 2012). Current work is focused to clinical and commercial translation. Translational Strategy The technology uses tissue-specific cells from the heart with expected advantages in integration, safety and efficacy. It also includes key advantages for manufacturing. The national situation for reimbursement in Germany in frame of the DRG system was analyzed. The product will be developed due to European ATMP regulation. In 1st generation, as autologous, 2nd as allogenic therapy. To support the commercial translation, CellServe GmbH was founded as a spin-off.
Conclusion: CAPcells are unique to treat cardiac diseases. Preclinical R&D continuously considered regulatory and commercial feasibility. A clinical study to treat cardiomyopathy is in preparation. Patent: CELLS FOR HEART TREATMENT; DE102007008650A, EP000002129774A1, US020100040587A1, Haag, M., Ringe, J., Tschöpe, C., Sittinger, M

75.4.06
Translation from research to GMP-compliant manufacturing: constructing a business model for a new stem cell source

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Cell based therapies promise to be a cure for many incurable diseases. However, despite extensive investments in the last two decades, most cell-based therapies have failed to deliver clinical and financial success. Particularly, autologous products are still too expensive compared to conventional therapies. We want to report on a newly developed and easily accessible stem cell source: Sweat Gland-derived Stem Cells (SGSC). Based on a patented cell isolation process, stem cells are effectively derived out of a small axillary skin biopsy that can be obtained in an ambulant procedure within few minutes. The cells are already well characterized and tested: we could show the potential for multilineage differentiation and conducted several preclinical tests using SGSCs in wound healing models. Highly proliferating stem cells were even derived from elderly patients, indicating that the cell source is suitable for autologous treatments of patients of all ages. Currently, we are developing a roadmap for transition into clinical practice: GMP-compliant process steps are being implemented and corresponding regulatory agencies are approached. Furthermore, we derived a business model combining an autologous stem cell treatment with stem cell banking for adult high risk patients, delivering an autologous "off-the-shelf" product. This business model might be particularly promising for treatment of chronic diseases from both, a clinical and economic perspective. In this talk, we present our experiences with this newly developed stem cell source and the strategies that a state-funded research organization like Fraunhofer pursues to commercialize regenerative therapies.

75.4.07
Early stage translational research of advanced therapies (part I)

TERMIS-EU INDUSTRY COMMITTEE, S Ellison and Y Bayon
Covidien - Sofradim Production; TERMIS-EU Industry Committee; NHS Blood & Transplant, UK

Ideas often start on pieces of paper and a test tube on the basis of basic science research, in academic and institutional laboratories, with the support of public and philanthropic research grants. Talented scientists drive these ideas and projects through various stages of early development that mostly result in significant scientific achievements, with the early proof concept of innovative and clinically promising advanced therapies, as illustrated by publications in peer-reviewed journals and, less often, by patent filings. Some regenerative medicine discoveries, when carefully evaluated, may have obvious and huge benefit for patients and the public, by satisfying massive unmet clinical needs, for a still large range of indications where no therapeutic approaches are really effective, today, in restoring an enjoyable level of quality of life (neurological: stroke, spinal cord injury; orthopaedic: large cartilage lesions; cardiovascular: critical limb ischemia, myocardial infarction; endocrine: diabetes; gastrointestinal: Crohn's disease, ulcerative colitis).But, the decision to move forward regenerative medicine discoveries into the clinic and to the market is certainly not an easy one. The path leading to commercialization – from promise to real delivery, from prototype to useful product. – is indeed challenging, with a number of obvious hurdles to overcome: eg. financing, intellectual property, regulatory requirements, GMP manufacturing.

75.4.08
Early stage translational research of advanced therapies (part II)

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Covidien - Sofradim Production, TERMIS-EU Industry Committee; NHS Blood & Transplant, UK

Meeting and learning from key players of the commercial translation of advanced therapies and sharing own translation project and experience, may certainly guarantee higher chances of success.Actively participating to this session 'Early Stage Translational Research of Advanced Therapies' of the Industry Symposium may also give a chance to meet future partners. With most funding sources pressuring and the complexity of the commercialization process of advanced therapies, there is an obvious need to increase the speed to the market, but without increasing risk. This may be achieved by a partnership approach. This session, a part of the full day Industry symposium, aims to highlight the translational research efforts or projects of in-house developed advanced therapies, managed by academic and/or academic spin-off companies and to discuss key technical/scientific, legal/IP, financing, regulatory and clinical advancements and current hurdles paving the road of the early stage commercialization process. Submitted abstracts should put more emphasis on the commercial translation management (see above) and less on detailed descriptions of scientific achievements. Speakers of the selected abstracts for oral communications will be invited, after their presentation, to join a round table with experienced players of commercial translation of advanced therapies, for Q&A.

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76. Special Technology Session

76.01
Engineering hydrophobin DewA to generate surface coatings that enhance adhesion of human but not bacterial cells

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Hydrophobins are fungal proteins with the ability to form immunologically inert membranes of high stability, properties that makes them attractive candidates for orthopaedic implant coatings. Cell adhesion on the surface of such implants is necessary for better integration with the neighbouring tissue; however, hydrophobin surfaces do not mediate cell adhesion. The aim of this project was, therefore, to investigate whether the Class I hydrophobin DewA from Aspergillus nidulans can be functionalised for use on orthopaedic implant surfaces. DewA variants bearing either one RGD sequence or the laminin globular domain LG3 binding motif were engineered. Surfaces of both variants showed significantly increased adhesion of mesenchymal stem cells (MSCs), osteoblasts, fibroblasts and chondrocytes; in contrast, the insertion of binding motifs RGD and LG3 in DewA did not increase Staphylococcus aureus adhesion to the hydrophobin surfaces. Proliferation of MSCs and their osteogenic, chondrogenic and adipogenic differentiation potential were not affected on these surfaces. The engineered surfaces, therefore, enhanced MSC adhesion without interfering with their functionality or leading to increased risk of bacterial infection.

76.02
Porous titanium as a high-strength scaffold for bone tissue engineering

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Over 70% of implant devices are made of metals. Of these titanium is widely implemented in dental and orthopaedic devices. Titanium plasma sprayed (TPS) surfaces on a Ti–6Al–4V core class as porous titanium with a surface roughness of Ra 20–30 μm and a porosity of $\sim$ 28%. It will be shown, that they can be modified either for an enhanced osteoconduction (bone ongrowth) or osteoinduction (de novo bone volume). Crucial for osteoconduction is the wettability of the surface. There are two forms of high wettability on titanium surfaces: ultrahydrophilicity [1] defined by contact angles $<10°$ and superhydrophilicity defined by novel imaginary contact angles $\sim$ 1i°–20i° [2]. Enhanced bone ongrowth in vivo can be demonstrated for ultrahydrophilic as well as seminally for superhydrophilic surfaces [3]. Crucial for osteoinduction are morphogens such as BMP-2, which can be immobilized on the TPS surface, while retaining biological activity and inducing bone volume in vivo [3]. A good performance in vivo is proof of principle that the scaffold is appropriate for supporting bone tissue engineering in vitro.

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76.03
Performance characterization and initial clinical experience using a filter-based, single-use, disposable system for procuring progenitor cells from peripheral blood

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Background: Peripheral blood (PB) mononuclear cells (MNC) contain progenitor cells that can stimulate angiogenesis and bone formation. They have been studied for their role in the treatment of critical limb ischemia (CLI), and bone repair following spinal degeneration. PBMNC are prepared by centrifugation limiting its application in some settings. We explore the performance, and clinical utility, of a single use disposable filter system that obviates the need for capital equipment and its associated space requirements.
Methods: One hundred millilitre of citrated WB was filtered and recovered with 12 or 20 ml of harvest solution using the CelerisTM WB System (Pall Corp. NY, USA). The cell types were measured and compared with influent whole blood (WB). Patients with CLI were sedated and treated four times at quarterly intervals by intramuscular injection of PBMNC.
Results: MNC recovery was (mean $\pm$ SD; $n = 12$) 54.9 $\pm$ 9.3% with a 12 ml harvest volume and 62.2 $\pm$ 7.1 with a 24 ml harvest volume. The fold-increase of MNC in the cell concentrate was 5.6 $\pm$ 0.9 and 2.9 $\pm$ 0.3 for the 12 ml and 24 ml volumes, respectively. Cell viability within the cell concentrate was greater than 99% using either harvest volume. Patients tolerated the injections without untoward effects.
Conclusion: Our experience confirms the procedure is effective for concentrating MNC and rapid, safe, and therefore conducive for clinical bed-side use. We will report clinical observations at this presentation.

76.04
Lipogems®: new device, method and human fat tissue product highly enriched in mesenchymal stem cells ready for autologous cell therapy and regenerative medicine

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Adipose tissue contains multipotent elements with phenotypic and gene expression profiles similar to Human mesenchymal stem cells (ADhMSCs). The multilineage potential of ADhMSCs, involves adipocyte, chondrocyte, osteoblast, neuronal, pancreatic, and myocardial-like pathways. Nevertheless, the chance of clinical translation is delayed by the poor/negligible cell survival within cryopreserved lipoaspirates, the difficulty of ex vivo expansion, and the need for GMP compliance of expanded cells. Hence, availability of a minimally

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manipulated, autologous, hMSC-enriched fat derivative would have obvious biomedical and clinical relevance. To this end, one of the Authors (CT) developed Lipogems, an innovative system providing a non-expanded, highly hMSC-enriched, ready-to-use fat product, starting from minimal amounts of lipoaspirates. Lipogems uses mild mechanical forces in a completely closed system, avoiding enzymes, additives and other manipulations. We present methodological details of the Lipogems system, and the phenotypic composition of the Lipogems product, both fresh and cryopreserved. Such product can also be easily expanded in culture for many passages. Lipogems-derived hMSCs shown remarkable multilineage potential in vitro and in vivo. Noteworthy, the Lipogems product harvested from human cadavers was found to encompass a viable stem cell population retaining phenotypic, yield, viability, and committal potential characteristic of the freshly isolated product.

76.05
Virusinactivation capacity of CTBA cleaning process

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Introduction: Prevention of virus transmission by allografts is an essential issue for safety of human allogenic bone transplants. Since donor testing alone may lack in serological diagnostics and therefore then leads to false negative results, an efficient virus cleaning process is crucial. On basis of ICH/Q5A (R1) and CPMP/BWP/268/95 guidelines regarding virus clearance studies, a virus clearance validation study was designed for the human bone allografts CTBA (Cells and Tissue Bank Austria) process. The aim of the study was a virus inactivation of >log 104 – according to German recommendations by A. Pruss.
Materials and methods: Besides common model viruses as BVDV (enveloped RNA virus), HSV (enveloped DNA virus), Adenovirus, PPV (not enveloped DNA virus) and Polio Typ 1 virus also HAV virus as a very high resistant virus type (not enveloped RNA virus) were tested. To ensure in depth efficacy of this process testing was conducted on large cancellous human bone blocks.
Results: The inactivation capacity was in a minimum log 6 and up to log 10 for all the tested viruses in large cancellous human bone blocks.
Conclusion: This long proofed technique has shown its capability of virus inactivation leaving the human allograft properties itself the same.
Keywords: virus; inactivation; human bone.

76.06
Human placental extracellular matrix as a platform technology for novel tissue engineered products

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Celgene Cellular Therapeutics (CCT), a wholly-owned subsidiary of Celgene Corporation, discovers and develops therapeutics from cells and biomaterials derived from the human placenta. CCT is a state-of-the-art research and development organization with in-house GMP cell manufacturing and GTP compliant tissue production, dedicated to developing cutting-edge products and therapies. CCT's cell therapy product candidate PDA-001, a novel culture expanded mesenchymal-like cell population that has broad therapeutic potential in areas such as immunology and inflammation, is currently evaluated in Phase II clinical studies. CCT has developed cost-efficient technology to extract placental extracellular matrix (ECM), which is a versatile material that can be molded into a variety of shapes and sizes. The availability of culture expanded cells from its GMP manufacturing facility allows the creation of combination products by growing placental cells on ECM matrix. CCT has produced a number of representative combination product prototypes using placental ECM and believes that such combination products will be effective in enhancement of wound repair and the control of scar formation after injury. In addition, CCT employs the placental ECM paste alone or as a composite for 3-dimensional bioprinting of tissue with cells. Placental ECM is a highly versatile pharmaceutical grade human matrix platform suitable for tissue products, tissue engineering products, and as a matrix for bioprinting.

76.07
Synergetic Cell Population – a powerful tool for autologous therapies

V Fulga
Hemostemix, Canada

Some tissue regeneration therapies are in clinical trials and expected to revolutionize medical practice. The healing process following tissue damage is a complex sequence of events involving synchronized activity of numerous factors and processes. It is achieved by cooperation between damaged tissue cells and circulating cells that are recruited and further activated in situ. Stem cells were shown to be capable to differentiate into a variety of tissues in addition to other potentially beneficial healing effects. Based on the concept that various cells need to be interacting with each other in order to induce an effective healing process, we hypothesized that peripheral blood leukocytes can serve as a source of various cell types if cultured under appropriate conditions. A blood-derived cell population named synergetic cell population (SCP) was detected. It is rich in cells expressing progenitor markers such as CD34 and CD31, in addition to other supportive cells and is capable of differentiation into a variety of cell lineages. Following differentiation in culture, these cells exhibit morphological, cyotchemical, and functional characteristics of angiogenic, neural and cardiomyocyte lineages. We took advantage of SCP's robust plasticity and its availability from peripheral blood and utilized it as a therapy for cardiovascular disorders in preliminary clinical studies. Encouraging safety and efficacy were observed.

76.08
Avoid pitfalls in your adoption of 3D cell culture

A Sim
AMS Biotechnology, UK

As the number of publications employing 3D cell culture increases massively, many researchers are still confused about which 3D system is right for their needs. As the leading 3D cell culture technology provider, AMSBIO can help you to define what factors are most important to consider. A variety of options including natural extracellular matrix proteins, recombinant mimetics, recombinant proteins, polystyrene scaffolds, biodegradable scaffolds and hydrogels are available. The talk will review the reasons why researchers should take care to consider long-term objectives early in the 3D cell culture adoption process. The whirlwind tour will include real examples where AMSBIO's offerings are used in many applications from angiogenesis to xenografts via spheroids, stem cells and regenerative medicine. The trip includes a view on adopting and translating research materials to GMP compliant device and therapy applications.

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