

3rd Conference on Impedance-Based Cellular Assays



August 9th – 12th, 2016
in Regensburg





August 9th – 12th, 2016 in Regensburg (Germany)

3rd Conference on Impedance-Based Cellular Assays



Four Days ...
20 Invited Talks
+ 2 Poster Sessions ...



... covering all Aspects
of Impedance-Based
Cellular Assays ...



... and a Keynote Address
by Nobel Laureate
Ivar Giaever.



www.ibca2016.net





Welcome to the 3^d Conference on Impedance-Based Cellular Assays

Dear Participants of IBCA 2016,

on behalf of the University of Regensburg and our sponsors I would like to welcome you in Regensburg for the 3rd *Conference on Impedance Based Cellular Assays (IBCA2016)*. After this conference series was started in Regensburg in 2011, the second meeting was held in Budapest (Hungary) in 2013 and it now returns to Regensburg. Quite many of you have attended all three events and even the *ECIS Research Meetings* in the years 2009 and 2010 which have been the predecessors of IBCA. Obviously an IBCA community is formed and it shapes up continuously. We should use this year's IBCA also to think about its future and its format in the years to come.

The scientific program of IBCA2016 with almost 40 talks and just as many posters organized in ten sessions addresses all aspects of impedance-based cellular assays, starting from new assay formats and technology developments to emerging applications like nanotoxicology or monitoring of cell differentiation. The list of presenters includes researchers from all levels of a scientific career, starting with students working for their master's degree and reaching as high as a nobel laureate. Besides the oral presentations every topic is also covered in the two poster sessions on Wednesday and Thursday since these are often the beating heart of scientific discussions and for the line-up of future collaborations. I hope it works out that way this year again.

The scientific program is rounded up by some evening events including a barbecue on campus (may the sun be with us), a city tour and of course our conference dinner which will be held in Parkhotel Maximilian close to the "Palais Thurn und Taxis" in downtown Regensburg. The hotel will catch the eye of everyone who has an interest in architecture. Also the rest of historic Regensburg invites you to take a stroll through the city and enjoy to the atmosphere in 'the most Italian city north of the Alps'.

As the host of IBCA 2016 I truly hope that the conference will be memorized for inspiring presentations, fruitful discussions and of course good times with close friends and colleagues. I am looking very much forward to IBCA 2016 and the opportunity to meet you all again - or for the first time.

Joachim Wegener

IBCA 2016 relies on the financial support of our sponsors. We gratefully acknowledge financial support by the companies and organizations listed below for providing the resources to organize this meeting. Thank you very much!!!



Scientific Program

Tuesday, August 9th 2016

Session 1

Drug Testing & Signal Transduction

Chair: **Joachim Wegener**

14:00 –
14:15 **Welcome**

14:15 – **T01 Judith Stolwijk (G):**
14:45 Novel aspects of impedance based signal transduction assays

14:45 – **T02 Scott Boitano (USA):**
15:15 Use of the xCELLigence real time cell analyzer in drug development for protease-activated receptor 2 (PAR2)

15:15 – **T03 Wen G. Jiang (UK):**
15:45 Hunting for anticancer compounds and medicines and exploring the mechanisms of anticancer drug actions, the potential implication of Electric Cell-Substrate Impedance Sensing

15:45 – **T04 Leonard Nelson (UK):**
16:15 ECIS reveals that low-dose acetaminophen induces early disruption of cell-cell tight junctions in human liver HepaRG cells - which correlates with an *in vivo* mouse liver model

16:15 –
16:45 **Coffee & Refreshments**

Session 2

Endothelial Physiology

Chair: **Christian Renken (USA)**

16:45 – **T05 Mohammed Trebak (USA):**
17:15 Calcium signaling is dispensable for receptor-regulated endothelial barrier function

17:15 – **T06 Peter Hordijk (NL):**
17:45 ECIS-based screening uncovers new regulators of endothelial barrier function



17:45 – 18:00	T07 Armin Bieser (G): Morphological changes of HUVEC under shear stress conditions
18:00 – 18:15	T08 Verena Küppers (SUI): Using a TEER-based screening to find novel mechanisms that regulate retinal endothelial barrier function
18:30	Barbecue & Get Together with Duo Ingravido

Wednesday, August 10th 2016

Session 3

Cells on Filters

Chair:

Joachim Wegener

08:30 –
09:00

T09 Dorothee Günzel (G):

Impedance spectroscopy in epithelial tissues and cell cultures

09:00 –
09:30

T10 Susanne Krug (G):

Application of two-path impedance spectroscopy to discriminate between trans- and paracellular epithelial ion transport

09:30 –
10:00

T11 Julia Bornhorst (G):

Impact of manganese and mercury species on and transfer across the blood-brain and blood-CSF barrier *in vitro*

10:00 –
10:15

T12 Kathrin Hajek (G):

Independent observation of two co-cultured cell layers grown on filter supports

10:15 –
10:45

Coffee & Refreshments

Session 4

Cell Migration & Chemotaxis

Chair:

Peter Hordijk (NL)

11:15 –
11:45

T13 Stefanie Michaelis (G):

Recent advances in impedance-based wound-healing assays to monitor cell migration in different experimental scenarios

11:45 –
12:15

T14 Fabian Bonetto (Arg):

Spectral impedance measurements during wound healing compared to standard ECIS measurements applied to cell differentiation

12:15 –
12:45

T15 Andreas Janshoff (G):

Cell locomotion monitored by impedance analysis - from Dictyostelium discoideum to primordial germ cells

12:45 –
13:00

T16 D.S.K. Nielson (DK):

Administration of protein kinase d1 induce migration of intestinal epithelial cells *in vitro*

13:00 –
14:00

Lunch & Poster Session 1

Session 5

New Technologies & Approaches

Chair: Pierre Bagnaninchi (UK)

- | | | |
|------------------|------------|--|
| 15:00 –
15:30 | T17 | Sven Ingebrandt (G):
Transistor-based impedimetric monitoring of single cells |
| 15:30 –
16:00 | T18 | Yama Abassi (USA):
Multi-parametric analysis of cardiomyocyte excitation-contraction coupling for pre-clinical safety assessment and investigating iPSC-cardiomyocyte disease models |
| 16:00 –
16:15 | T19 | S.M. Weiz (G):
Impedimetric microtomography in rolled-up tubes |
| 16:15 –
16:30 | T20 | E. Lajko (H):
Impedance-based analysis and holographic phase imaging of the GnRH-III-based drug targeting in melanoma cells |
| 16:30 –
16:45 | T21 | Christian Götz (G):
PEDOT/PSS as transparent electrode material for impedance-based cell analysis |
| 16:45 –
17:00 | T22 | Bastian Böttcher (G):
A versatile lab-on-a-chip-system for optical and impedance-based cytotoxicity assays |

18:00	Optional City Tour starting @ Parkhotel Maximilian guided by a city mouse
-------	--

Thursday, August 11th 2016

Keynote Lecture

08:45 –
09:15

Charles R. Keese (USA):

Impedance-Based Cellular Assays: how it all began

Session 6

Chair:

Cell Differentiation

Andrea Robitzki (G)

09:15 –
09:45

T23

Pierre Bagnaninchi (UK):

Real-time monitoring of stem cell based disease models with Electric Cell-substrate Impedance Sensing

09:45 –
10:15

T24

Orsolya Lang (H):

Impedimetric characterisation of cell physiological responses in oral biology

10:15 –
10:30

T25

S. Gomez (TW):

Use of cross-linked polypeptide multilayer-coated electrodes to monitor cardiac differentiation of human mesenchymal stem cells

10:30 –
10:45

T26

H.-G. Jahnke (G):

Impedimetric monitoring of neural stem cell differentiation

10:45 –
11:15

Coffee & Refreshments

Session 7

Chair:

Specialized Impedance-Based Assays

Mohammed Trebak (USA)

11:15 –
11:45

T27

Chun-Min Lo (TW):

Impedance analysis of cadherin-mediated cell adhesion

11:45 –
12:15

T28

Verena Charwat (A):

Monitoring cellular stress responses using integrated high-frequency impedance spectroscopy and time-resolved ELISA

12:15 –
12:30

T29

K. Szaszi (CA):

Role of claudins in tumor necrosis factor-induced permeability and migration changes in tubular epithelial cells

12:30 –
12:45

T30

Monique F. Stins (USA):

Brain endothelial activation and neuronal damage (during cerebral malaria)

12:45 –
14:30

Lunch Break & Poster Session 2

Session 8 Sponsors' Presentations & Questions to Manufacturers

Chair: Joachim Wegener

14:30 – 14:45	Applied BioPhysics Inc (USA)
14:45 – 15:00	Nanion GmbH (G)
15:00 – 15:15	Ibidi GmbH (G)
15:15 – 15:30	Acea Bioscience (USA)
15:30 – 15:45	nanoAnalytics (G)
15:45 – 16:00	Cellasys GmbH & HP-Med GmbH (G)
16:00 – 16:15	ScioSpec GmbH (G)

16:15 – 16:45	Coffee & Refreshments
------------------	----------------------------------

Session 9 3D Cultures & Tissue Models

Chair: Peter Ertl (A)

16:45 – 17:15	T31	Andrea Robitzki (G): A novel multiparametric bioelectronic measurement system for long-term monitoring of human stem cell derived human 3D cardiomyocyte clusters
17:15 – 17:45	T32	Frank Alexander (USA): RTEMIS: Real-time microTissue and environment monitoring using impedance spectroscopy and pH Sensing
17:45 – 18:00	T33	R.M. Owens (F): Impedance analysis of cells using organic electronics: focus on 3D cellular models
18:00 – 18:15	T34	M. Schweinlin (G): Impedance spectroscopy: A new tool to characterize reconstructed epidermis

19:30	Conference Dinner in Parkhotel Maximilian
-------	--

Friday, August 12th 2016

Session 10

Cell Response to Nanomaterials & Hot Topics

Chair:

Joachim Wegener

09:00 –
09:30 **T35**

Christian Renken (USA):

Automated statistical analysis of an siRNA Screen of kinases involved in TNF- α signalling

09:30 –
10:00 **T36**

Peter Ertl (A):

Impact of nanomaterial perfusion on cellular uptake and nanotoxicity at physiological barriers

10:00 –
10:30 **T37**

Grigore Rischitor (I):

Label-free monitoring of cellular interaction with the surface modified nanoparticles by Electric Cell-substrate Impedance Sensing (ECIS)

10:30 –
11:00 **T38**

Christina Hupf (G):

Cells in Contact to Nanomaterials: A Multi-modal Perspective based on Impedance Analysis

11:00

Coffee & Farewell



Oral Presentations

Novel Aspects of Impedance-Based Signal Transduction Assays

J.A. Stolwijk¹; M. Trebak²; C.W. Renken³; J. Wegener¹

¹University of Regensburg, Regensburg (Germany);

² Department of Cellular and Molecular Physiology, Penn State University College of Medicine, Hershey, PA (USA); ³Applied BioPhysics Inc., Troy, NY (USA).

judith.stolwijk@chemie.uni-regensburg.de

Label-free impedance-based assays have evolved as a valuable tool in drug discovery and screening. Especially G-Protein Coupled Receptors (GPCRs) are promising targets as they are involved in a multitude of human diseases. GPCRs can couple to different downstream pathways, depending on the cell type and the receptor's conformation(s) stabilized by the ligand. Recognizing these concepts has become extremely important for the development of effective, low side-effect drugs and explains the increasing popularity of label-free, so-called holistic assays [1]. Rather than quantifying only one pathway-specific event as obtained by most biochemical assays (e.g. Ca^{2+} , cAMP, specific kinase activity), impedance assays measure the integrated response to all activated signaling pathway(s) that evoke changes in cell morphology. Moreover, impedance assays are capable of detecting GPCR activity at endogenous receptor levels of non-transfected and primary cells in a non-invasive and time-resolved manner.

However, the cell-type specific impedance response profiles to GPCR agonists are not well enough understood yet to assign general rules for identifying respective pathway activities, to unravel the time-dependent sequence of signaling events or to quantify their individual contribution. In our opinion closing this gap in knowledge can only be achieved by the combination of two approaches: (i) Using pathway inhibitors, activators and genetic manipulation as well as comparison with biochemical assays is a straight-forward concept for deconvolution of the impedance response profiles. (ii) An important prerequisite, however, is the correct interpretation of cell-type dependent impedance signals in response to GPCR agonists and pathway activators. This requires substantiated understanding of the very basic experimental parameters of the assay technique that influence signal profile and intensity.

In our study we used primary human dermal endothelial cells and the U373 astrocytoma cell line in Electric Cell-Substrate Impedance Sensing (ECIS) measurements [2] to illustrate how cell-type specific morphological parameters influence the impedance signal upon GPCR stimulation and how proper selection of experimental parameters including monitoring frequency can improve assay stability and sensitivity as well as data interpretation [3].

References:

- [1] C.W. Scott and M.F. Peters, *Drug Discovery Today* 15 (2010) 704–716.
- [2] I. Giaever & C.R. Keese, *PNAS USA* 81 (1984) 3761–3764.
- [3] J.A. Stolwijk, K. Matrougui, C.W. Renken, M. Trebak, *Pflügers Archiv* 467 (2015) 2193– 218.

Use of the xCELLigence real time cell analyzer in drug development for protease-activated receptor 2 (PAR₂)

S. Boitano, J. Hoffman, C.L. Sherwood, C. Rivas, A.N. Flynn, T.J. Price and J. Vagner

University of Arizona, Tucson, Arizona (USA), The University of Texas at Dallas, Dallas, Texas (USA)

sboitano@email.arizona.edu

Protease-activated receptor-2 (PAR₂) belongs to a four-member family of G protein-coupled receptors (GPCRs) that contain internal ligands exposed following exogenous or endogenous protease cleavage of the extracellular amino terminus. PAR₂ is associated with a variety of inflammatory conditions, including asthma and pain. PAR₂ activation typically leads to G_q-dependent Ca²⁺ and mitogen activated protein kinase (MAPK) signaling as well as a β-arrestin-dependent MAPK signaling. Elucidation of PAR₂ signaling contributions to disease has been hindered by the lack of potent, efficacious antagonists, and/or biased-ligand signaling agonists and antagonists. We have used the xCELLigence impedance-based cellular assay to develop several series of peptidomimetic PAR₂ ligands based on the primary trypsin cleavage sequence (SLIGRL) or the primary Kallikreins 4/16 cleavage sequence (SSKGRS) exposed in the N-terminus of PAR₂ [1-5] Viable candidates from xCELLigence screening are then subjected to Ca²⁺ and MAPK signaling *in vitro* assays, followed by *in vivo* assays. Using this paradigm we have developed the most potent PAR₂ full agonists (compound 484, C484), full antagonist (C391) and two biased-signaling antagonists (C595 and C608) that can be used to evaluate physiological consequences of PAR₂ full and biased ligand signaling

References:

- [1] S. Boitano, A.N. Flynn, S.M. Schulz, J. Hoffman, T.J. Price and J. Vagner *J. Med. Chem.* 54 (2011) 1308-1313.
- [2] S. Boitano, J. Hoffman, A.N. Flynn, M.N. Asiedu, D.V. Tillu, Z. Zhang, C.L. Sherwood, C.M. Rivas, K. DeFea, J. Vagner and T.J. Price, *Br. J. Pharmacol.* 172 (2015) 4535-4545.
- [3] S. Boitano, J. Hoffman, D.V. Tillu, M.N. Asiedu, Z. Zhang, C.L. Sherwood, Y. Wang, X. Dong, T.J. Price and J. Vagner, *PLoS One* 9 (2014) e99140.
- [4] A.N. Flynn, J. Hoffman, D.V. Tillu, C.L. Sherwood, Z. Zhang, R. Patek, M.N. Asiedu, J. Vagner, T.J. Price and S. Boitano, *FASEB J.* 27 (2013) 1498-1510.
- [5] A.N. Flynn, D.V. Tillu, M.N. Asiedu, J. Hoffman, J. Vagner, T.J. Price and S. Boitano, *J. Biol. Chem.* 286 (2011) 19076-19088.

Hunting for Anticancer Compounds and Medicines and Exploring the Mechanisms of Anticancer Drug Actions, the Potential Implication of Electric Cell-Substrate Impedance Sensing

W.G. Jiang, S. Owen, F. Ruge, A.J. Sanders, L. Ye, M.D. Mason

Wales Cancer Metastasis Research Platform and Cardiff China Medical Research Collaborative, Cardiff University School of Medicine, Cardiff, Wales, UK

Jiangw@Cardiff.ac.uk

Cancer is the leading cause of death in most Western countries. Searching for new targets and new drugs for the disease have been an ongoing task and challenge in the fight against cancer. Here, we have adopted Electric Cell-Substrate Impedance Sensing (ECIS) as a key component in a platform for screening anticancer compounds and explored the mode of action of anticancer medicines.

A range of human cancer cells that have covered more than 12 different types of human cancers were used. Gene array and protein kinase array, together with clinical tumour cohorts were used to search for and validate potential targets. High throughput ECIS Z0 units were employed to screen compounds for their actions on the matrix-adhesion and cellular migration of cancer cells. Traditional protein blot, histology, immunocytochemistry and immunofluorescence (IFC), scratch wounding and AVOS methods were used in conjunction with ECIS to validate the targets and test the compounds. *In vivo* tumour models were used to evaluate the antitumour growth effects.

1. Potential new targets in prostate cancer. We have validated that the prostate transglutaminase, also known as transglutaminase-4 (TGase-4), was a potential target in human prostate cancer, a clear contrast from other cancer types. This was seen by the action of the transglutaminase molecule on the aggressiveness of prostate cancer cells. From a chemical library of 35k, a few hundreds of structurally relevant compounds were further screened using ECIS and a small cohort of the compound were found to be unique in inhibiting transglutaminase-4 induced cell migration and cell adhesion in prostate cancer. Ongoing work is focused on refining the compounds and improving their efficacy. **2. Exploring the mode of action of anticancer medicines.** One part of our recent work has been focused on the mode of action of a known anticancer formula, known as *YangZheng XiaoJi* (YZXJ), a herbal medicine that has been shown to have clinical benefit in patients with solid cancers. First, protein kinase array (Kinexus 850) screening had demonstrated that a number of intracellular signalling pathways were markedly influenced by this medicine. Focal adhesion kinase (FAK) and protooncogen cMET were amongst the kinases most significantly inhibited by YZXJ. Using IFC and protein blotting, it was confirmed that the phosphorylation of both FAK and cMET were inhibited by YZXJ in a number of cancer types, human lung cancer and human osteosarcoma being amongst the sensitive ones. Using ECIS based methods, we went on to demonstrate that YZXJ worked in synergy with small inhibitor to FAK and small inhibitors to cMET (the hepatocyte growth factor receptor) by suppressing the aggressive nature of cancer cells including reduction of cell migration and invasiveness. The inhibitory effect on cancer cells was able to be translated in the *in vivo* tumour models, in that YZXJ, small kinase inhibitors (both to FAK and cMET) markedly reduced the rate of growth of a number of tumour types. The combination of the kinase inhibitors and YZXJ further enhanced the reduction of tumour growth, compared with using the agents alone. In these tumours, the level of activation of FAK and cMET were found to be down-regulated by YZXJ.

The combined use of Electric Cell-Substrate Impedance Sensing (ECIS) and other modern technologies have provided us with a highly flexible, versatile, high throughput and unique platform in drug discovery and mechanism exploration in cancer research. Using the platform, we have already identified new compounds for some of newly discovered cancer targets.

ECIS reveals that low-dose acetaminophen induces early disruption of cell-cell tight junctions in human liver HepaRG cells - which correlates with an *in vivo* mouse liver model

W. Gamal¹, P. Treskes², K. Samuel³, K. Morgan², A. Bryans², A. Kozłowska², A. Koulovasilopoulos², J. del-Pozo⁴, S. Moss⁴, A.I. Thompson⁵, N.C. Henderson⁵, P.C. Hayes², J.N. Plevris², P.O. Bagnaninchi¹ and *L.J. Nelson²

¹MRC Centre for Regenerative Medicine, SCRM Building, The University of Edinburgh (UoE),

²Hepatology Laboratory, UoE; ³Scottish National Blood Transfusion Service, Edinburgh;

⁴The Roslin Institute (Vet Pathology), UoE; ⁵MRC Centre for Inflammation Research, The Queen's Medical Research Institute, UoE, Edinburgh (all UK).

l.nelson@ed.ac.uk

Tight junction (TJ) barrier dysfunction is a main feature in the pathogenesis of various diseases, including, hepatitis, cancer, inflammatory bowel disease and drug-induced liver injury. Hepatic TJs are cell-cell adhesions that preserve cellular polarity by delimiting functional bile canaliculi structures, forming the blood-biliary barrier. Although acetaminophen (APAP) toxicity remains the leading cause of acute liver failure (ALF), temporal and quantitative effects of direct APAP toxicity on adhesion structures have not been previously explored. Here, we investigated the effects of APAP on the stability, phenotype and distribution of hepatic TJ-associated structures in hepatocytes, and *in vivo* mouse liver. We show temporal, dose-dependent disruption of TJs and cell-matrix adhesion structures in both functionally polarized human hepatic HepaRG cells. Associated modulation of hepatic ultrastructure, cytoskeletal-F-actin, TJ-associated protein ZO-1 and integrin expression was demonstrated. Real-time impedance biosensing (ECIS) combined with HepaRG cells also quantified early, dose-dependent decrease in intercellular TJ and cell-substrate adhesion structures. *In vivo*, a dose-dependent decrease in ZO-1 staining was observed in mouse liver, which further validates our *in vitro* findings. Treatment with the protein kinase C (PKC)-activator, phorbol-12-myristate-13-acetate, similarly induced early (<5 hours) disruption of TJs, implicating involvement of the PKC pathway in APAP-induced TJ destabilization. Whereas treatment with purified N-acetyl-p-benzo-quinoneimine, the highly reactive metabolite of APAP, directly disrupted TJ structures, possibly through reactive oxygen species effects on TJ-associated cytoskeletal-F-actin structures.

Using ECIS, we demonstrate an undescribed feature of APAP - temporal, dose-dependent disruption of intercellular tight junction/ adhesion structures, even at low dose - which correlates with an *in vivo* model of APAP toxicity. This observation may have relevance in the clinical syndrome of APAP-induced ALF and may inform future therapeutic strategies.

References:

- [1] R.K. Rao, G. Samak, *Tissue barriers* 1 (2013) e25718.
- [2] T.B. Andersson, K.P. Kanebratt, J.G. Kenna, *Expert Opin. Drug Metab. Toxicol.* 8 (2012) 909-920.
- [3] J. Wegener, J. Seebach, *Cell Tissue Res.* 355 (2014) 485-514.

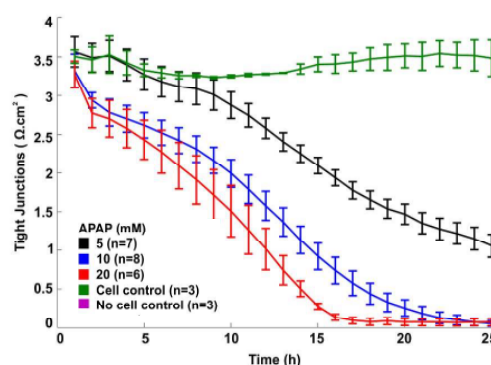


Fig. 1: Impedance monitoring shows early dose-dependent disruption of Rb parameter (TJ) induced by APAP in human liver HepaRG cells:

Calcium Signaling is Dispensable for Receptor-Regulated Endothelial Barrier Function

J.A. Stolwijk, W. Zhang, K. Matrougui, C. Renken and M. Trebak

Department of Cellular and Molecular Physiology, Penn State University College of Medicine;
Department of Physiological Sciences, East Virginia Medical School, Norfolk, VA; and Applied
Biophysics Inc., Troy NY

mtrebak@psu.edu

Endothelial barrier function is tightly regulated by plasma membrane receptors and is crucial for tissue fluid homeostasis; its dysfunction causes disease, including sepsis and inflammation. The ubiquitous activation of Ca^{2+} signaling upon phospholipase C (PLC)-coupled receptor ligation leads quite naturally to the assumption that Ca^{2+} signaling is required for receptor-regulated endothelial barrier function. This widespread hypothesis draws analogy from smooth muscle and proposes the requirement of G protein coupled receptor (GPCR)-generated Ca^{2+} signaling in activating the endothelial contractile apparatus and generating inter-endothelial gaps. Notwithstanding endothelia being non-excitable in nature, the hypothesis of “ Ca^{2+} -induced endothelial contraction” has been invoked to explain actions of GPCR agonists that either disrupt or stabilize endothelial barrier function. Here, we challenge this correlative hypothesis by showing complete lack of causal link between GPCR-generated Ca^{2+} signaling and changes in human microvascular endothelial barrier function. We used three endogenous GPCR agonists; thrombin and histamine which disrupt endothelial barrier function; and Sphingosine-1-phosphate (S1P) which stabilizes barrier function. The qualitatively different effects of these three agonists on endothelial barrier function occur independently of Ca^{2+} entry through the ubiquitous store-operated Ca^{2+} entry (SOCE) channel Orai1, global Ca^{2+} entry across the plasma membrane and Ca^{2+} release from internal stores. However, disruption of endothelial barrier function by thrombin and histamine requires the Ca^{2+} sensor STIM1, while S1P-mediated enhancement of endothelial barrier function occurs independently of STIM1. We conclude that the “ Ca^{2+} -induced endothelial contraction” hypothesis should be abandoned.

RhoGTPases and their regulators: a large scale siRNA screen to identify new regulators of the endothelial barrier

J. Amado-Azevedo^{1,4}, J. Bezu^{1,4}, R.X. de Menezes², V.W. van Beusechem³, V.W. van Hinsbergh¹, G.P. van Nieuw Amerongen¹, P.L. Hordijk^{1,4}

¹Department of Physiology, Institute for Cardiovascular Research, VU University Medical Center, Amsterdam, The Netherlands

²Department of Epidemiology and Biostatistics, VU University Medical Center, Amsterdam, The Netherlands

³Department of Medical Oncology, RNAinterference Functional Oncogenomics Laboratory, VU University Medical Center, Amsterdam, The Netherlands

⁴Research cluster Microvascular Disease, Amsterdam Cardiovascular Sciences, The Netherlands

p.hordijk@vumc.nl

Vascular leakage is a hallmark of many chronic diseases. Despite its importance, no specific therapies are available to prevent or reduce it. RhoGTPases, their regulators and effectors are central to many cellular processes exerting both positive and negative effects on the integrity of the endothelial barrier. Precise knowledge about this mechanism remains fragmentary as data is limited to a few family members. Combining RNAi and electrical resistance measurements, we quantified the relevance of RhoGTPases and of Rho-regulatory proteins for endothelial barrier function.

HUVECs of 12 donors were isolated, pooled, seeded on ECIS® arrays and transfected with a customized library of 270 siRNAs targeting all known RhoGTPases, GAPs, GEFs and Effectors. The effect of the loss of each target on the endothelial barrier was evaluated by recording the absolute endothelial resistance at defined time-points. Statistical analysis identified 10 candidate genes, six of which promoted endothelial barrier function (i.e. induced higher electrical resistance compared to the controls) whereas four induced a loss of barrier function. Two of these hits (a RhoGAP and a RhoGEF) were studied in more detail and characterized by biochemical and immunofluorescence analyses.

Thus, using this approach, we identified new positive and negative regulators of endothelial barrier formation, inflammatory dysfunction and/or re-establishment of cell-cell contacts.

Morphological changes of HUVEC under shear stress conditions

A. Bieser, H. Wagner, C. Fey, A. Reiser

ibidi GmbH, 82152 Martinsried, Germany

abieser@ibidi.de

In vivo endothelia cells like HUVEC develop and differentiate under shear stress conditions. Despite this most in vitro studies are still performed under steady state conditions. Therefore the aim of this study was to establish an **in vitro model** for long term experiments of HUVEC under shear stress.

Up to now it is unknown which shear range is responsible for an in vivo like cell behavior. Regarding the physiological properties and morphological characteristics of the cell layer shear stress values between 0.7 and 30 dyn/cm² were investigated over 96 hours.

Results:

1. Morphology

- Cell orientation is significantly changed at shear stress higher than 0.7 dyn/cm²
- Elongation of cell shape depends on shear stress parameters
- The adherens junction protein VE-Cadherin undergoes a remarkable change during shear stress conditions

2. Cell Physiology- Impedance measurements

- Resistance and Capacitance of the confluent cell layer display strong dependency on shear stress conditions
- According to the tested parameters, shear stress of 30 dyn/cm² proved to be far from physiological conditions. A leakage of the cell layer indicated by the sharp and inhomogeneous increase of C (capacitance) and the disturbed optical coherence of the cell layer was observed

Conclusion:

In long term in vitro experiments HUVEC displayed a dynamic modification in cell morphology and cell-cell contacts leading to a dramatic change in the physiological behavior of the cell monolayer. Low shear stress of 0.7 dyn/cm² had no effect on tested parameters and correlates to steady state observations.

The results highlight the importance of the exact definition of shear stress parameters since only a range between >0.7 and <30 dyn/cm² seems to mimic physiological flow conditions for HUVEC under in vitro conditions.

Using a TEER-based screen to find novel mechanisms that regulate retinal endothelial barrier function

V. Küppers¹, M. Tzouros¹, D. W. Avila¹, L. Badi¹, M. Ebeling¹, J. D. Zhang¹, O. Eidam¹, P. Turowski², G. Hartmann¹

¹ Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

² UCL Institute of Ophthalmology, London, United Kingdom

verena.kueppers@roche.com

The retina is one of the metabolically most active tissues in the body and is supplied with nutrients and oxygen by the choroidal vasculature and the retinal vasculature. While choroidal endothelial cells (ECs) are highly fenestrated and do not form a tight barrier, retinal ECs exhibit a high barrier function and form the inner blood-retinal barrier (iBRB). Conditions like hyperglycemia and hypoxia, which can be found in diabetic patients, weaken the endothelial barrier. Breakdown of the iBRB, followed by vascular leakage and neovascularization, is one of the main drivers in diabetic retinopathy (DR) and diabetic macular edema (DME), which both lead to vision loss and blindness. An intact and tightly regulated blood-retinal barrier is thus crucial to prevent disease progression and to avoid vision loss. The main target for treating DME/DR is currently vascular endothelial growth factor (VEGF). Since 30% of patients do not fully or not at all benefit from those therapies, there is an important unmet medical need for the discovery of additional therapeutics. The identification of new target proteins and pathways which regulate blood-retinal barrier integrity is therefore of crucial importance.

One of the signaling pathways that influence endothelial barrier function apart from VEGF signaling is the angiopoietin/Tie system. Angiopoietin-1 (Ang-1) and Ang-2 both bind the receptor tyrosine kinase Tie-2. Ang-1 signaling leads to barrier stabilization and increased vessel integrity, while Ang-2 is only a weak agonist. It is at the same time an antagonist of Ang-1 and overall promotes barrier destabilization, proliferation and angiogenesis. Using transendothelial electrical resistance (TEER) measurements, we could show that Ang-1 counter-regulates VEGF-induced barrier breakdown in HUVECs.

In order to dissect the differential signaling upon angiopoietin stimulation, HUVECs were stimulated with Ang-1, Ang-2 and the combination of both, and analyzed with Tandem Mass Tags (TMT) based quantitative phosphoproteomics. The initial discovery hits were further bioinformatically evaluated on single peptide/protein level as well as on pathway level. We identified biological processes and signaling pathways which are differentially enriched in the respective treatment conditions. Together with additional sites from the literature, a list of 500 distinct and candidate phosphosites was assembled, which will now be verified using a targeted phosphoproteomics approach.

The 500 hits were additionally mapped on an integrated kinase-substrate-interaction network, which identified central signaling proteins likely involved in the regulation of barrier function. Those signaling proteins are now analyzed functionally in more detail using TEER as a direct readout for endothelial barrier function to confirm their role in barrier regulation. Small-molecule inhibitors specifically targeting these proteins are applied to unstimulated, VEGF stimulated and Ang-1 stimulated HUVECs, respectively. Using this setup, it is possible 1) to elucidate the role of the protein in baseline endothelial barrier function, 2) to detect a possible effect of the protein on barrier restoration after VEGF-induced barrier breakdown, and 3) to unravel the interplay of this protein with Ang-1 induced barrier stabilization. This screen will thus result in a list of potential new candidates and pathways that efficiently regulate endothelial barrier function. The most promising candidates will be verified and analyzed in more detail and could later on be used as targets for new therapeutics in DME and DR.

Impedance spectroscopy in epithelial tissues and cell cultures

D. Günzel¹, S.M. Krug¹, M. Fromm¹, T. Nacke², T. Schmid³ and M. Bogdan³

¹Institut für Klinische Physiologie, Charité, Campus Benjamin Franklin, Berlin (Germany)

²Institut für Bioprocess- und Analysenmesstechnik e.V, Heilbad Heiligenstadt (Germany)

³Abteilung Technische Informatik, Universität Leipzig, Leipzig (Germany)

dorothee.guenzel@charite.de

Epithelia and endothelia are tissues that form barriers between different compartments of the body. In contrast to non-epithelial tissues, epithelia (and endothelia) form cell-cell contact complexes called tight junctions (TJs) that regulate and restrict paracellular transport between neighbouring cells. Many epithelia and some endothelia fulfill the definition by Schultz [1] for a tight epithelium, i.e. that the paracellular conductance is lower than the transcellular conductance. Such tight epithelia are the prerequisite for building up and maintaining concentration differences in adjacent compartments.

Diseases such as infection or inflammation may affect various parameters of epithelia. In the intestine, loss of barrier function as well as stimulation of transcellular transport may cause diarrhea. In parallel, the subepithelial tissue may increase in thickness and the effective epithelial surface area may be altered, e.g. by crypt hyperplasia or loss of villi.

A detailed impedance analysis of cultured cells as well as of epithelial tissues such as human intestinal biopsies allows to identify and characterize epithelial and subepithelial components in basic and translational biomedical research (Fig.). Whereas conventional “one-path” impedance spectroscopy only separates epithelial from subepithelial components, our new developments combine fast multisinus impedance spectroscopy, mathematical modeling and machine learning algorithms and enable us to quantify paracellular and transcellular pathways and to determine apical and basolateral membrane properties of the epithelial cell layers [2-4].

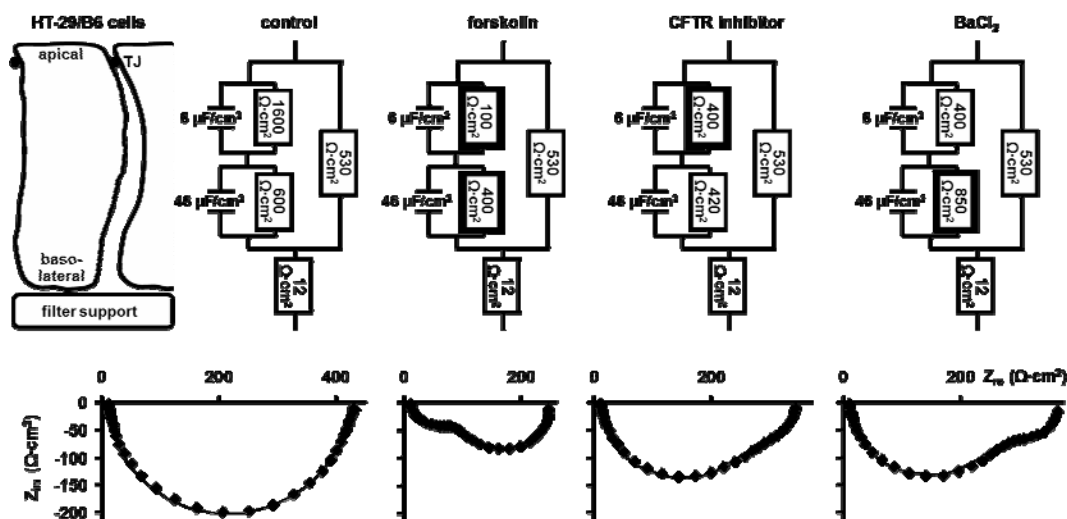


Figure: Modelling impedance spectra from confluent monolayers of the human colon cell line HT-29/B6. Forskolin application decreases both apical and basolateral resistance. Apical effects are due to CFTR Cl^- channel activation as they are reduced by CFTR(inh)-172, basolateral effects are due to Ba^{2+} -sensitive K^+ channels.

References:

- [1] S.G. Schultz, *J. Gen. Physiol.* 59 (1972) 794-798.
- [2] S.M. Krug, M. Fromm, and D. Günzel, *Biophys. J.* 97 (2009) 2202-2211.
- [3] D. Günzel, S. Zakrzewski, T. Schmid, M. Pangalos, J. Wiedenhoef, C. Blasse, C. Ozboda, and S.M. Krug, *Ann. N.Y. Acad. Sci.* 1257 (2012) 142-151.
- [4] T. Schmid, M. Bogdan M, and D. Günzel, *PLOS One* 8 (2013) e62913.

Application of two-path impedance spectroscopy to discriminate between trans- and paracellular epithelial ion transport

S.M. Krug, M. Fromm and D. Günzel

Institut für Klinische Physiologie, Charité, Campus Benjamin Franklin, Berlin (Germany)

susanne.m.krug@charite.de

Transport of solutes across epithelia can occur via transcellular and paracellular pathways. While transcellular passage is a transport through the apical and basolateral membranes of cells, the paracellular passage takes place through the cleft of neighboring cells and is regulated by the tight junction. The tight junction is composed of more than 30 different transmembranal proteins comprising the claudin family and the tight junction-associated MARVEL protein family, called TAMPs. Most of these proteins strengthen the barrier, but in contrast some claudins form specific paracellular ion channels [1]. Under pathological conditions, expression of tight junction proteins is altered leading to an impaired epithelial barrier [2].

Conventional transepithelial resistance (TER) provides only a rough idea of the characteristics of an epithelium e.g. in intestinal inflammation diseases, for at least two reasons. First, a defective barrier is reflected by a decrease in epithelial resistance. Unfortunately, this is usually masked by an increased resistance of subepithelial tissue layers, because both add together in TER. Second, transmembrane ion transporters and paracellular channels may be altered independent of each other. The most simple impedance technique, "one-path impedance spectroscopy", distinguishes between epithelial and subepithelial fraction of TER, thus resolving the first problem. The more complex method of "two-path impedance spectroscopy" allows to resolve the second problem, as it measures trans- and paracellular separately [3,4].

We present practical data of both impedance techniques for different epithelia and cell culture models which were used for characterization of barrier-forming as well as for channel-forming tight junction proteins, but also for characterization of the action of different treatments affecting the epithelial barrier.

Also in analysis of diseases like ulcerative colitis or Crohns disease impedance techniques are applied. A typical outcome of such measurements is an increased paracellular ion conductivity due to an upregulation of the paracellular cation and water channel claudin-2 and/or a downregulation of barrier-forming claudins. As a consequence, ions and water excessively diffuse from blood to lumen and result in a so-called leak-flux diarrhea.

In conclusion, comparison of results of conventional TER with data from impedance spectroscopy show that the latter allows for deeper mechanistic insight into epithelial/subepithelial as well as transcellular/paracellular characteristics of epithelia in health and disease.

References:

- [1] D. Günzel and M. Fromm, *Compr. Physiol.* 2 (2012) 1819-52.
- [2] S.M. Krug, J.D. Schulzke, and M. Fromm, *Semin. Cell Dev. Biol.* 36 (2014) 166-176.
- [3] S.M. Krug, M. Fromm, and D. Günzel, *Biophys. J.* 97 (2009) 2202-2211.
- [4] D. Günzel, S.M. Krug, R. Rosenthal, and M. Fromm, *Current Topics in Membranes* 65 (2010) 39-78.

Impact of manganese and mercury species on and transfer across the blood-brain and blood-CSF barrier *in vitro*

¹J. Bornhorst, ¹H. Lohren, ²S. Hüwel, ²H.-J. Galla, ¹T. Schwerdtle

¹Institute of Nutritional Science, Department of Food Chemistry, University of Potsdam, Germany

²Institute of Biochemistry, Westphalian Wilhelms-University Muenster, Germany

Julia.bornhorst@uni-potsdam.de

Manganese (Mn) is essential for the development and function of the central nervous system (CNS) in mammalian systems. However, chronic occupational and dietary overexposure to excessive levels of Mn results in neurological consequences with symptomatology analogous to Parkinson's disease (PD). The pathways of Mn uptake in the CNS and the underlying mechanisms of toxicity are not yet fully understood. The use of primary porcine *in vitro* models of the two brain regulating interfaces, the blood-brain barrier (BBB) and the blood-cerebrospinalfluid (CSF) barrier allows for the first time a direct comparison of Mn-induced effects on the integrity of the barriers as well as Mn transfer across these two barriers in both directions. The data pointed out a stronger Mn sensitivity of the *in vitro* blood-CSF barrier as well as a site-directed, most probably active Mn transport towards the brain-facing compartment. In contrast to the general assumption in the literature, the data suggest that the blood-CSF barrier might be a major route for Mn into the CNS after oral Mn intake [1].

Exposure to organic mercury (Hg) compounds promotes primarily neurological effects. Humans are exposed to organic Hg mainly in the form of methylmercury (MeHg) *via* the consumption of contaminated fish and other seafood products. In terrestrial food sources Hg is mostly found as inorganic Hg. Thiomersal is a further organic Hg compound which is used as a preservative in medical preparations. Although MeHg is recognized as a potent neurotoxicant, its transfer into the CNS is not fully evaluated. The understanding of transfer mechanisms regarding the CNS is an important precondition for an evaluation of Hg species-induced neurotoxicity. Thus, primary porcine *in vitro* models of the BBB and the blood-CSF barrier were used to investigate effects of MeHgCl, thiomersal and HgCl₂ on the barriers as well as transfer into and out of the CNS *in vitro*. The results show significant transfer differences of the various incubated species as well as in the different barrier systems. Regarding the BBB in case of MeHgCl and thiomersal incubation, mercury crossed the barrier in both directions, with a slight accumulation in the brain-facing compartment, after simultaneous incubation in both compartments. For HgCl₂, our data provide first evidence that the BBB transfers mercury out of the brain. In the blood-CSF barrier model the organic Hg species are transferred actively in the contrary direction by the blood-CSF barrier as compared to the BBB. These results are the first to demonstrate an efflux of organic mercury compounds regarding the CNS and provide a completely new approach in the understanding of mercury compounds specific transport. Additionally, the results present a new basis for further research regarding the interaction between mercury compounds and the blood-CSF barrier [2].

References:

- [1] J. Bornhorst, C. A. Wehe, S. Hüwel, H.-J. Galla, U. Karst, T. Schwerdtle, *The Journal of Biological Chemistry*, 287/21 (2012) 17140-17451.
- [2] H. Lohren, J. Bornhorst, H.-J. Galla, T. Schwerdtle, *Metallomics*, 7/10 (2015) 1420-1430.

Independent Observation of Two Co-Cultured Cell Layers Grown on Filter Supports

K. Hajek and J. Wegener

University of Regensburg, Regensburg (Germany)

Kathrin.Hajek@chemie.uni-regensburg.de

Measuring transepithelial, electrical resistances (TEER) is an established non-invasive and label-free method to quantify the permeability of barrier-forming cell layers (skin tissue, cells of the intestine or vessels). Highly permeable polycarbonate membranes (pore density $10^8/\text{cm}^2$, pore- $\varnothing$ 400 nm) are commonly used as culture substrate in standard transfilter measurements for 2D cell layers. A confluent cell layer, cultured upon these porous polymer membranes, represents a diffusion barrier between the two fluid compartments in a typical measurement chamber. Electrodes individually placed in either compartment of the chamber are necessary to determine the TEER as well as the frequency-dependent impedance of the cell layer. The latter can be analyzed by means of equivalent circuit modelling.

By depositing a thin gold film on one side of the filter support which is used as an additional, third electrode enables independent observation of two cell layers co-cultured on opposite sides of the filter by impedance analysis (Fig. 1). As the filter membrane is thin ($10\text{ }\mu\text{m}$) and highly porous, cell-to-cell communication between the cells on opposite sides of the filter is possible and its influence on the individual barrier properties of the two cell populations is accessible. Moreover, this setup allows studying the influence of environmental pollutants or drugs on both cell layers in a single experiment.

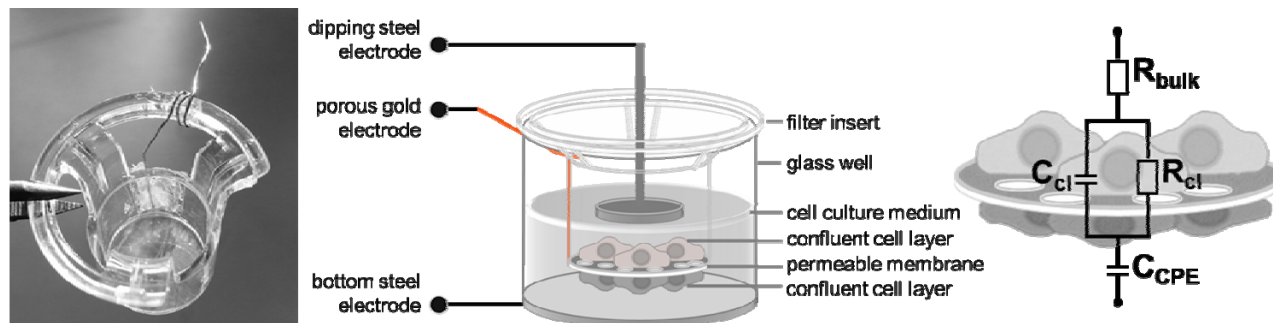


Fig. 1: Photograph of a modified transwell filter insert (left). The scheme in the middle panel represents the experimental setup to measure the impedance contribution of each cell layer in a co-culture. Transepithelial electrical resistance (TEER) can be obtained by fitting the experimental data to the equivalent circuit shown in the scheme on the right side.

Recent Advances in Impedance-Based Wound-Healing Assays to Monitor Cell Migration in Different Experimental Scenarios

Stefanie Michaelis

Universität Regensburg, Regensburg (Germany)

Stefanie.Michaelis@chemie.uni-regensburg.de

Collective cell migration plays essential roles in a wide spectrum of biological processes, such as embryogenesis, tissue regeneration, and cancer metastasis. Numerous wound-healing assays based on mechanical, chemical, optical, and electrical approaches have been developed to create model “wounds” in cell monolayers to study the collective cell migration processes [1]. These approaches can result in different microenvironments for cells to migrate and possess diverse assay characteristics in terms of simplicity, throughput, reproducibility, and multiplexability. This presentation will provide an overview of advances in wound-healing assays and discuss their advantages and limitations in studying collective cell migration.

Special emphasis will be focused on impedance-based approaches, their physical principles and a thorough validation [2]. The performance of the assay is illustrated by several published examples and a chemosensitivity study that experimentally addresses the question whether widely applied chemotherapeutics might affect collective cell migration. The human lung carcinoma cell line A549 serves as a model system that will be replaced by biopsy material in the future. The wound healing data is correlated with proliferation assays.

References:

- [1] R. Riah; Y. Yang; D. Zhang and P.K. Wong, *Journal of Laboratory Automation* 17/1 (2012) 59-65.
- [2] C.R. Keese; J. Wegener; S.R. Walker and I. Giaever, *Proc. Natl. Acad Sci USA* 101/6 (2004) 1554-1559.

Spectral Impedance Measurements during Wound Healing Compared to Standard ECIS Measurements applied to Cell Differentiation

F.E. Giana, F.J. Bonetto, M.I. Bellotti

Instituto Balseiro, CAB/CNEA, San Carlos de Bariloche, RN, Argentina

fabian.bonetto@gmail.com

We measured the spectral impedance with high voltage pulses (Wound Healing Technique) in a MDCK cell monolayer. Then we monitored the effect on the cell monolayer using the standard ECIS technique. We computed the ratio between the two values (high voltage divided by low current impedances). We computed a figure of merit for the MDCK cell monolayer. We repeated the experiment for the IOVA cell line. We obtained characteristic curves for each cell lines.

Cell locomotion monitored by impedance analysis - from *Dictyostelium discoideum* to primordial germ cells

T. Baronsky, E. Schaefer, M. Tarantola, A. Janshoff

Georg-August-Universität Göttingen, Göttingen (Germany)

ajansho@gwdg.de

Morphological changes of *Dictyostelium discoideum* cells during chemotaxis under conditions of starvation have been scrutinized by impedance spectroscopy and total internal reflection microscopy. Amebas were seeded on gold electrodes displaying impedance oscillations that were analyzed by optical video microscopy to relate synchronous changes in cell density, morphology, and distance from the surface to the transient impedance signal. We found that starved amebas periodically reduce their overall distance from the surface producing a larger impedance and higher total fluorescence intensity in total internal reflection fluorescence microscopy. We propose that the dominant sources of the observed impedance oscillations on electric cell-substrate impedance sensing electrodes are periodic changes of the overall cell-substrate distance of a cell. These synchronous changes of the cell-electrode distance were also observed in oscillating signal of acoustic resonators covered with amebas. We also found that periodic cell-cell aggregation into transient clusters correlates with changes in the cell-substrate distance and therefore also contributes to the impedance signal. Both, cell-cell as well as cell-substrate contacts form in phase during chemotaxis of *Dictyostelium discoideum* cells.

Another type of cell locomotion was addressed by examining the migration of primordial germ cells from *Xenopus*. The transition from passive to active migration of primordial germ cells in *Xenopus* embryos correlates with a reduction in overall adhesion to surrounding endodermal cells as well as with reduced E-cadherin expression. Single cell force spectroscopy, in which cells are brought into brief contact with a gold surface functionalized with E-cadherin constructs, allows for a quantitative estimate of functional E-cadherin molecules on the cell surface. The adhesion force between migratory PGCs and the cadherin-coated surface was almost identical to cells where E-cadherin was knocked down by morpholino oligonucleotides (180 pN). In contrast, non-migratory PGCs display significantly higher adhesion forces (270 pN) on E-cadherin functionalised surfaces. On the basis of these observations, we propose that migration of PGCs in *Xenopus* embryos is regulated via modulation of E-cadherin expression levels, allowing these cells to move more freely if the level of E-cadherin is reduced.

References:

- [1] T. Baronsky, A. Dzementsei, M. Oelkers, J. Melchert, T. Pieler, A. Janshoff, *Integr. Biol.* 8 (2016) 349-358.
- [2] A. Dzementsei, D. Schneider, A. Janshoff, T. Pieler, *Biology Open* 2 (2013) 1279-1287.
- [3] E. Schäfer, M. Tarantola, E. Polo, C. Westendorf, N. Oikawa, E. Bodenschatz, B. Geil, A. Janshoff, *PLOS ONE* 8/1 (2013), e54172.
- [4] E. Schäfer, C. Westendorf, E. Bodenschatz, C. Beta, B. Geil, A. Janshoff, *Small* 7 (2011) 723-726.

Administration of protein kinase d1 induce migration of intestinal epithelial cells *in vitro*

D.S.G. Nielsen; M. Fredborg and S. Purup

Department of Animal Science, Aarhus University, DK-8830 Tjele, Denmark

ditteg.nielsen@anis.au.dk

Significant increases in prevalence rates of inflammatory bowel diseases (IBD), characterized by a compromised epithelial barrier function, requires development of new, innovative and cost-effective drugs to optimize patient treatment and the economic burden [1]. Protein kinase d1 (PKD1) is rapidly activated in intestinal epithelial cells *in vitro* upon wounding, promoting migration and wound healing [2]. Here we investigate whether administration of recombinant human PKD1 to rat and human intestinal epithelial cells *in vitro* induce migration of cells into the wounded area, besides studying the potential signaling mechanisms behind these effects.

Rat and human intestinal epithelial cells (IEC-6, FHs 74 Int cells) were used for scratch wound healing assays, as previously reported [3]. Cells were treated for 6 hours with human recombinant PKD1 at 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10, 50 and 100 ng/ml. Assays were performed in triplicates with twelve repetitions for each treatment and migration presented relative to the negative control (0 ng/ml PKD1). Intracellular signaling proteins were studied by sensitive Amplified Luminescence Proximity Homogenous Assay (AlphaScreen®). FHs 74 Int cells were scratched and treated with 0.1 and 100 ng/ml of PKD1 for 2, 5 and 15 minutes alone or in presence of a G protein-coupled receptor agonist, either 100 nM of phorbol 12,13-dibutyrate (PDBu) or angiotensin II (ANGII).

Administration of human recombinant PKD1 to FHs 74 Int cells increased migration at 0.0001 to 10 ng/ml PKD1 ($P<0.05$) compared with the negative control. Specifically, PKD1 at 0.01 and 0.1 ng/ml increased migration by >60%. A similar effect was observed in IEC-6 cells where PKD1 at 0.00001 to 10 ng/ml increased migration ($P<0.05$) compared with the negative control. Preliminary studies revealed deactivation of the Erk signaling pathway following PKD1 treatment, whereas no effects were observed on Akt and NFκB signaling pathways. Treatment with PDBu caused a marked activation of the Erk signaling pathway ($P<0.05$), yet PKD1 tended to deactivate this pathway even in the presence of PDBu ($P=0.0506$). Both Akt and NFκB signaling pathways were deactivated by PDBu ($P<0.05$). This is in accordance with a previous study showing early activation of Erk followed by subsequent activation of Akt and NFκB signaling pathways [4]. The agonist ANGII had no notable effect on these pathways. These results demonstrate that administration of recombinant human PKD1 induce wound healing in intestinal epithelial cells *in vitro*, suggesting that PKD1 might be a possible approach for future treatment of IBD.

References:

- [1] G.G. Kaplan, *Nat. Rev. Gastroenterol. Hepatol.* 12/12 (2015) 720-727.
- [2] S.H. Young, et al., *Am. J. Physiol. Gastrointest. Liver Physiol.* 303/3 (2012) G356-366.
- [3] S. Purup and T.S. Nielsen, *Animal* 6/3 (2012) 423-432.
- [4] Li, J., et al., *J. Cell Sci.* 126 (2013) 5005-5017.

Transistor-based impedimetric monitoring of single cells

S. Ingebrandt

University of Applied Sciences Kaiserslautern, Zweibrücken (Germany)

sven.ingebrandt@hs-kl.de

In recent years we developed a technique using open-gate, ion-sensitive field-effect transistor (ISFET) devices for impedimetric recordings from cell cultures. This novel technique has the potential to supplement the classical ECIS method using metal microelectrodes with a novel and powerful technique, which has proven to provide a single cell and even a subcellular lateral resolution.

We fabricated ISFET array devices and the readout circuits for this direction of research in house and applied it in several applications and proof-of-concept experiments in recent years. In ECIS usually the very high input impedance of the metal microelectrodes is complicating the miniaturization of the sensors, since very tiny currents need to be transported via long contact lines and wires to the readout units. In contrast to this the ISFET devices are brought to their working point by applying an appropriate bias voltage to the source and drain contacts and to the reference electrode (most of the time Ag/AgCl), which is immersed into the electrolyte solution. In this condition a constant current of about 0.5 mA is flowing through our ISFETs (I_{DS}) and can easily be amplified and read out by a transimpedance amplifier circuit. To the constant gate-source voltage an additional, very small stimulation voltage v_{stim} is overlaid, which is converted by the ISFET and carried as small modulation on top of the strong I_{DS} current. With this method a frequency scan of ISFET and first amplifier stage can be done, which reflects the transfer function of the circuit. The attachment of a cell is influencing this transfer function mainly in the low pass characteristics and the spectra of the device can reveal attachment, detachment, spreading, migration, and effects to chemical stimuli on individual cells.

In recent years we developed an equivalent circuit model with an analytical solution [1] to explain the spectra, which was also applied to improve the design of the devices [2]. We named our technique in accordance to the ECIS nomenclature the Field-Effect Transistor Cell-substrate Impedance Sensing (FETCIS) and applied it to sense effects of anti-cancer drugs to cancer cells [3], toxic effects of hydrogen peroxide to neurons [4], effects of silica nanoparticles ('dust') to lung epithelial cells [5] and used the technique to study the adhesion and migration of individual, human T lymphocytes [6].

This presentation provides an overview to the FETCIS technique and to the past works where many members of our research group were involved and to the fascinating and manifold future possibilities of FETCIS.

References:

- [1] A. Susloparova, D. Koppenhöfer, J.K.Y. Law, X.T. Vu and S. Ingebrandt, *Lab on a Chip* 15 (2015) 668-679.
- [2] A. Susloparova, X.T. Vu, D. Koppenhöfer, V. Pachauri, J.K.Y. Law and S. Ingebrandt, *physica status solidi (a)* 211 (2014) 1395-1403.
- [3] A. Susloparova, D. Koppenhöfer, X.T. Vu, M. Weil and S. Ingebrandt, *Biosensors and Bioelectronics* 40 (2013) 50-56.
- [4] D. Koppenhöfer, F. Kettenbaum, A. Susloparova, J.K.Y. Law, X.T. Vu, T. Schwab, K.H. Schäfer and S. Ingebrandt, *Biosensors and Bioelectronics* 67 (2015) 490-496.
- [5] D. Koppenhöfer, A. Susloparova, D. Docter, R.H. Stauber and S. Ingebrandt, *Biosensors and Bioelectronics* 40 (2013) 89-95.
- [6] J.K.Y. Law, A. Susloparova, X.T. Vu, X. Zhou, F. Hempel, B. Qu, M. Hoth and S. Ingebrandt, *Biosensors and Bioelectronics* 67 (2015) 170-176.

Multi-Parametric Analysis of Cardiomyocyte Excitation-Contraction Coupling for Pre-Clinical Safety Assessment and Investigating iPSC-Cardiomyocyte Disease Models

Y.A. Abassi and X. Zhang

ACEA Biosciences, Inc. San Diego, California (USA)

yabassi@aceabio.com

Drug toxicity is a pervasive issue in pharmaceutical drug development that is difficult to avoid but needs to be identified and well mitigated. Failure to recognize such toxicities during the course of drug development can pose great risk not only to the target population but can also be of tremendous financial burden to drug developers due to drug withdrawals from the market and legal ramifications. The potential for clinical manifestation of ventricular arrhythmias, known commonly as “Torsade de Pointes” (TdP), by pharmaceutical drugs continue to be a major concern for both pharmaceutical industry and regulatory agencies. In this presentation we will discuss a platform technology which combines cellular impedance measurement together field potential recording using human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) as a predictive assay system for screening and identification of pro-arrhythmic and cardio-modulating compounds. The impedance readout allows measurement of hiPSC-CM contraction which field potential measurement allows for measurement and assessment of integrated ion channel activity of the cardiomyocytes. We will share our results which provide evidence that drugs which modulate ventricular repolarization through the hERG channel can be readily detected by this dual readout system. Furthermore, we will discuss the utility of this multi-parametric readout for assessment of certain cardiac arrhythmias that may be manifested due to genetic mutations. In summary, our data demonstrates that hiPSC-CMs in conjunction with simultaneous measurement of ion channel activity and contractility can be a reliable approach for risk assessment of arrhythmia.

Impedimetric microtomography in rolled-up tubes

S.M. Weiz; M. Medina-Sánchez and O.G. Schmidt

Institute for Integrative Nanosciences, IFW Dresden (Germany)
Chemnitz University of Technology, Chemnitz (Dresden)

s.m.weiz@ifw-dresden.de

m.medina.sanchez@ifw-dresden.de

Electrical impedance tomography (EIT) is a label-free and non-invasive imaging technique that is suitable to study the conductivity distribution of an object. Recently, millimeter-sized EIT setups have been reported [1,2]. Smaller devices with sizes of few tens of microns would be desirable to study, for instance, cell-cell, cell-material or cell-drug interactions.

Hence, microtubular EIT (μ EIT) systems were fabricated by rolled-up technology [3]. Using sequential lithography and electron beam deposition steps, an array of 12 electrodes (Cr/Au) was patterned on top of a TiO_2 strained layer and a Ge sacrificial layer. Upon selective removal of the sacrificial layer, the TiO_2 thin films rolled up to form tubes with diameters of ca. 30 μm (Fig. 1a). Tube characteristics like diameter and number of windings can easily be defined by tuning the fabrication parameters.

For characterization measurements, a partially Au-coated SiO_2 microparticle was introduced into the μ EIT system as a phantom. Cyclic voltammetry and impedance measurements were conducted and revealed clear differences with and without the introduced particle (Fig. 1b, c), showing that the presence of a structure inside the tube can be detected. Furthermore, different results were obtained for electrodes in proximity to the dielectric or the conductive part of the Janus particle, respectively. In order to obtain cross-sectional tomography images, strategies for appropriate data acquisition and plotting are being developed. First cell insertion tests were carried out with a HeLa cell, demonstrating the feasibility of the introduction and removal of cells into the μ EIT structure. Future experiments will be conducted with different types of cells, e.g. neuronal stem cells.

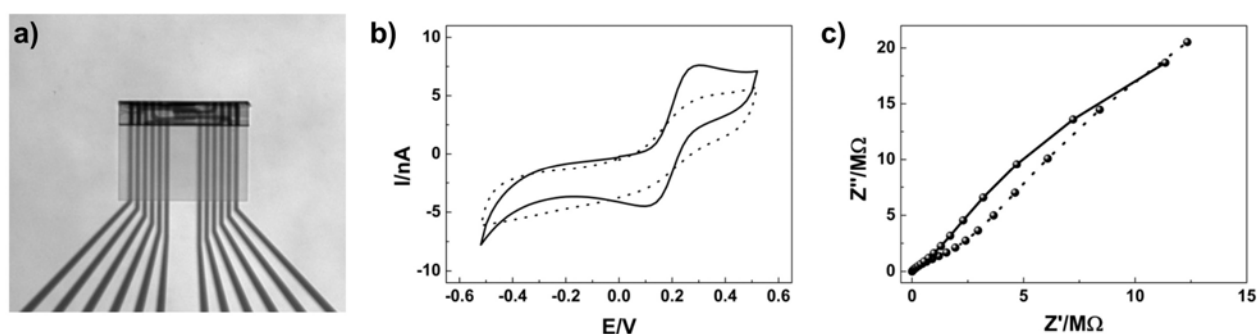


Figure 1: a) Tubular μ EIT structure. b) Cyclic voltammetry and c) Nyquist plot obtained with (dotted lines) and without (solid lines) particle inside the μ EIT device.

References:

- [1] T. Sun, S. Tsuda, K.-P. Zauner and H. Morgan, *Biosens. Bioelectron.* 25 (2010) 1109–1115.
- [2] T.A. York, T.N. Phua, L. Reichelt, A. Pawlowski and R. Kneer, *Meas. Sci. Technol.* 17 (2006) 2119–2129.
- [3] Y. Mei, G. Huang, A.A. Solovev, E.B. Ureña, I. Mönch, F. Ding, T. Reindl, R.K.Y. Fu, P.K. Chu and O.G. Schmidt, *Adv. Mater.* 20 (2008) 4085–4090.

Impedance-based Analysis and Holographic Phase Imaging of the GnRH-III-based Drug-targeting in Melanoma Cells

E. Lajkó¹, S. Spring^{1,2}, O. Láng¹, S. Ingebrandt², G. Mező³ and L. Köhidai¹

¹ Impedimetry MERILL Core Facility/Chemotaxis Research Group, Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest (Hungary)

² Department of Informatics and Microsystem Technology, University of Applied Sciences Kaiserslautern, Zweibrücken (Germany)

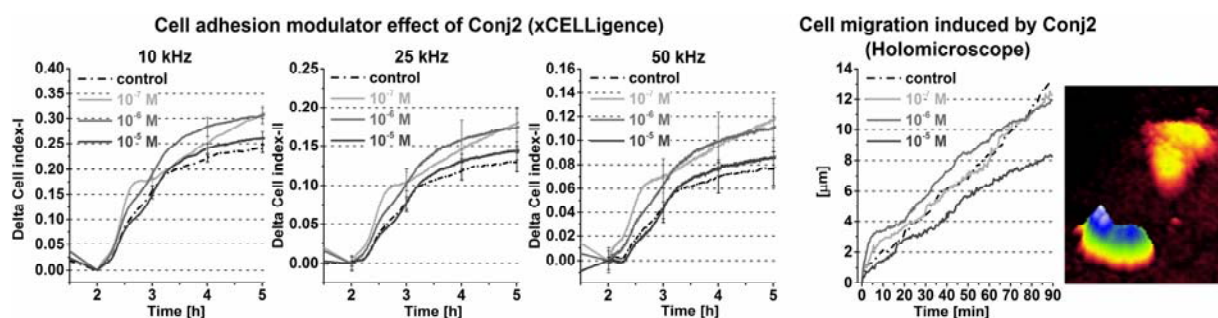
³ MTA-ELTE Research Group of Peptide Chemistry, Eötvös Loránd University, Budapest
lajesz@gmail.com

Peptide hormone based targeted tumor therapy is an approved strategy to selectively block the tumor growth and spreading. The gonadotropin-releasing hormone receptors (GnRH-R) over-expressed on different tumors (e.g. melanoma) could be utilized for drug-targeting by application of a GnRH analog as a carrier to deliver a covalently linked chemotherapeutic drug directly to the tumor cells [1]. In this study our aim was (i) to analyze the effects of GnRH-drug conjugates on melanoma cell proliferation, adhesion and migration; (ii) to study the mechanisms of the tumor cell responses, and (iii) to compare the activities of the conjugates with the free drug.

In the conjugates, the daunorubicin (Dau) was coupled to GnRH-III (conj3) or its derivatives modified in position 4 with Lys(Ac) (conj1) or Lys(nBu) (conj2) [2]. The adhesion and proliferation of A2058 melanoma model cell was detected by xCELLigence SP. The holographic microscope, HoloMonitor M4 was used for the morphometry and migration studies. To investigate molecular biological background of the melanoma responses, flow cytometry, quantitative PCR and inhibition assay with PI3K inhibitors were employed.

All conjugates elicited irreversible, tumor growth inhibitory effect (IC₅₀: conj1: 6.4×10^{-6} M, conj2: 5.4×10^{-6} M, conj3: 1.45×10^{-5} M) mediated via the PI3K sensible pathway. The conj1 and 3 proved to be cytostatic (G2/M arrest), while a stronger, cytotoxic effect of conj2 was verified by apoptosis measurement after annexin staining and morphometry analysis. In short term, the melanoma cell adhesion was increased by conj1 (10^{-6} – 10^{-5} M: 122.8–131%) and conj2 (10^{-8} M– 10^{-6} M: 131.7–121.9%). The uptake of conjugates by A2058 cells proved to be time dependent. The modification of the GnRH-III in position 4 was accompanied with an increased cellular uptake, higher cytotoxic and cell adhesion inducer activity, as well. In general, the migratory behavior of the melanoma cells was increased by conj1, while the conj2 decreased this activity. Expression study of different apoptosis markers also referred to structure-dependent mechanisms induced by the conjugates.

The internalization, the toxic and apoptotic activities of the conjugates show that the GnRH-III peptides could guard the Dau to melanoma cells and promote antitumor activity. The model system was shown to be sensitive enough to test small structural differences (e.g. length of the side chain in ⁴Lys). Conj2 proved to be the best candidate for targeted tumor therapy due to its cytotoxicity and immobilizing effect on tumor cell spreading.



References:

- [1] P. Limonta, M.M. Montagnani, S. Mai et al. *Endocr. Rev.* 33 (2012) 784-811.
- [2] R. Hegedűs, M. Manea, E. Orbán et al. *Eur. J. Med. Chem.* 56 (2012) 155-65.

PEDOT/PSS as transparent electrode material for impedance-based cell analysis

C. Götz^{1,2}, S. Trupp¹, and J. Wegener²

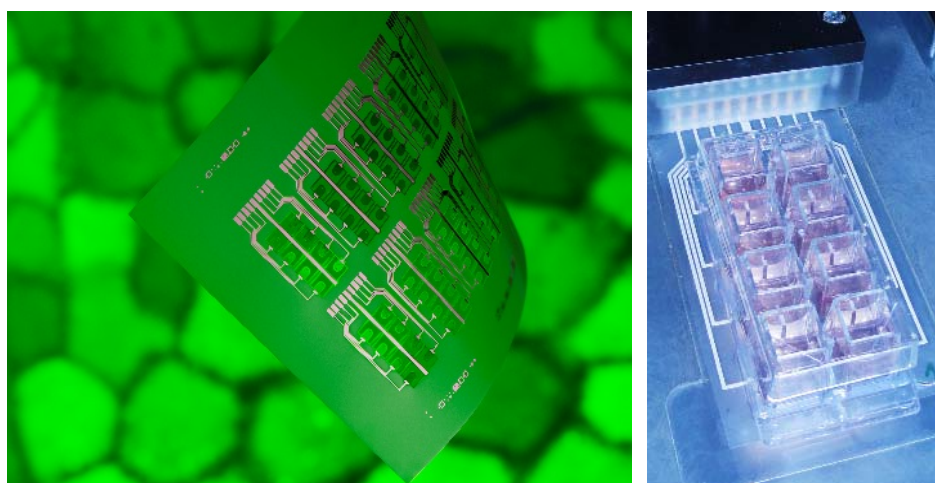
¹Fraunhofer EMFT, Business Area Sensor Materials, Munich, Germany

²Universität Regensburg, Regensburg, Germany

christian.goetz@chemie.uni-regensburg.de

Conducting polymers have increasingly found their way into products of everyday life. Established materials like gold and indium tin oxide are being replaced as conducting polymers stand out not only because of their low cost, but also some particular material characteristics like mechanical elasticity, optical transparency and their dielectric properties. Among all conducting polymers PEDOT/PSS shows the most prominent application potential. It can be dispersed in aqueous solutions and hence makes it accessible for large scale manufacturing techniques like screen printing. Layers of PEDOT/PSS show swelling in aqueous solutions, thus increase the interface capacitance between electrode and electrolyte, rendering it interesting for its application as a transparent transducer in impedimetric biosensors [1]. This research is focused on the use of PEDOT/PSS as electrode material in impedimetric whole-cell biosensors (Electric Cell-substrate Impedance Sensing, ECIS). 8-well electrode arrays have been fabricated by screen printing, showing great potential for commercial application. A comparison between screen printed PEDOT/PSS and the commonly used gold electrodes shows possibilities and limitations of this electrode material. Different applications are being investigated.

The distinct dielectric properties of PEDOT/PSS make investigation of the cell-cell and cell-substrate contacts highly sensitive and may even enable impedimetric measurements of cell lines formerly not accessible. Low cost and easy processing allow for electrode fabrication in industrial scales.



References:

- [1] A.S. Karimullah, D.R.S. Cumming, M. Riehle, N. Gadegaard, *Sensors and Actuators B* 176 (2013) 667– 674.

A versatile lab-on-a-chip system for optical and impedance based cytotoxicity assays

B. Böttcher; M. Büttner; S. Eisenhuth; B. Büttner and K.-H. Feller

Ernst-Abbe University of Applied Sciences Jena, Jena (Germany)

Bastian.Boettcher@eah-jena.de

Due to the rapid development of novel substances as nanoparticles as drug delivery cargos or drugs itself efficient and sensitive systems to test possible cytotoxic side effects are required. A solution for this problem is the application of lab-on-a-chip-systems since they can integrate many functional fluidic structures and analytical methods.

Therefore a lab-on-a-chip system was developed that combines optical and impedimetric measurement methods to determine cytotoxicity of substances with human cells. The chip consists of three layers, each made of TOPAS® COC 6015 which are bonded via hot-melt foils. This allows the generation of complex fluidic structures e.g. micromixers. For cultivation of cells, nine reservoirs are integrated whereat three of these wells each are connected via microfluidic channels forming one lane. Two lanes are connected with to micromixers each allowing the addition of the test or a reference substance. For impedance measurement, gold electrodes are deposited on the bottom of each well. The optical assay is based on cell stress induced expression of a heat shock protein (HSP72/70b) coupled with green fluorescent protein (GFP) via genetic modification. The high sensitivity of the method was proven by the measurement of dose-dependent increase of HSP-GFP-expression in modified HaCaT cells at sub lethal concentrations of CdCl_2 . Although this range of concentrations is interesting, it is also limited [1].

To detect cellular reactions at higher toxin concentration, ECIS measurement is applied within the chip system. With this extension of possible measurement concentration, also chemosensitivity tests are possible. As an example, chemotherapeutic agent paclitaxel and doxorubicin were added to unmodified A549 cells showing different cytotoxic effects. Adapting this chemosensitivity test to primary cells from cancer patients, a possibility for tumor therapy optimization is given.

The measurements showed that the developed lab-on-a-chip system is well suited for determination of the cytotoxicity of substances in a wide range of concentrations and offers also applications with unmodified cells due to the non-invasive character of ECIS.

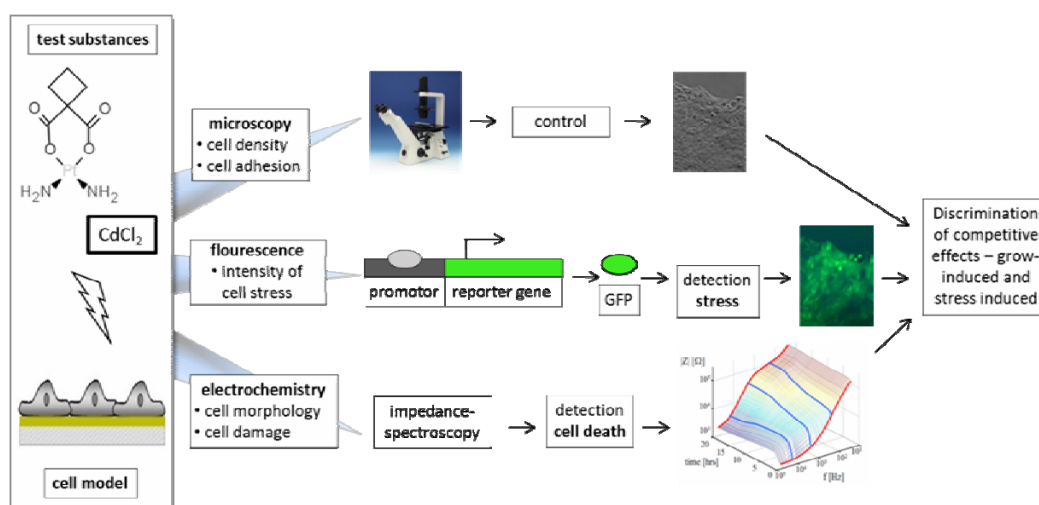


Figure: Overview of the analysis methods in the lab-on-a-chip system for cytotoxicity determination

References:

- [1] U. Hofmann; S. Michaelis; T. Winckler; J. Wegener and K.-H. Feller, *Biosensors and Bioelectronics* 39 (2013) 156-162.

Impedance-Based Cellular Assays: how it all began

Charles R. Keese

Applied BioPhysics Inc. (USA)

keese@biophysics.com

A personal account of the invention, growth and commercialization of impedance-based cellular assays by one of the co-inventors of the original technology referred to as Electric Cell-substrate Impedance Sensing (ECIS). From the first experiments at the General Electric Research and Development Center in the early 1980s to the outgrowth of applications and finally the incorporation of Applied BioPhysics, Inc., the talk provides an historical and often amusing account of the evolution of ECIS.

Real-time monitoring of stem cell based disease models with Electric Cell-substrate Impedance Sensing

P. Bagnaninchi

The University of Edinburgh, Scottish Centre for Regenerative Medicine, Edinburgh,
EH16 4UU

Pierre.Bagnaninchi@ed.ac.uk

In vitro cell-based disease models are essential tools to help us better understand disease mechanisms, to test new drugs and novel therapies. Ideally, these models are based on primary human cells which are more physiologically relevant when compared to animal cells or immortalised cell lines. However, human primary cells are limited, difficult to source, and often unstable in culture. Moreover standard end-point biochemical assays generally impede comprehensive time-course studies of the dynamics associated with disease, tissue injury and recovery. To overcome both these limitations we have recently developed in our group two disease models, based on stem cell technology, directly on Electric Cell-Substrate impedance sensing microelectrodes: i) An induced pluripotent stem cells (IPSCs) model of age-related macular degeneration (AMD) and ii) a stem cell based human liver model to study drug induced liver injuries (DILI) and liver diseases. The in vitro diseases models were characterised throughout stem cell differentiation with both impedance sensing and biochemical assays. We finally demonstrated that these tissue-on-chip approaches have informed basic discoveries in addition to their potential for drug discoveries.

Impedimetric characterisation of cell physiological responses in oral biology

L. Kőhidai¹ and O. Láng¹

Impedimetry MERILL Core Facility/Chemotaxis Research Group, Department of Genetics, Cell- & Immunobiology, Semmelweis University (Budapest, Hungary)

kohlasz2@gmail.com

Background: Impedimetric characterization of cell physiological responsiveness in oral target cells has an emerging significance in the last decade of oral medicine. The real-time HTS technique provides a cutting edge technology to evaluate qualitative and quantitative characteristics of cell populations prepared/cultured from healthy individuals or from a wide range of dental disorders (gingivitis, periodontitis, oral malignancies etc.). Evaluation of (i) drug candidates, (ii) quality control of dental fillings, as well as (iii) characterization of dental auxiliaries (e.g. components of irrigatives and toothpastes) are also effectively supported by introduction of impedimetry to dental research.

Objectives: In the last decade our research group has intensively worked on different topics of oral biology, as a pioneer lab on the field our very goal was to objectify cell physiological responses (e.g. adhesion, migration, proliferation) by application impedimetry. Our main objectives were: (i) to characterize dental stem cells as targets of drug candidates; (ii) to evaluate effects elicited by irrigative substances and toothpastes; (iii) to characterize peptide type molecules as surface coating candidates of dental implants.

Material and Methods: A variety of representative cell cultures were investigated including cells prepared from patients representing different clinical categories and cell cultures of stem cells (PDLSC, DPLSC) as well as cell lines of gingival epithelium (HGEP). The investigated oligonucleotide library was Tp5-Tp55 oligomers. Polylysine-based (AK and SAK) peptides were used as coating. Impedimetric analysis was carried out in ECIS Theta Z and xCELLigence SP and DP. For supplementary experiments (i) immune-phenotyping (FACSCalibur); (ii) holographic microscopy (Holo M4); (iii) wound-healing assays (ibidi inserts); (iv) apoptosis assay were also applied. For statistical analysis of data Origin Pro 8.0 and the built-in statistical routines of equipments were used.

Results: (i) Tp derivatives proved to influence cell adhesion of crevicular cells in a disease-dependent manner. In PDLSC and HGEP cells Tp20-25-30 proved to be the most effective, while proliferation was induced in a wide range (Tp20-50) of derivatives. Considering migratory and morphometric effects dental cells proved to be highly sensible to even slight structural alterations of the peptide carrier. (ii) Investigation of 10 irrigative commercial compounds of the market showed that these substances may elicit disadvantageous effects (direct toxic, apoptotic) not only on the target bacteria but even on the cellular components of the host (e.g. gingival epithelium). Testing of 45 commercial toothpastes leads to the conclusion that: they have proliferative effects on PDL and HGEP cells, however, this advantageous effect may be accompanied by cell adhesion inhibitor properties. (iii) Surface coating materials (poly-L-lysine based 'RGD' motif containing polypeptides) of implants proved to have adhesion and proliferation activator in PDLSCs. SAK-cRGDfC and AK-cRGDfC peptides enhanced PDLSCs migratory activity while SAK-cRGDfC showed most significant adherent and migratory effects. The two peptides increased amount of calcium containing deposits in PDLSCs during osteogenic induction. In long term (1 week) all the 3 tested peptides increased expression of CD105 (endoglin) and CD146 while surface integrin α 1 was increased only in short-term (1 day) relation.

Conclusion: Our results support further the professional opinion of dental research that impedimetric analysis is a highly reliable, novel member of qualitative and quantitative analysis of dental model cells as well as effects of active ingredients of dentistry.

Use of cross-linked polypeptide multilayer-coated electrodes to monitor cardiac differentiation of human mesenchymal stem cells

S. Gomez, Y.-R. Chen, C.-C. Hsu and C.-M. Lo

Department of Biomedical Engineering, National Yang-Ming University, Taipei (Taiwan)

sofiag594@hotmail.com

Polyelectrolyte multilayer film is an emerging method for substrate coating to control stem cell proliferation and differentiation [1]. This material allows for modulation of thickness, stiffness, and adhesiveness [2,3]. In this study, human mesenchymal stem cells (hMSCs) were cultured on polypeptide multilayer films fabricated by the alternate deposition of cationic poly-L-lysine and anionic poly-L-glutamic acid, cross-linked with EDC/sulfo-NHS, and coated with gelatin. The characteristics of film thickness, roughness, Young's modulus, and adsorbed film mass were measured by AFM and QCM. Cardiac differentiation was evaluated by RT-PCR analysis and immunostaining of cardiac markers, and monitored by cross-linked polypeptide multilayer-coated electrodes using electric cell substrate impedance sensing (ECIS). The results showed that thicker native films exhibited lower Young's moduli than thinner native films, whereas the Young's moduli of cross-linked films was much larger than native films. Furthermore, hMSCs demonstrated better cell attachment and spreading on even number layer of films, where the top layers are poly-L-glutamic acid films. The cardiac differentiation of hMSCs showed a significantly higher differentiation rate on cross-linked films than on culture dishes or cover glasses. Our observations indicate a possible way to facilitate hMSCs toward cardiac differentiation using cross-linked polypeptide multilayer films.

In the ECIS monitoring of stem cell differentiation, hMSCs were seeded into electrode wells and allowed to form confluent cell layers, and the impedance time courses of the cell-covered electrodes throughout the induction towards cardiomyocytes were measured up to 10 days at 11 frequencies ranging from 62.5 Hz to 64 kHz [4]. Compared with the data of undifferentiated hMSCs, significantly lower impedance time courses of hMSCs treated with cardiac differentiation medium were observed. In addition, these two distinct time course profiles were detected as early as a few hours after induction and lasted for 10 days. To detect subtle changes in cell morphology in the early stage of cardiac differentiation, the frequency-dependent impedance data were analyzed with a theoretical cell-electrode model [5]. The results showed that the decrease of measured impedance of differentiated cells was mainly due to a decrease of the junctional resistance between cells (R_b) and an increase of the cell-substrate separation (h). We also analyzed the impedance changes between the differentiated groups with the cross-linked polypeptide multilayer film and without it. The results showed that hMSC differentiation towards cardiomyocytes is augmented when cross-linked polypeptide multilayer films were used.

References:

- [1] G. Decher, *Science* 277 (1997) 1232-1237.
- [2] L. Richert, A. J. Engler, D. E. Discher and C. Picart, *Biomacromolecules* 5 (2004) 1908-1916.
- [3] T. Boudou, T. Crouzier, K. Ren, G. Blin and C. Picart, *Advanced Materials* 22 (2010) 441-467.
- [4] P.O. Bagnaninchi and N. Drummond, *Proc. Natl. Acad. Sci. USA* 108 (2011) 6462-6467.
- [5] I. Giaever and C.R. Keese, *Proc. Natl. Acad. Sci. USA* 88 (1991) 7896-7900.

Impedimetric monitoring of neural stem cell differentiation

H.-G. Jahnke, D. Seidel and A.A. Robitzki

Center for Biotechnology and Biomedicine (BBZ), Universität Leipzig, Germany

heinz-georg.jahnke@bbz.uni-leipzig.de

According to the needs of assays and screenings, human pluripotent stem cells are a unique source for functional human cell types. Especially human neural stem cell-derived neuronal networks represent the only accessible in vitro model that resembles a functional primary human phenotype. However, differentiation and maturation of these cells are a very complex and time-consuming process. In this context, non-invasive label-free techniques to monitor the neuronal differentiation and maturation of human stem cells are highly demanded. Our aim was to demonstrate the successful use of impedance spectroscopy for the sensitive label-free monitoring of the complete differentiation process from progenitor cells to neuronal networks.

Therefore, we used optimized high-dense microelectrode arrays (MEAs) in combination with high precision impedance analyzers to identify specific impedimetric characteristics for the neuronal differentiation process [1]. Moreover, we performed detailed correlative molecularbiological analyses to validate the identified impedimetric parameters. First, we identified the optimum MEA to analyze the differentiation of reference human neural stem/progenitor cell models by impedance spectroscopy. In this context, we needed to optimize the surface of the MEAs for achieving a long-term stable cell-electrode interface. Afterwards, we identified distinct cell dependent impedimetric parameters that could specifically be associated with the progress and quality of neuronal differentiation. Applying an adapted equivalent circuit model, we were able to understand the identified empiric impedimetric parameters. For validation, we analyzed the expression of progenitor versus mature neural marker and typical structural changes. The correlation analysis revealed a strong predictive power of the identified impedimetric parameters. More strikingly, we could demonstrate the capability of our impedimetric differentiation monitoring system to identify neuronal differentiation accelerating compounds.

For the first time, we proved that non-invasive impedance spectroscopy can be used for the specific quantitative monitoring of neuronal differentiation processes. Therefore, this technique will be a useful tool for quality control of neuronal differentiation, neurogenic compound identification and moreover, high-content screening demands in the field of safety assessment as well as drug development.

References:

- [1] D. Seidel, J. Obendorf, B. Englich, H.-G. Jahnke, V. Semkova, S. Haupt., M. Girard, M. Peschanski., O. Brüstle, A.A. Robitzki, *Biosens. & Bioelec.*, doi:10.1016/j.bios.2016.06.056

Impedance Analysis of Cadherin-Mediated Cell Adhesion

C.-M. Lo¹ and Y.-S. Chu²

¹Department of Biomedical Engineering, National Yang-Ming University, Taipei (Taiwan)

²Brain Research Center, National Yang-Ming University, Taipei (Taiwan)

cmlo@ym.edu.tw

Catenins are cytosolic proteins which play essential roles in cadherin-mediated cell adhesion [1]. Here electric cell-substrate impedance sensing (ECIS) was applied to assess cell junctional disassembly and reassembly of Eph4 mouse mammary epithelial cell monolayers subjected to a calcium switch. Wild-type (Eph4WT), p120-catenin knockout (Eph4C3), Utrophin knockout (Eph4U7), and alpha-catenin knockout (Eph4A3) Eph4 cells were used in this study. Stable clones with specific gene knockout, relevant to cadherin-mediated intercellular adhesion, were obtained through genome editing technology by CRISPR/Cas9 [2]. Cells were first seeded into 8W1E electrode wells and grown to confluence in DMEM. After 36 hours the medium was changed to low-calcium medium for 45 minutes and then restored to normal DMEM. Cell recovery was monitored by ECIS multiple frequency time (MFT) course for 24 hours. To detect subtle changes in cell morphology, the frequency-dependent impedance data of the cell monolayer were measured and analyzed with a theoretical cell-electrode model [3,4]. The junctional resistance between cells (R_b) and the distance between the basal cell surface and substratum (h) are $51 \Omega \cdot \text{cm}^2$ and 0.6 nm for Eph4WT, $18 \Omega \cdot \text{cm}^2$ and 2.0 nm for Eph4C3, $27 \Omega \cdot \text{cm}^2$, 0.6 nm for Eph4U7, and $2.1 \Omega \cdot \text{cm}^2$ and 26 nm for Eph4A3 respectively. These differences in cell-cell and cell-substrate adhesion were accompanied by different cell recovery rates (Eph4WT > Eph4U7 > Eph4C3) in calcium switch assay monitored by ECIS. Despite these changes, there were little differences in the migration rate observed by ECIS wound healing assay or the cell morphology viewed by a regular optical microscope. These data suggest that the loss of alpha-catenin or p120-catenin significantly disrupts the barrier function of Eph4 epithelial cell layers and that ECIS can serve as a useful tool to investigate the functional roles of different catenins in cadherin-mediated cell adhesion.

References:

- [1] J. R. Miller and D. R. McClay, *Developmental Biology* 192 (1997) 323–339.
- [2] Y.-S. Chu et al., unpublished data
- [3] I. Giaever and C.R. Keese, *Proc. Natl. Acad. Sci. USA* 88 (1991) 7896-7900.
- [4] D. Opp, B. Wafula, J. Lim, E. Huang, J.-C. Lo, and C.-M. Lo, *Biosensors and Bioelectronics* 24 (2009) 2625-2629.

Monitoring cellular stress responses using integrated high-frequency impedance spectroscopy and time-resolved ELISA

V. Charwat^{1,2}; M. Jokschi³; D. Sticker¹; M. Purtscher^{1,4}; M. Rothbauer^{1,5} and P. Ertl^{1,5}

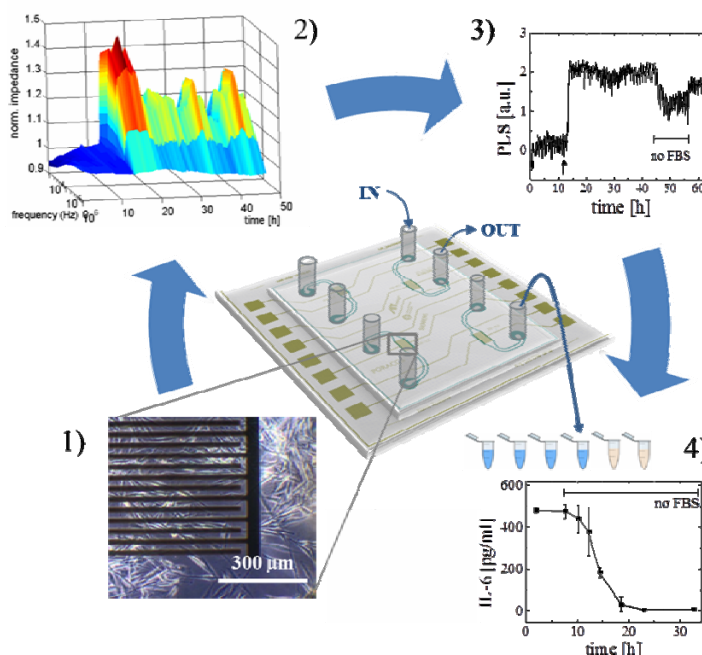
¹AIT Austrian Institute of Technology GmbH, Vienna (Austria); ²University of Natural Resources and Life Sciences, Vienna (Austria); ³Siemens AG, Vienna (Austria);

⁴FH Technikum, Vienna (Austria); ⁵Vienna University of Technology, Vienna (Austria)

verena.charwat@boku.ac.at

We have developed a lab-on-a-chip system for continuous and non-invasive monitoring of microfluidic cell cultures using integrated high-frequency contactless impedance spectroscopy [1]. Electrically insulated microfabricated interdigitated electrode structures (1) were embedded into four individually addressable microchambers to reliably and reproducibly detect cell-substrate interactions, cell viability and metabolic activity. While silicon nitride passivated sensor substrates provided a homogeneous cell culture surface that minimized cell orientation along interdigitated electrode structures, the application of high frequency AC fields reduced the impact of the 300 nm thick passivation layer on sensor sensitivity. The additional implementation of multivariate data analysis methods such as partial least square (3) for high-frequency impedance spectra (2) provided unambiguous information on intracellular pathway activation, up and down-regulation of protein synthesis as well as global cellular stress responses. A comparative cell analysis using connective tissue fibroblasts showed that high-frequency contactless impedance spectroscopy and time-resolved quantification of IL-6 secretion using ELISA (4) provided similar results following stimulation with circulating pro-inflammatory cytokines IL-1 β and TNF α . The combination of microfluidics with contactless impedance sensing and time-resolved quantification of stress factor release will provide biologist with a new tool to (a) establish a variety of uniform cell culture surfaces that feature complex biochemistries, micro- and nanopatterns; and (b) to simultaneously characterize cell responses under physiologically relevant conditions using a complementary noninvasive cell analysis method.

In a recent addition to this work [2] we have established zirconium dioxide (15 nm) passivated impedance electrodes that proved useful for identification of stem cell differentiation.



References:

- [1] V. Charwat, M. Jokschi, D. Sticker, M. Purtscher, M. Rothbauer and P. Ertl, *Analyst* 139 (2014) 5271-5282.
- [2] D. Sticker, M. Rothbauer, V. Charwat, J. Steinkühler, O. Bethge, E. Bertagnolli, H. Wanzenböck and P. Ertl, *Sensors and Actuators B: Chemical* 213 (2015) 35-44.

Role of claudins in Tumor Necrosis Factor-induced permeability and migration changes in tubular epithelial cells

Y. Amoozadeh, S. Anwer, E. Branchard, Q. Dan and K. Szaszi

Keenan Research Center, St. Michael's Hospital and University of Toronto

szaszik@smh.ca

Tumor Necrosis Factor- α (TNF) is a key pathogenic factor in kidney disease. TNF alters tubular epithelial permeability and promotes migration but the underlying mechanisms are not well known. Using filter-based Electric Cell-substrate Impedance Sensing (ECIS) we demonstrate that TNF causes a biphasic transepithelial resistance (TER) change in LLC-PK1 tubular cells. This effect involves an early drop in TER followed by recovery (1-3h), and a late increase (>8h) [1]. In search for the underlying mechanisms, we found that TNF α alters the expression of several claudin (Cldn) family members. Claudins are tight junction proteins with unique properties. The combination of various claudin isoform expressed in a given cell determines paracellular permeability. Interestingly, TNF causes a biphasic change in Cldn-2 and 3 expression: an initial transient increase due to reduced degradation (1-3h) is followed by a decrease in mRNA and protein levels (>8h). Prolonged TNF treatment also increases claudin-1, 4 and 7 protein and mRNA levels. The initial increase in Cldn-2 levels is mediated by the RhoA, ERK and JNK pathways. ERK and JNK are also required for the late expression changes of Cldn-1, 4 and 7, and have a key role in the TNF-induced late TER increase. To correlate TNF-induced claudin expression and TER changes, we silenced each claudin and monitored TER using ECIS. Cldn-1 is necessary for the early TNF-induced TER change. In contrast, Cldn-2 decrease appears to be the main contributor to the late TER increase, with only minor roles for Cldn-1, 4 and 7.

Claudins have also been implicated in cell migration, and TNF also enhances epithelial cell migration. Therefore, we used the ECIS wound healing assay to assess the role of the various claudins in the TNF-induced increased migration. Silencing Cldn-1, 2 or 3 significantly slowed wound healing. Our ongoing studies explore the role of these claudins in migration-associated cytoskeleton remodeling. Thus, TNF-induced altered claudin expression may have consequences beyond permeability changes. By affecting epithelial regeneration following injury, altered claudin expression could contribute to the pathogenesis of kidney disease.

References:

- [1] Y. Amoozadeh, Q. Dan, J. Xiao, F. Waheed, K. Szászi, *Am. J. Physiol. Cell Physiol.* 309/1 (2015) C38-50 .

Brain Endothelial Activation and Neuronal Damage

M.F. Stins^{1,2}, A. Tripathi², M. Motari¹, and D. Sullivan²

¹ Department of Neurology, Johns Hopkins School of Medicine

² Malaria Research Institute, Bloomberg School of Public Health, Baltimore MD, U.S.A.

mstins@jhmi.edu

Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection, especially in children, exposed non immune travelers and military personnel. Clinically, CM includes seizures, reversible coma and often death. Upon clearance of the infection, patients are often left with neurologic sequelae, such as seizures, learning and behavioral disorders. Recent data also show that ADHD can be linked to CM. Post malaria syndrome can also include psychotic or acute confusional episodes and tremor.

CM pathology is characterized by sequestration of Plasmodium infected erythrocytes (PRBC) to human blood brain barrier (BBB) endothelium without invasion into the brain. It is unclear as to how these PRBC that are confined to the brain vasculature induce neurological dysfunction. In CM, the BBB lies at the interface of the events occurring in blood and brain. The BBB is part of the neurovascular unit (NVU), a concept that emphasizes homeostatic interactions between its components to ensure optimal functioning of the central nervous system. It is hypothesized that activation of the BBB endothelium disturbs the homeostasis between the astro-glial and neuronal components of the NVU leading to neurological dysfunction.

Using an *in vitro* model for the human BBB, PRBC exposure increased ICAM-1 expression on the cells and decreased the integrity (barrier function) of monolayers in a dose dependent manner. Microarray and Gene Ontology (GO) analysis indicated a predominance of the NFκB mediated proinflammatory responses among the host signaling pathways. RT-PCR and protein analysis confirmed the increase in transcripts and release of cytokines and chemokines.

To assess whether BBB secretions could affect the brain, BBB models were constructed on TW inserts. Upon confluence, TW-BBB models were placed in the Cellscope and exposed to PRBC. Media was collected from the basal side of BBB models and added to astroneuronal cultures. These basal secretions caused dose-dependent abnormal astroneuronal morphology and cell death. Determination of the underlying pathogenesis of observed BBB activation and astroneuronal effects may lead to development of adjunctive neurotherapeutics to ameliorate neurologic sequelae.

References:

- [1] A.K. Tripathi, D.J. Sullivan and M.F. Stins *Infection and Immunity* 74/6 (2006) 3260-3270.
- [2] A.K. Tripathi, D.J. Sullivan and M.F. Stins, *Journal of Infectious Disease* 195/7 (2007) 942-950. Editorial commentary, JID Vol 195 (7)
- [3] A.K. Tripathi, W. Sha, V. Shulev, M.F. Stins, D.J. Sullivan, *Blood* 114/19 (2009) 4243-4252

A novel multiparametric bioelectronic measurement system for long-term monitoring of human stem cell derived human 3D cardiomyocyte clusters

A.A. Robitzki; S. Fleischer, F. Zitzmann, D. Seidel, H.-G. Jahnke

Center for Biotechnology and Biomedicine (BBZ), Universität Leipzig, Germany

andrea.robitzki@bbz.uni-leipzig.de

Over the last decades, bioelectronic and bio-electrochemical measurement systems evolved to novel useful tools for the label-free and non-invasive monitoring of cellular alterations. Many individual measurement setups were developed focusing on a single biophysical analysis technique like electrical impedance spectroscopy (EIS), field potential recording (FPR) or electrochemistry, which also mostly included electrode systems and multi-electrode arrays (MEA) optimized for each individual technique and most suitable for 2D cultures. Today, there is an increasing demand for multiparametric analysis systems to avoid parallel experiments on proprietary measurement setups and to obtain the most comprehensive data from the same cell or tissue sample by direct correlation of multiple bioelectronic and even optical read-outs. EIS and FPR perfectly match the demands for label-free detection and real-time monitoring of electrogenic cells like cardiomyocytes in high content screening systems. Due to the oppositional nature of both techniques with EIS as an active and FPR as a passive measurement method until today, there were no combined systems available.

To overcome these limitations, we developed a hybrid measurement system for the sensitive non-invasive monitoring of 3D cardiomyocyte cultures that is based on the microcavity arrays [1]. In contrast to already described systems, our novel hybrid measurement system connects both measurement circuit paths to the same measurement electrodes. By the use of a novel microcontroller based switching system, loss of sensitivity or gain of noise as well as interference for both methods are prohibited. Moreover, our indium tin oxide based (ITO) microelectrode arrays allow the combination with optical read-out systems. To demonstrate the performance of our system we bioelectronically characterized human induced pluripotent stem cell (hiPS) derived cardiomyocyte clusters for more than 100 days. Using reference compounds we successfully demonstrated the sensitive quantification of drug effects in physiological relevant ranges.

In conclusion, our novel hybrid multiparametric bioelectronic measurement system is a promising tool for the label-free and non-invasive monitoring of 3D hiPS derived cardiomyocyte cultures and therefore, offers great advantages in the field of *in vitro* safety assessment for cardiotoxicity as well as drug development for cardiomyopathies [2, 3].

References:

- [1] D. Kloß, M. Fischer., A. Rothermel., J.C. Simon, A.A. Robitzki, *Lab on a Chip*, 8 (2008) 879-884.
- [2] A.A. Robitzki, H-G. Jahnke, T. Friedriszik, S. Fleischer, D. Seidel, V. Rosas, M. Peschanski, SEURAT 3 (2013) ISBN : 978-2-9539634-2-7, „Toxicology in the 21st century: Mechanism-driven Toxicology defines the safe dose“
- [3] H-G. Jahnke, D. Steel, S. Fleischer, D. Seidel, R. Kurz, S. Vinz, K. Dahlenborg, P. Sartipy, A.A. Robitzki, *PLoS One* (2013) 8/7: e68971.

RTEMIS: Real-time microTissue and Environment Monitoring Using Impedance Spectroscopy and pH Sensing

F.A. Alexander Jr.; S. Eggert and J. Wiest

cellasys GmbH, Munich (Germany)

alexander@cellasys.com

Toxicologists are increasingly turning to 3D cellular models as an intermediate step to bridge results from planar *in vitro* models with *in vivo* animal models in an effort to more accurately predict the effects in humans. Sensor-based label-free techniques, such as impedance spectroscopy, are also receiving more attention due to a decreased probability of interference in native function by removing colorimetric stains from testing schemes. As a result, multiple commercial impedance monitoring systems provide solutions for noninvasive, label-free monitoring of cellular morphology and viability in several conformations, including scaffold-free microtissues and scaffolded constructs [1,2]. Although impedance spectroscopy (IS) is an effective method to monitor real-time changes in overall cellular vitality and structural changes in response to toxic agents, valuable information relating to cellular metabolic activity cannot be monitored via IS and are ultimately lost. Cellular microphysiometry, a parallel sensor-based monitoring technique, combined with impedance spectroscopy using on-chip metabolic sensors to supplement structural impedance data with metabolic information gives a clearer picture of whole-cell response to a given test substance.

In the presented work, two approaches to creating a modular cellular microphysiometry platform for online, label-free monitoring of cellular microtissues are described. A microfluidic, multi-electrode impedance sensor was designed, fabricated, and evaluated for making impedance measurements with multicellular (Hs578t) tumor spheroids and a candidate 3D printed spheroid encapsulations was designed and tested with HepG2 liver spheroids for real-time pH monitoring. The final spheroid encapsulation design and methodology can be applied to a multitude of spheroidal microtissues and serves as a proof-of-concept study of cellular microphysiometry performed on 3D tissues. This combination of pH and impedance sensing provides a useful platform for in depth understanding of microtissue responses to foreign substances.

References:

- [1] A. Robitzki, H. Thielecke, and A. Reininger-Mack, "Development of a novel microcapillary array: Characterization of in vitro 3D tissue models by bioimpedance spectroscopy," 2002.
- [2] C. Canali, C. Mazzoni, L.B. Larsen, A. Heiskanen, Ø. Martinsen, A. Wolff, *et al.*, *Analyst* 140 (2015) 6079-6088.

Impedance Analysis of Cells using Organic Electronics; focus on 3D cellular models

S. Inal, M. Ferro, A. Hama, V. Curto and R.M. Owens

Dept. of Bioelectronics, Ecole des Mines de St. Etienne, Gardanne (France)

owens@emse.fr

The development of electrical techniques for monitoring of biological phenomena is a field that is fast gathering pace. Advantages of electrical techniques are manifold, including the fact that they are label-free, and have the potential to be very efficient transducers, since the signal measured is already in an electrical readout format.

Electronic methods for live-cell sensing can be applied to applications involving extracellular recording of electrical activity from electrically active cells (neurons/myocytes), but also for monitoring of non-electrically active cells and tissue assemblies. Electrical impedance sensing (EIS) has emerged as a dynamic method, with demonstrated potential for use in monitoring barrier function, cellular adhesion, proliferation, and micro motion [1]. Until recently however, EIS has been restricted to monitoring of 2D cultures. We have successfully demonstrated the use of conducting polymer devices for monitoring a variety of *in vitro* models with the goal of developing physiologically relevant systems with integrated monitoring for use in diagnostics, toxicology or drug development [2,3,4]. CPs are 'smart materials' that have gained considerable attention of late for their use in interfacing with tissues *in vivo* and *in vitro* [3]. Mixed ionic/ electronic conduction, along with an ideal biocompatible surface and soft tissue-like mechanical properties, have contributed to the successful use of this material for integration with biological components. One key advantage of these materials is their amenability for processing in a variety of formats, including in 3D scaffolds [5]. A second major advantage of conducting polymers is their compatibility with high resolution imaging, allowing correlation of electrical data with time lapse imaging of the cells in real time [6]. In this presentation I will show recent work on the integration of 3D tissue models with conducting polymers to allow real time monitoring of cellular function.

References:

- [1] I. Giaever, C.R. Keese, *Proc. Natl. Acad. Sci. USA* 81 (1984) 3761.
- [2] M. Huerta, J. Rivnay, M. Ramuz, A. Hama, R.M. Owens, *Appl. Vitro Toxicol.* 2 (2016) 17.
- [3] X. Strakosas, M. Bongo, R. M. Owens, *J. Appl. Polym. Sci.* 132 (2015) 41735.
- [4] M. Huerta, J. Rivnay, M. Ramuz, A. Hama, R.M. Owens, *APL Mater.* 3 (2015) 30701.
- [5] A.M.-D. Wan, S. Inal, T. Williams, K. Wang, P. Leleux, L. Estevez, E.P. Giannelis, C. Fischbach, G.G. Malliaras, D. Gourdon, *J. Mater. Chem. B* 3 (2015) 5040.
- [6] M. Ramuz, A. Hama, J. Rivnay, P. Leleux, R.M. Owens, *J. Mater. Chem. B* 3 (2015) 5971.

Impedance Spectroscopy: A new tool to characterize reconstructed human epidermis

M. Schweinlin, L. Engelhardt, R. Seliger, F. Schmied, H. Walles, F. Groeber

GRK 2157, University of Würzburg, Würzburg (Germany)

Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Würzburg (Germany)

Fraunhofer Translational Center Würzburg 'Regenerative Therapies in Oncology and Musculoskeletal Disease', Würzburg (Germany)

matthias.schweinlin@uni-wuerzburg.de

Despite advances in the development of *in-vitro*-tissue-models such as reconstructed human epidermis (RHE), the number of endpoints in toxicity-testing, which can be addressed with these models are limited. The analysis of the models are still dependent on invasive methods such as histological processing or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining.

As an alternative for destructive methods, we established a non-destructive technology to analyze the integrity of the epidermal barrier based on impedance spectroscopy. RHE typically exhibits characteristic impedance spectra in a frequency ranging between 1 Hz and 100 kHz, which is comparable to the spectra of freshly isolated human epidermal biopsies. From these spectra, we extracted electrical parameters of the RHE such as the capacitance and the ohmic resistance. These parameters change significantly during epidermal differentiation and were used to quantify the effects of mechanical and chemical disruption of the epidermal integrity. Most relevant, impedance spectroscopy shows a sufficient sensitivity to detect a transient decreased ohmic resistance caused by 2-propanol, which is classified as a non-irritant by MTT assays. This result indicates that impedance spectroscopy can be employed as an additional method to assess mild irritative effects.

In our work we developed a non-invasive assay for analysis of tissue-models which is a vital requirement to increase the success of *in-vitro*-test-methods.

References:

- [1] F. Groeber, L. Engelhardt, J. Lange, S. Kurdyn, F.F. Schmid, C. Rücker, S. Mielke, H. Walles, J. Hansmann, ALTEX. 2016

Automated Statistical Analysis of an siRNA Screen of Kinases Involved in TNF- α Signalling

S. Umlauf¹, M. Kluger², C. Renken³

¹Yale University, New Haven, CT

²Yale School of Medicine, New Haven, CT

³Applied BioPhysics Inc., Troy (NY)

renken@biophysics.com

Phenotypic assays are the original drug development platform in which cells, organs or organisms expressing a certain disease state are assayed for compounds that could alter their phenotype. However for a phenotypic HTS assay to be feasible, the detection metric for the cell response needs to be easily quantifiable. Electric Cell Impedance Sensing (ECIS) exploits the fact that cell morphological changes are intimately linked with functional changes. ECIS non-invasively converts complex morphological changes into a continuous real time electrical signal. Microvascular endothelial cells residing in capillaries form a homeostatic barrier that governs the systemic exchange of fluids, solutes and macromolecules between blood and tissue, but barrier dysfunction is a normal inflammatory response that when left unresolved leads to life-threatening complications in systemic sepsis, cardio-pulmonary bypass and viral hemorrhagic fevers. We have shown that human dermal microvascular endothelial cells (HDMEC) model tight junction-dependent capillary barriers in vitro, that the inflammatory cytokine Tumor Necrosis Factor (TNF) disrupts HDMEC barriers, and that ECIS instruments can detect this disruption in a continuous real-time assay. The ECIS output of the HDMEC to TNF stimulation is a complex tri-phasic curve of impedance over time, $Z(t)$, sampled at 5 minute intervals for 20 hours. Differences from the control curve can occur in time, magnitude and combination thereof which indicate the MOA for compounds showing significant differences. For a pooled siRNA screen targeting the individual genes of the 715 kinases comprising the human kinome (within the Dharmacon siGENOME[®] siRNA Library), we needed a rapid method of classifying a few thousand such curves into groups of siRNAs that have no effect, that are similar to siRNA targeting the TNF receptor and then those that act in unknown manners. The 715 kinase library was completely assayed twice across 24 96 well plates. Each plate had 8 wells of non-targeting siRNA as the negative control (normal phenotype) and 8 wells of siRNA targeting the TNF receptor 1 as a positive control. MagCor calculates the standardized magnitude and correlation distance between each well within a plate as a function of time. For exploratory analysis the average Z distance (Mag) and correlation distance (Cor) from in-plate negative controls are recorded in a database. The database is then easily searchable to return time points having a maximum diversity, identify outliers at any time point or integrate over the entire curve to identify targets with maximum deviation from both the magnitude of negative controls as well as the response of negative controls. Based on preliminary analysis we have identified 6 broad groups of kinases; Those that have no effect. Those that change baseline values yet have no effect on TNF response. Those that act similar to loss of TNF receptor. Those that effect short term (< 1hr) responses, or medium term (between 1 and 8 hours), or those that effect the long term response (> 8 hours).

Impact of Nanomaterial Perfusion on cellular uptake and Nanotoxicity at physiological barriers

M. Rothbauer¹, V. Charwat² and P. Ertl^{1*}

¹ Vienna University of Technology – Faculty of Technical Chemistry

² University of Life Sciences, Vienna – Department of Biotechnology

peter.ertl@tuwien.ac.at

In the last decade, the application of nanomaterials (NMs) in technical products and biomedicine has become a rapidly increasing market trend. As the safety and efficacy of NMs are of utmost importance, new methods are needed to study the dynamic interactions of NMs at the nano-bio-interface. However, evaluation of NMs based on standard and static cell culture end-point detection methods does not provide information on the dynamics of living biological systems, which is crucial for the understanding of physiological responses. To bridge this technological gap, we here present a microfluidic cell culture system containing embedded impedance micro-sensors to continuously and non-invasively monitor the effects of NMs on adherent cells under varying flow conditions.

As a model, the impact of silica NMs on the vitality and regenerative capacity of human lung cells after acute and chronic exposure scenarios was studied over an 18-h period following a four-hour NM treatment. The observed relationship between fluid flow velocity and NP uptake strongly suggests that fluid mechanical forces also need to be accounted for in order to generate physiologically relevant data for nanotoxicological and nanomedical evaluations.

To gain a deeper understanding on nanoparticle distribution and accumulation within the human vascular system, we have further employed both an perfused microfluidic cell culture system and an in silico CFD simulations to identify the critical shear force above which the cellular capacity to actively up take nanoparticles is predominantly governed by fluid dynamic forces. Results of the study revealed that cellular up take rates of 250 nm polystyrene nanoparticles already declines sharply above the critical shear force of 3.1 dyn/cm² or 1.4 µl/ min resulting in a 90% decreased uptake at 10 dyn/ cm² or 6 µL/ min.

Results of the study demonstrated that the developed systems are applicable to reliably analyze the consequences of dynamic NM exposure to physiological cell barriers in both nanotoxicology and nanomedicine.

Label-free monitoring of cellular interaction with the surface modified nanoparticles by Electric Cell-substrate Impedance Sensing (ECIS)

G. Rischitor; A. Valsesia; I. Ojea Jimenez and P. Colpo

European Commission, Directorate General Joint Research Centre
Directorate F – Health, Consumers and Reference Materials
Unit F.2 Consumer Products Safety

grigore.rischitor@ec.europa.eu

The constant increase of the use of nanomaterials in consumer products makes very urgent the need of robust, standardized and reliable in vitro test methods for toxicity screening.

Most of the nanomaterials are inducing cell death in unrealistically high doses and the cellular responses generated by low doses exposure are difficult to assess. Electric Cell-substrate Impedance Sensing (ECIS) advantage in monitoring toxic effect of the nanoparticles was demonstrated over classical toxicity methods assessing cell death at specific end points.

Being a continuous monitoring technique the risks of missing the cellular responses to nanoparticles exposure by analyzing just one end point is eliminated. We can certainly monitor and compare the hormesis (transduction) peaks, the length of cellular micromotion and other proliferation parameters. Furthermore after modeling the ECIS frequencies we can get valuable information of adherence, cell-cell interactions and cell membrane integrity after nanoparticles exposure.

Our specific interest of our lab was in ECIS cell recovery test development- the time of cell recovery after nanoparticles exposure. Cancer cell are recovering faster after inert nanoparticles (e.g. Au) exposure and slower after ion releasing silver nanoparticles treatment. We found out also that some nanoparticles can induce cancer cell stasis where the cells appear to be death and they are recovering faster after nanoparticles removal from culture media.

Another significant finding of our team was the ability of ECIS system to monitor the dissolution of silver nanoparticles. The release of the silver ions in the culture media from silver nanoparticles is size dependent as well as dose dependent. Addition of antioxidants in the media or surface protection of nanoparticle can prevent ions release.

The surface of nanoparticles can be chemically modified by carboxylation, hydroxylation, sulfonation, amination, etc. The nanoparticles toxicity induced by the surface modification can as well be monitored by ECIS. After exposing the cells with the same amount of nanoparticles having different surface modifications we observed that the carboxylated nanoparticles are impairing cell proliferation more than hydroxylated or sulfonated ones.

Cellular uptake and intracellular nanoparticles localization are influenced by the surface modification of the NPs. A new scanning electron microscope (SEM) based technique was developed to confirm the ECIS results.

Cells in Contact to Nanomaterials: A Multi-modal Perspective based on Impedance Analysis

C. Hupf, M.-M. Lemberger, M. Sperber and J. Wegener

Universität Regensburg, Regensburg (Germany)

Christina.Hupf@chemie.uni-regensburg.de

Nanomaterials find increasingly access to our daily lives. Unfortunately, a detailed understanding about their harmfulness to human health and the environment lags behind our knowledge about nanomaterial synthesis and manipulation. Label-free monitoring of the nanomaterial-cell encounter might shed light on the biological response to nanomaterials.

Among the established label-free techniques to monitor cell-based assays, impedance analysis is the farthest developed with respect to the available assay formats, throughput and information content of the raw data. This presentation will introduce various assay formats that can be used to unravel the biological response to nanoscale materials on different levels of cell physiology. When these assays are used sequentially to examine one particular type of nanomaterial, a dynamic response profile can be established that provides a multi-dimensional perspective on the nanomaterial cell interaction [1]. This talk will highlight such a multi-model approach using carbon dots (Cdots) as a model material. Cdots represent a relatively new allotropic form of carbon with interesting material properties such as photo-luminescence with high quantum yield and long-term photostability [2]. These interesting bioanalytical properties are, however, worthless when the material proves to be non-biocompatible.

References:

- [1] M. Sperber, C. Hupf, M. Lemberger, B. Goricnik, N. Hinterreiter, S. Lukic, M. Oberleitner, J. A. Stolwijk, J. Wegener, *Bioanalytical Reviews* 2016.
- [2] S. Sahu, B. Behera, T.K. Maiti, S. Mohapatra, *Chem. Commun.* 48 (2012) 8835–8837.



Poster Presentations

Investigation of the Effects of the Cholestatic Drug Chlorpromazine in Human HepaRG cells using ECIS

A. Kozłowska¹, W. Gamal², K. Morgan¹, A. Bryans¹, P. Treskes¹, P.C. Hayes¹, J.N. Plevris¹, P.O. Bagnaninchi², L.J. Nelson^{*1}

¹Hepatology Laboratory, ¹Centre for Biomedical Engineering, University of Edinburgh, Edinburgh, United Kingdom

l.nelson@ed.ac.uk

Introduction: Cholestatics such as chlorpromazine (CPZ), an anti-psychotic drug, can cause severe liver injury including intrahepatic cholestasis (*jaundice*). Preclinical *in vitro* models including hepatocyte couplets or sandwich cultures of rat and primary human hepatocytes (PHHs) are used to analyze hepatic drug/ bile transport processes. Dysregulation of bile canaliculi-associated transporters is recognized as a molecular initiating event in cholestasis. However existing models have limitations - notably that animal models do not accurately predict cholestasis in humans, whilst PHHs are scarce, expensive and prone to batch variation. Therefore, new methods of detecting cholestatic liver injury caused by candidate compounds under development is critical. Cholestatics can also have profound effects on tissue architecture. For example, CPZ and cyclosporine-A have recently been shown to destabilize intercellular tight junctions (TJs) via oxidative stress-mediated effects on TJ-associated cytoskeletal pericanalicular F-actin distribution in a 2D human hepatic HepaRG cell model [1-2]. HepaRG cells correctly localize hepatobiliary transporters to canaliculi structures, comparable with primary human hepatocytes, and exhibit functional polarity [3].

Aims & Methods: Our aim was to develop mediated cholestatic liver injury, using impedance-based biosensing. Differentiated HepaRGs were cultured to confluence on 8-well (8W10E+) gold micro-electrode arrays. On day 8, CPZ time-/dose-response [0-100 μ M] was monitored with quantitative impedance, $|Z|$, measurements (180s intervals; multiple frequencies: 4-64kHz) for 24h; to detect the TJ parameter (R_b), using $|Z|$ -spectra modeling. Correlative hepatotoxicity/ phenotypic assays were performed: i) ATP-depletion; Prestoblu (PB: live-cell viability); and i) F-actin/phalloidin fluorescent staining; and morphological analysis performed

Results: Real-time $|Z|$ monitoring showed highly-sensitive/ temporal dose-response to CPZ, with a decrease of impedance at all frequencies, indicating a global decline in cellular health. Subsequent $|Z|$ -spectra modelling reflected significant early (1h) disruption of the TJ (R_b), and in the cell-substrate adhesion parameters (z -alpha), at all CPZ doses [25, 50, 100 μ M]. Endpoint ATP-depletion and PB assays (24h) correlated with $|Z|$ changes only at the lethal [100 μ M] dose. TJ disruption was confirmed using immunofluorescence imaging of the cytoskeletal F-actin/ TJ-associated protein ZO-1 at 50-100 μ M CPZ (**Fig.1**).

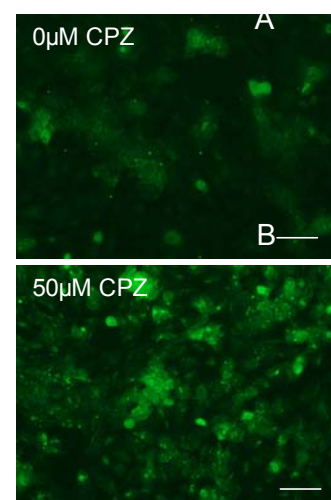


Figure 1: Immunofluorescent staining of ZO-1 in Day 7 HepaRG controls (**A**) or following 50 μ M CPZ for 24 hours (**B**). Scale bars 200 μ m.

Conclusion: Our human liver Biosensor provides a continuous/ quantitative real-time indicator of hepatic TJ integrity; revealing early, dose-dependent disruption of TJs even at sub-toxic CPZ levels (25 μ M). This non-invasive platform may help detection and provide mechanistic insight into effects of putative cholestatic compounds in preclinical drug testing.

References:

- [1] Anthérieu, et al., *Hepatology*, 57/4, (2013) 1518-1529.
- [2] Sharanek, et al., *Toxicol. Sci.* 141/1 (2014) 244-253.
- [3] Bachour-El Azzi et al., *Tox. Sciences* (2015).

AMAP, the putative 'non-toxic' isomer of acetaminophen, is shown to be toxic in primary human hepatocytes using ECIS

A. Bryans¹, W. Gamal², K. Morgan¹, P. Treskes¹, P.C. Hayes¹, J.N. Plevris¹, P.O. Bagnaninchi², L.J. Nelson^{*1}

¹Hepatology Laboratory, ¹Centre for Biomedical Engineering, University of Edinburgh, Edinburgh, United Kingdom.

l.nelson@ed.ac.uk

Acetaminophen (APAP) toxicity is a major cause drug-induced liver injury (DILI). N-acetyl-meta-aminophenol (AMAP) is a regioisomer of APAP and often considered a suitable non-toxic control compound for toxicology studies. However, inter-species variation in response to AMAP has been observed between mouse, rat and human liver samples [1], which may limit the application of AMAP in comparative toxicology investigations. AMAP toxicity has been associated with mitochondrial dysfunction [2] and we recently demonstrated tight junction (TJ) disruption in low dose APAP-treated primary human hepatocytes (PHHs) (srep; under review). This study examined the dose-response of PHHs to both APAP and AMAP using the ECIS platform and hepatotoxicity assays.

Impedance monitoring was performed in PHHs cells treated with APAP (0-40 mM) or AMAP (1-40 mM). Metabolic competency and cell viability were also assessed in PHHs by the PrestoBlue assay and ATP cell viability assays, respectively. PHHs were seeded on a collagen ECIS biosensor (8W10E+ PET, Ibidi®) pre-coated with Collagen-I, and induced with rifampicin for 24 hours, prior to treatment with either APAP or AMAP for a further 24 hours. Impedance analysis revealed a significant and more pronounced drop in resistance in AMAP- vs APAP-treated PHHs, notably at low doses. PHHs treated with only 5mM AMAP demonstrated significantly greater hepatotoxicity (ATP depletion) vs APAP ($p < 0.0001$, unpaired t-test) with a concomitant dose-dependent reduction in metabolic competency (PrestoBlue, Fig.1).

These findings suggest that AMAP is not a suitable as a 'control compound' or non-toxic regioisomer for studying mechanisms of hepatotoxicity/ DILI, or as a pharmacological comparator drug in pre-clinical drug screening strategies. The ECIS biosensor data suggests that loss of cell-cell interactions and TJ integrity may contribute to PHH toxicity. The results also reinforce the need for appropriate validation of drug screening compounds using human liver models to yield physiologically-relevant insights into the mechanisms of hepatotoxicity.

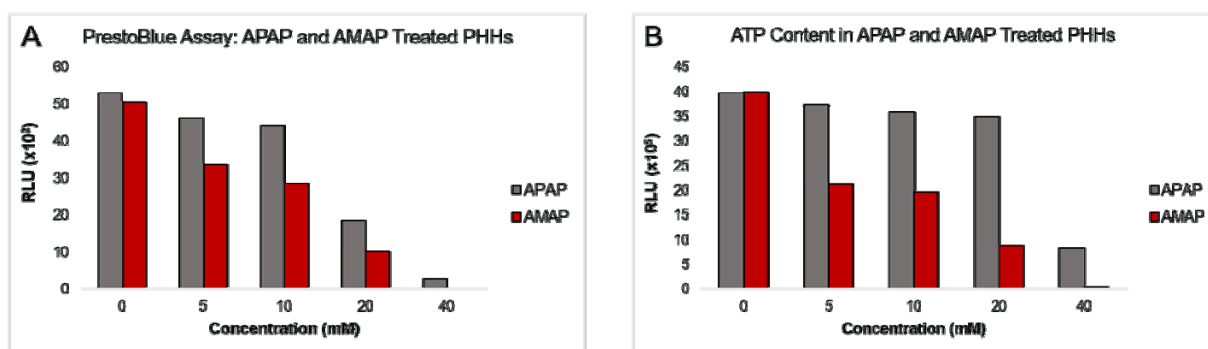


Figure.1 A: PrestoBlue assay in PHHs treated with increasing doses of AMAP and APAP. **B:** ATP content in PHHs treated with increasing doses of APAP and AMAP. Measured in Relative Luminescence Units (RLU). N=2 biological replicates with 3-8 technical replicates

References:

- [1] M. Hai; S. Dragovic; R. van Swelm; B. Herpers; B. van de Water; F.G.M. Russel; J.N.M. Commandeur and G.M.M. Groothuis, *Archives of Toxicology* 87 (2013) 155-165.
- [2] Y. Xie; M.R. McGill; K. Du; K. Dorko; S.C. Kumer; T.M. Schmitt; W. Ding and H. Jaeschke, *Toxicology and Applied Pharmacology* 289 (2015) 213-222.

Label-Free Analysis of GPCR-Stimulation: The Critical Impact of Cell Adhesion

S. Lieb¹; S. Michaelis¹; N. Plank¹; G. Bernhardt¹; A. Buschauer¹ and J. Wegener¹

¹Universität Regensburg, Regensburg (Germany)

sebastian.lieb@chemie.uni-regensburg.de

Label-free cell-based assays have been attracting growing attention in drug research [1]. Optical approaches based on evanescent electric fields and electrochemical impedance analysis are by far the most widespread techniques for such purposes.

We compared three label-free approaches (ECIS, RWG/DMR and SPR) with respect to the activation of the human histamine H₁ receptor (H₁R) expressed by U-373 MG glioblastoma and genetically engineered HEK293T-CRE-Luc cells. HEK293T-CRE-Luc cells were either expressing the H₁R alone or in combination with the adhesion protein human macrophage scavenger protein (hMSR1) [2]. The β_2 -adrenergic receptor (β_2 -AR) expressed by bovine aortic endothelial cells (BAEC) served as a second cell model.

Reduced cell adhesion to the surface of the sensing devices affected both, the optical and the impedance-based readout, but became much more obvious in case of RWG- or SPR-based assays. By contrast, the co-expression of hH₁R and hMSR1 in HEK293T-CRE-Luc cells strongly enhanced the signal compared to hH₁R expression alone.

As the sensitivity of the optical readouts is confined to a distance of 100-200 nm [3,4] from the surface, depending on the wavelength of the incident light, this observation is in accordance with tighter adhesion of the co-transfectants, inducing a shorter distance between the cell membrane and the substrate.

Combining ECIS and SPR, allowing for simultaneous registration of both signals for a single cell population, provided a direct correlation of both readouts, when H₁R or β_2 -AR stimulation was investigated for the same cell populations.

Cell adhesion was found to have a critical impact on the results of label-free cell monitoring, in particular when techniques based on evanescent electric fields are applied.

References:

- [1] R. Schröder et al., *Nat. Biotechnol.* 28 (2010) 943-949.
- [2] A. Matsumoto et al., *Proc. Natl. Acad. Sci. USA* 23 (1990) 9133-9137.
- [3] Y. Fang et al., *Cambridge University Press* (2009) 206-222.
- [4] M.A. Cooper, *Drug Discov. Today* (2006) 1061-1067.

Impedance-Based Theranostics in Tumor Research

S. Michaelis¹; M. Skiba¹, M. Büttner², B. Büttner², K.-H. Feller² and J. Wegener¹

¹Universität Regensburg, Regensburg (Germany)

²Institute of Microsystem and Precision Manufacturing Engineering, Ernst-Abbe-University of Applied Sciences Jena, Carl-Zeiss-Promenade 2, 07745 Jena (Germany)

Stefanie.Michaelis@chemie.uni-regensburg.de

Cancer is a highly heterogeneous disease whose causes, pathogenesis, metastatic potential and response to treatment can be very different among individuals even for the same type of cancer [1]. Thus, drug therapy is usually only effective in a fraction of those treated, while most patients suffer adverse drug reactions. In order to improve both, therapy and the quality of life for cancer patients, rapid clinical diagnostics of tumor cells as well as a better prediction of drug efficacy are crucial factors.

Personalized medicine is a new paradigm in state-of-the-art cancer treatment. It is the idea of this approach to test biopsy material of a tumor *ex vivo* with respect to its sensitivity for a given chemotherapy before the patient gets involved directly. This approach is scientifically and technically rather challenging, as all testing has to be performed fast and on a rather small cell population with a maximum of reliability.

This project aims to develop a microfluidic platform as low volume perfusion culture dish for primary tumor cells with all technical components to perform a multimodal, impedance-based monitoring of chemosensitivity assays with medium throughput [2]. Within the microfluidic channels the tumor cells will be exposed to a given drug candidate and changes in cell vitality will be recorded non-invasively and in real time by impedance measurements (ECIS). The impedimetric assays are based on planar gold-film electrodes established on the bottom of the microfluidic chip by photolithography.

This study shows an impedimetric characterization of the cell-free microfluidic platform with respect to electrode parameters, device operation and flow features, giving access to parameters like dead volume, retention time and flow rate distribution.

Furthermore, a sequence of impedimetric cell-based assays was developed (yet in off-chip assays) to assess key parameters of cell vitality both, before and after application of chemotherapeutic agents. Impedance-based assays were applied to monitor (i) the adhesion of cells to the electrode surface as a measure for their cell-matrix interactions; (ii) their motility as a measure for cell body dynamics, (iii) their ability to migrate laterally and (iv) their proliferation behavior in presence or absence of selected cytostatics. The time- and dose-dependent answer of the tumor cells provides a first line of evidences for a tailored cancer therapy with reduced side effects. The experiments are based on the well-established lung carcinoma cell line A549.

References:

- [1] M. Diamandis, N.M.A. White and G.M. Yousef, *Mol. Cancer Res.* 8/9 (2010) 1175-1187.
- [2] U. Hofmann, S. Michaelis, T. Winckler, J. Wegener and K.-H. Feller, *Biosens. Bioelectron.* 39 (2013) 156-162.

Measurement of Endothelial Permeability based on the Electric Cell-substrate Impedance Sensing (ECIS) method

A. Selmi¹, S. Chlopicki^{1,2}

¹Jagiellonian Centre for Experimental Therapeutics (JCET), Bobrzynskiego 14, 30-348 Krakow, Poland

²Department of Experimental Pharmacology, Chair of Pharmacology, Jagiellonian University, Medical College, Grzegorzeczka 16, PL 31-531 Krakow, Poland

anna.selmi@jcet.eu

The endothelium as a semipermeable barrier between blood and tissue, is closely regulated under physiological conditions. A variety of physiological signals induce specific changes in endothelial barrier function. On the second hand, endothelial dysfunction is linked with increased endothelial permeability. Therefore, a deeper understanding of endothelial barrier function and of its physiological and pathological regulations is of importance.

Lately, ECIS® (Electric Cell-substrate Impedance Sensing) the novel method to describe the barrier properties of endothelium in vitro has been developed. This method is based on changes of electrical impedance endothelial cells in culture grown on gold-film electrodes it is a real-time, label-free approach to measure endothelial permeability. The ECIS-based measurement allows to analyze morphological changes of endothelium, wound healing response and migration, cellular adhesion to endothelium, measuring and modeling of endothelial cell barrier function also under flow conditions.

The impedance of cells on electrode is measured at low or high frequencies. Ions passage through cell body is measured under high frequency and is mainly determined by the three-dimensional shape of the cells. Ions passage around cells is measured under low frequency and is mainly determined by the barrier function of endothelium.

In this work we present changes in endothelial permeability in human umbilical vein cell line, EA.hy926, human umbilical vein endothelial cells (HUVEC), human lung microvascular endothelial cells (HLMVEC) and dermal microvascular endothelial cells (HMEC-1). We analyzed effects following pharmacological agents known to increase endothelial permeability: histamine, forskolin, hyaluronidase. We also tested whether PAPA-NO and cPGI protect endothelial barrier function compromised by histamine.

References:

- [1] A. Fallarero; A.E. Batista-González and A.K. Hiltunen, *Int. J. Mol. Sci.* (2015) 27044-27057.
- [2] I. Bischoff; M.C. Hornburger and B.A. Mayer, *Sci. Rep.* 6 (2016) 23671.

Impedance Sensing of HUVECs after Tacrolimus Treatment

S. Thoms, E. Friehs, R. Jonczyk, A. Lavrentieva, T. Scheper, C. Blume

Institut für Technische Chemie, Leibniz Universität Hannover, Hannover (Germany)

thoms@iftc.uni-hannover.de

Immunosuppressants are used to inhibit adverse reactions of the immune system after the transplantation of organs to prevent organ rejection. Unfortunately, these substances have a broad range of undesired side effects, especially when higher levels are used, therefore limiting the long time application. Tacrolimus is a widely used immunosuppressant as it inhibits the enzyme calcineurin that plays a central role in T-lymphocyte dependent immune response (especially the so called T-cell mediated rejections = TCMR). For a successful integration of transplanted organs and tissues into the new organism, a good vascularization is necessary which indicates a good nutrient supply of the transplanted organ. Therefore, our research interest is focused on understanding the impact of Tacrolimus on endothelial cells that are lining the inner walls of blood vessels.

Human umbilical vein endothelial cells (HUVECs) have been used as model system for all experiments. The cytotoxic effect of Tacrolimus and the EC_{50} was determined by dose response curve generated by screening of different concentrations of Tacrolimus with CellTiter-Blue® assay. A difference between necrosis and apoptosis was determined by LDH release and Caspase 3/7 activity. The obtained EC_{50} value of Tacrolimus for HUVECs was $26.48 \mu\text{g}\cdot\text{mL}^{-1}$ and a lethal dose of $34 \mu\text{g}\cdot\text{mL}^{-1}$ was determined. Additionally, non-invasive impedance sensing was used to verify these results and monitor cell death dynamics. We present data from HUVECs cultured in ECIS single electrode arrays (seeding 250.000 cells) treated with Tacrolimus EC_{50} and lethal doses. After 40 hours of cultivation, confluence was reached and the immunosuppressant was added. The effect was monitored over a frequency range of 64 Hz to 64.000 Hz and 140 hours. A cytotoxic effect of tacrolimus resulting from damage of the cell membrane was demonstrated. Moreover, an angiogenesis assay was performed to analyze the effect of Tacrolimus on formation of tubular like structures as a preform of vessels. With higher concentration of Tacrolimus the HUVECs showed a lower formation of tubular like structures in the angiogenesis assay due to its cytotoxicity.

Using a TEER-based screen to find novel mechanisms that regulate retinal endothelial barrier function

V. Küppers¹, M. Tzouros¹, D. W. Avila¹, L. Badi¹, M. Ebeling¹, J. D. Zhang¹, O. Eidam¹, P. Turowski², G. Hartmann¹

¹ Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd., Basel, Switzerland

² UCL Institute of Ophthalmology, London, United Kingdom

verena.kueppers@roche.com

The retina is one of the metabolically most active tissues in the body and is supplied with nutrients and oxygen by the choroidal vasculature and the retinal vasculature. While choroidal endothelial cells (ECs) are highly fenestrated and do not form a tight barrier, retinal ECs exhibit a high barrier function and form the inner blood-retinal barrier (iBRB). Conditions like hyperglycemia and hypoxia, which can be found in diabetic patients, weaken the endothelial barrier. Breakdown of the iBRB, followed by vascular leakage and neovascularization, is one of the main drivers in diabetic retinopathy (DR) and diabetic macular edema (DME), which both lead to vision loss and blindness. An intact and tightly regulated blood-retinal barrier is thus crucial to prevent disease progression and to avoid vision loss. The main target for treating DME/DR is currently vascular endothelial growth factor (VEGF). Since 30% of patients do not fully or not at all benefit from those therapies, there is an important unmet medical need for the discovery of additional therapeutics. The identification of new target proteins and pathways which regulate blood-retinal barrier integrity is therefore of crucial importance.

One of the signaling pathways that influence endothelial barrier function apart from VEGF signaling is the angiopoietin/Tie system. Angiopoietin-1 (Ang-1) and Ang-2 both bind the receptor tyrosine kinase Tie-2. Ang-1 signaling leads to barrier stabilization and increased vessel integrity, while Ang-2 is only a weak agonist. It is at the same time an antagonist of Ang-1 and overall promotes barrier destabilization, proliferation and angiogenesis. Using transendothelial electrical resistance (TEER) measurements, we could show that Ang-1 counter-regulates VEGF-induced barrier breakdown in HUVECs.

In order to dissect the differential signaling upon angiopoietin stimulation, HUVECs were stimulated with Ang-1, Ang-2 and the combination of both, and analyzed with Tandem Mass Tags (TMT) based quantitative phosphoproteomics. The initial discovery hits were further bioinformatically evaluated on single peptide/protein level as well as on pathway level. We identified biological processes and signaling pathways which are differentially enriched in the respective treatment conditions. Together with additional sites from the literature, a list of 500 distinct and candidate phosphosites was assembled, which will now be verified using a targeted phosphoproteomics approach.

The 500 hits were additionally mapped on an integrated kinase-substrate-interaction network, which identified central signaling proteins likely involved in the regulation of barrier function. Those signaling proteins are now analyzed functionally in more detail using TEER as a direct readout for endothelial barrier function to confirm their role in barrier regulation. Small-molecule inhibitors specifically targeting these proteins are applied to unstimulated, VEGF stimulated and Ang-1 stimulated HUVECs, respectively. Using this setup, it is possible 1) to elucidate the role of the protein in baseline endothelial barrier function, 2) to detect a possible effect of the protein on barrier restoration after VEGF-induced barrier breakdown, and 3) to unravel the interplay of this protein with Ang-1 induced barrier stabilization. This screen will thus result in a list of potential new candidates and pathways that efficiently regulate endothelial barrier function. The most promising candidates will be verified and analyzed in more detail and could later on be used as targets for new therapeutics in DME and DR.

Independent Observation of Two Co-Cultured Cell Layers Grown on Filter Supports

K. Hajek and J. Wegener

University of Regensburg, Regensburg (Germany)

Kathrin.Hajek@chemie.uni-regensburg.de

Measuring transepithelial, electrical resistances (TEER) is an established non-invasive and label-free method to quantify the permeability of barrier-forming cell layers (skin tissue, cells of the intestine or vessels). Highly permeable polycarbonate membranes (pore density $10^8/\text{cm}^2$, pore- $\varnothing$ 400 nm) are commonly used as culture substrate in standard transfilter measurements for 2D cell layers. A confluent cell layer, cultured upon these porous polymer membranes, represents a diffusion barrier between the two fluid compartments in a typical measurement chamber. Electrodes individually placed in either compartment of the chamber are necessary to determine the TEER as well as the frequency-dependent impedance of the cell layer. The latter can be analyzed by means of equivalent circuit modelling.

By depositing a thin gold film on one side of the filter support which is used as an additional, third electrode enables independent observation of two cell layers co-cultured on opposite sides of the filter by impedance analysis (Fig. 1). As the filter membrane is thin ($10\text{ }\mu\text{m}$) and highly porous, cell-to-cell communication between the cells on opposite sides of the filter is possible and its influence on the individual barrier properties of the two cell populations is accessible. Moreover, this setup allows studying the influence of environmental pollutants or drugs on both cell layers in a single experiment.

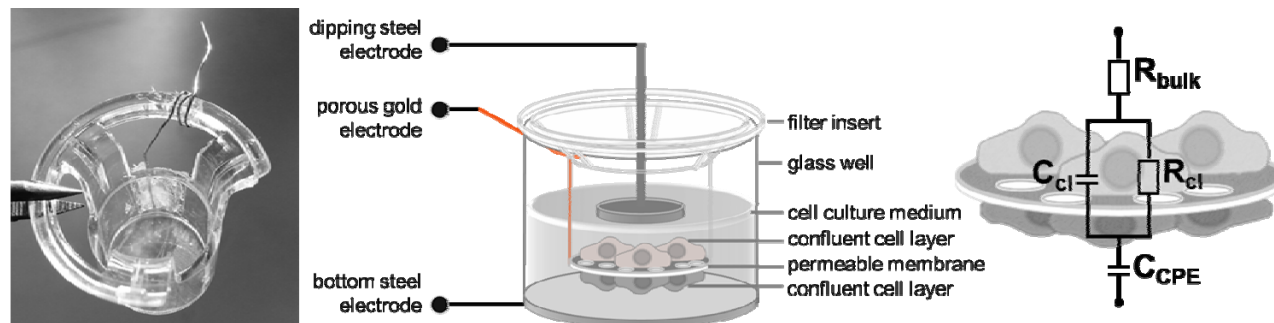


Fig. 1: Photograph of a modified transwell filter insert (left). The scheme in the middle panel represents the experimental setup to measure the impedance contribution of each cell layer in a co-culture. Transepithelial electrical resistance (TEER) can be obtained by fitting the experimental data to the equivalent circuit shown in the scheme on the right side.

An Automated Perfusion System for Monitoring Complex Transepithelial Electrical Resistance (TEER) of Membrane-bound Cultures

F.A. Alexander Jr.; S. Eggert and J. Wiest

cellasys GmbH, Munich (Germany)

alexander@cellasys.com

Monitoring the transepithelial electrical resistance, or TEER, of three dimensional and Organ-on-a-chip models has received increased attention in the past 5 years due to its potential as a label-free method for screening barrier properties of cell layers in response to stimulation [1]. The majority of current TEER measurement systems rely upon manually-positioned “chopsticks” electrodes that require users to remove cells from the incubator for measurements and produce highly variable measurements. Chamber-style electrodes can overcome this limitation, but still require users to manually dispense the measurement solution and test reagents. Automated measurements can not only drastically reduce user work load but also improves the reproducibility of measurements by eliminating sample disturbances.

To enable automated screening of tissue cultures on membrane inserts, a novel, PC-controlled system was created that 1) preserves the air-liquid interface of porous membrane cultures; 2) periodically dispenses and removes phosphate buffered saline (PBS) or a test reagent onto the surface of the membrane culture; and 3) seamlessly measures the real and imaginary impedance of a membrane embedded on BioChips without requiring removal from the incubator and manual positioning of electrodes. The recorded impedance can then be utilized as a TEER measurement to indicate the barrier strength of various membrane cultures. Briefly, the apical (top) side of membrane cultures was exposed to PBS for 5 minutes once each hour, to acquire complex TEER values. The basal side of the membrane culture was then exposed to medium with 1% SDS at 37 hours as a positive control. Measured real and imaginary values decreased shortly after the addition of SDS indicating cellular death on the membrane. With this system, more reproducible TEER measurements serve as the basis for a new generation of fully-automated, label-free screenings of membrane culture-based organ-on-a-chip devices.

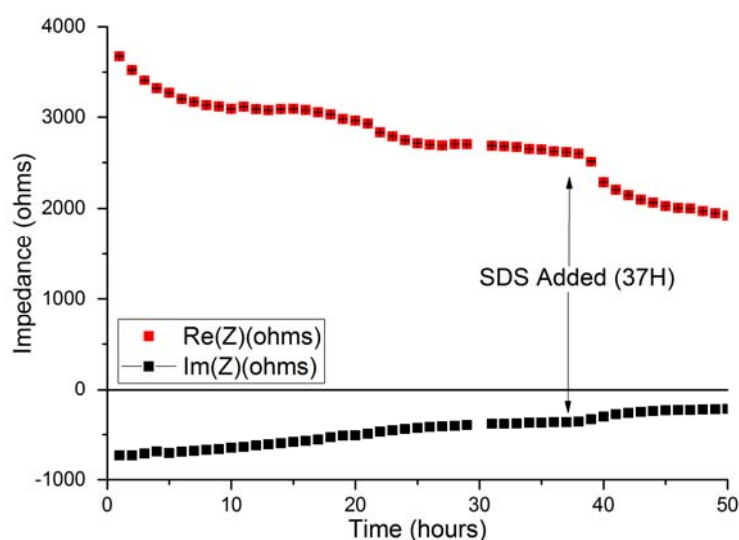


Fig: Measured complex impedance values for cellular layer immobilized on porous membrane insert

References:

- [1] M.W. van der Helm, M. Odijk, J.-P. Frimat, A. D. van der Meer, J. C. T. Eijkel, A. van den Berg et al., *Biosensors and Bioelectronics*, 85 (2016) 924-929.

Administration of protein kinase d1 induce migration of intestinal epithelial cells *in vitro*

D.S.G. Nielsen; M. Fredborg and S. Purup

Department of Animal Science, Aarhus University, DK-8830 Tjele, Denmark

ditteg.nielsen@anis.au.dk

Significant increases in prevalence rates of inflammatory bowel diseases (IBD), characterized by a compromised epithelial barrier function, requires development of new, innovative and cost-effective drugs to optimize patient treatment and the economic burden [1]. Protein kinase d1 (PKD1) is rapidly activated in intestinal epithelial cells *in vitro* upon wounding, promoting migration and wound healing [2]. Here we investigate whether administration of recombinant human PKD1 to rat and human intestinal epithelial cells *in vitro* induce migration of cells into the wounded area, besides studying the potential signaling mechanisms behind these effects.

Rat and human intestinal epithelial cells (IEC-6, FHs 74 Int cells) were used for scratch wound healing assays, as previously reported [3]. Cells were treated for 6 hours with human recombinant PKD1 at 0.00001, 0.0001, 0.001, 0.01, 0.1, 1, 10, 50 and 100 ng/ml. Assays were performed in triplicates with twelve repetitions for each treatment and migration presented relative to the negative control (0 ng/ml PKD1). Intracellular signaling proteins were studied by sensitive Amplified Luminescence Proximity Homogenous Assay (AlphaScreen®). FHs 74 Int cells were scratched and treated with 0.1 and 100 ng/ml of PKD1 for 2, 5 and 15 minutes alone or in presence of a G protein-coupled receptor agonist, either 100 nM of phorbol 12,13-dibutyrate (PDBu) or angiotensin II (ANGII).

Administration of human recombinant PKD1 to FHs 74 Int cells increased migration at 0.0001 to 10 ng/ml PKD1 ($P<0.05$) compared with the negative control. Specifically, PKD1 at 0.01 and 0.1 ng/ml increased migration by >60%. A similar effect was observed in IEC-6 cells where PKD1 at 0.00001 to 10 ng/ml increased migration ($P<0.05$) compared with the negative control. Preliminary studies revealed deactivation of the Erk signaling pathway following PKD1 treatment, whereas no effects were observed on Akt and NFκB signaling pathways. Treatment with PDBu caused a marked activation of the Erk signaling pathway ($P<0.05$), yet PKD1 tended to deactivate this pathway even in the presence of PDBu ($P=0.0506$). Both Akt and NFκB signaling pathways were deactivated by PDBu ($P<0.05$). This is in accordance with a previous study showing early activation of Erk followed by subsequent activation of Akt and NFκB signaling pathways [4]. The agonist ANGII had no notable effect on these pathways. These results demonstrate that administration of recombinant human PKD1 induce wound healing in intestinal epithelial cells *in vitro*, suggesting that PKD1 might be a possible approach for future treatment of IBD.

References:

- [1] G.G. Kaplan, *Nat. Rev. Gastroenterol. Hepatol.* 12/12 (2015) 720-727.
- [2] S.H. Young, et al., *Am. J. Physiol. Gastrointest. Liver Physiol.* 303/3 (2012) G356-366.
- [3] S. Purup and T.S. Nielsen, *Animal* 6/3 (2012) 423-432.
- [4] Li, J., et al., *J. Cell Sci.* 126 (2013) 5005-5017.

Impedance Analysis of pH-dependent Cell Migration

L. Sauer; C. Schmittlein and J. Wegener

Universität Regensburg, Regensburg (Germany)

Lisa.Sauer@chemie.uni-regensburg.de

In contrast to healthy cells, cancer cells show a different intracellular pH_i value as a consequence of an altered cellular metabolism. This change of pH_i affects several key features of cell physiology like proliferation, migration or wound healing efficiency [1]. It is noteworthy that differences in migration are closely linked to enhanced invasive and metastatic properties. Monitoring the speed of migration serves an important experimental parameter to describe metastatic potential as well as to screen drugs and toxins with respect to their impact on cell dissemination. As has been shown recently, Electric Cell-Substrate Impedance Sensing (ECIS) may serve as a useful tool for an automated and label-free analysis of cell migration even in 96well format.

In our study we used ECIS® to monitor pH-dependent cell migration of two mammalian cell lines. Adenocarcinoma human alveolar basal epithelial cells (A549) and rat kidney epithelial cells (NRK) were grown to confluence in 8W2LE arrays while cell adhesion and spreading was monitored by ECIS. When the cells reached confluency, resulting in a stable impedimetric signal, they were incubated with buffers varying in pH value. The cell monolayer was then wounded by application of an invasive voltage pulse of several volts amplitude and several tens of seconds duration. The pulse leads to lysis of all cells residing on the electrode surface. As the cells in the electrode periphery were not compromised, the area of the free electrode is repopulated by migration and proliferation of neighboring cells. We observed that the healing rate was strongly pH-dependent; with increasing pH value of the applied buffer, wound closure was observed to be faster. However, lower pH values resulted in a drastically decreased healing behavior, ultimately leading to cell death. In contrast to NRK cells, A549 cells exhibited substantially longer healing times. In summary, we showed that ECIS® can be used as a tool to monitor pH-dependent wound healing of two different cell lines and may help to analyze specific aspects of tumorigenesis.

References:

- [1] Z. Ge and S. Liu, *Chem. Soc. Rev.* 42 (2013) 7289-7325.

Quartz Crystal Microbalance as a Transducer in Cell-Based Assays

S. Ruckdäschel; S. Michaelis and J. Wegener

Universität Regensburg, Regensburg (Germany)

Simone.Ruckdaeschel@chemie.uni-regensburg.de

The *Quartz Crystal Microbalance* (QCM) technique is often used as a non-invasive and label-free biosensor to monitor biological adsorption processes or to follow changes of cell morphology during exposure to chemical substances. The core component of this technique is a circular piezoelectric 5 MHz AT-cut quartz disk with two evaporated gold-electrodes on each side. By applying an AC potential difference between the surface electrodes the shear-wave resonator is excited to perform mechanical oscillations parallel to the resonator's faces at its resonance frequency. If mass is deposited on the quartz surface, e.g. during cell adhesion, the parameters of the resonant oscillation respond in a very sensitive way. This change in shear wave parameters serves as an indicator reporting on mass deposition as a function of time (*impedance analysis*).

For the sensitivity and the information content of QCM measurements, the magnitude of the penetration depth of the shear wave is a crucial parameter. This amplitude depends on the density and viscosity of the environment close to the resonator surface. It varies between 250 nm for water and 21 μm for honey at room temperature. It may also be affected by the rigidity of a pre-adsorbed mass layer [1]. Since a cell monolayer forms a non-rigid, viscoelastic film on the quartz disk, a linear dependence between frequency shift and mass loading as established by Sauerbrey is not valid [2,3].

In almost all cell-based studies in literature, the observed changes of the QCM parameters were attributed to the cell-substrate adhesion zone and the substrate-facing membrane. In this project we wanted to identify subcellular contributions especially from the apical cell membrane and their individual impact on the integral QCM signal. One study in literature reports on QCM parameter changes in response to adsorption processes at the apical membrane. Here, Pei et al. described measurements in which receptor-ligand interactions occurring at the apical membrane surface were monitored after fixing the cells with formaldehyde. The authors claim a change in the penetration depth of the shear wave after having increased the cells' rigidity, so that measurements along this protocol may get analyzed according to the Sauerbrey equation [4].

In order to determine the penetration depth of the shear wave for a cell monolayer and to check the claims of Pei et al., three cell-based assays are applied in this study, focusing on cellular contributions from the apical membrane. MDCK II cells are grown to confluence on the resonator surface before they are either stiffened (with glutardialdehyde or paraformaldehyde) or left untreated and further processed as follows: (i) The lectin concanavalin A, which binds to selective receptors on the apical membrane, is added to the cells, (ii) the viscosity of the medium overlaying the cells is increased by adding different concentrations of polyvinylpyrrolidone, (iii) silica particles are added to the cell layers. Readings of the characteristic shear wave parameters for these three experimental scenarios should report on potential contributions of the apical cell surface to the overall QCM signal.

References:

- [1] J. Wegener; A. Janshoff and C. Steinem, *Cell Biochemistry and Biophysics* 34 (2001) 121-151.
- [2] J. Wegener; A. Janshoff and H. J. Galla, *European Biophysics Journal* 28/1 (1998) 26-37.
- [3] G. Sauerbrey, *Zeitschrift für Physik* 155 (1959) 206-222.
- [4] Z. Pei et al., *Biosensors and Bioelectronics* 35/1 (2012) 200-205.

Impedimetric / Luminescent Dual Mode Readout for multiparametric online Analysis of Adherent Cells

C. Schmittlein¹; R.J. Meier² and J. Wegener¹

¹Universität Regensburg, Regensburg (Germany)

²Presens GmbH, Regensburg (Germany)

Carina.Schmittlein@chemie.uni-regensburg.de

Assays using adherent cells are an established and widely applied tool in both basic research and screening of potential drugs or cytotoxic compounds. Recently, non-invasive, label-free readout technologies (impedance, refractive index) that measure passive physical properties have been introduced as monitoring tools for cell-based assays [1].

In order to gain multiparametric information about cell behavior and the cellular response to exposure to different stimuli, we combined impedimetric and optical readout concepts in one sensor substrate. Therefore planar gold film electrodes with a thickness of 100 nm were generated on top of the surface of a planar oxygen-sensitive polymer foil with incorporated luminescent dyes for ratiometric readout of oxygen levels. Photolithography which would lead to bleaching of the sensor dyes was avoided and replaced by sputter coating through a corresponding mask created by laser beam cutting. Cells can directly adhere to the surface of these substrates enabling label-free monitoring of impedance on the electrode area and oxygen levels via luminescence quenching on the electrode free areas (**Fig. 1**). The dual sensor allows gaining information about changes in cell morphology and electrode coverage via impedance measurements while the changes in oxygen concentration yield information about the oxygen consumption rate (OCR) of the cells.

Proof-of-concept experiments include characterization of the dual sensor substrates, characterization of the electrode layout, as well as single mode measurements (not yet dual mode) using impedimetric and luminescent readout separately.

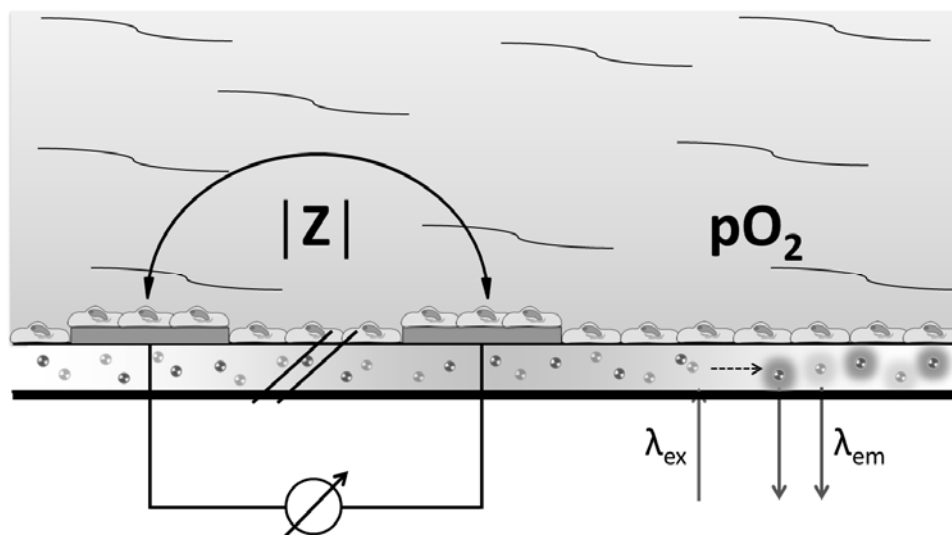


Fig. 1 Schematic of dual sensor layout with planar gold film electrodes for impedimetric monitoring sputtered on top of the culture substrate which contains luminescent dyes for the ratiometric readout of oxygen levels.

References:

- [1] S. Lukic; J. Wegener, *eLS* (2015), DOI: 10.1002/9780470015902.a0025710

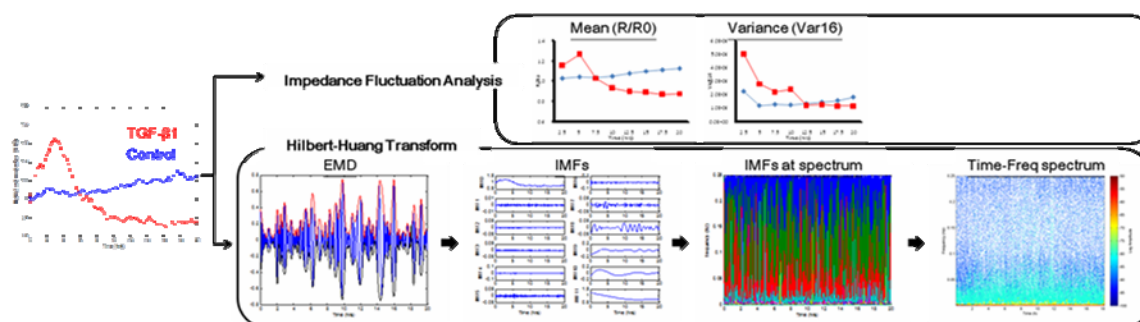
Use of Hilbert-Huang Transform to Characterize Impedance Time Series of TGF- β Induced Cellular Activity of MDA-MB-231 Cells

Y.T Lai and C.-M. Lo

Department of Biomedical Engineering, National Yang-Ming University, Taipei (Taiwan)

g39904002@ym.edu.tw

Transforming growth factor- β 1 (TGF- β 1) is one of the key regulators in tumor formation, progression, and metastasis; however its dual roles (inhibition or promotion at different time of tumor development) in tumor progression render effects of TGF- β 1 beyond a clear biophysical description in cell biology context. In this study, we aim to provide a plausible solution by applying novel analytical methods for real-time monitoring of cell adhesion activity. Electric cell-substrate impedance sensing (ECIS) was used to examine the effect of TGF- β 1 on MDA-MB-231 cells, a highly invasive breast cancer cell line. Impedance time series measured by ECIS were analyzed by Hilbert-Huang Transform (HHT) to extract various frequency components, called intrinsic mode functions (IMFs). These IMFs were used to calculate fractal correlation of time series and were further represented as a time-frequency spectrum. Our results show that a reproducible transient peak over impedance measurement is manifested within 10 hours after MDA-MB-231 cells were treated with 10 ng/mL TGF- β 1. HHT spectral analysis reveals that treatment of TGF- β 1 enhances high frequency field energy, in particular at the second and the third of total instantaneous energy (IE2, IE3 respectively), and both IE2 and IE3 are slowly decreased within time regime of activation. Additionally, the fractal scaling components of impedance fluctuations range from 2.1 to 1.9 ($p > 0.05$), suggesting that impedance time series of MDA-MB-231 cells induced by TGF- β 1 are persistent. Further correlative examination is required for unveiling the link between the change of impedance fluctuations and the biological processes of cells. Our work highlights the necessity of using detailed HHT analyses for ECIS measurement, which can decipher impedance fluctuations in response to cell membrane adhesion and dynamics.



References:

- [1] A.V. Bakin, et al. *Molecular Biology of the Cell* 15 (2004) 4682-4694.
- [2] D. Schneider, M. Tarantola, A. Janshoff, *Biochimica et Biophysica Acta* 1813 (2011) 2099-2107.
- [3] N.E. Huang, et al., *Proceedings of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences* 454 (1998) 903-995.
- [4] C.-M. Lo, C. R. Keese and I. Giaever, *Experimental Cell Research* 204 (1993) 102-109.

Discrete Wavelet Transform Algorithms for Multi-Scale Analysis of Impedance Fluctuation Obtained from Cellular Micromotion

T.H. Tung; C.M. Lo

Department of Biomedical Engineering, National Yang-Ming University, Taipei (Taiwan)

tsehuatung@gmail.com

We applied electric cell-substrate impedance sensing (ECIS) to assess in vitro cytotoxicity of human umbilical vein endothelial cells (HUVECs) and 3T3 fibroblasts exposed to different concentrations of CdCl₂ and H7 dihydrochloride, a protein kinase C inhibitor. To detect the alteration of cell micromotion in response to the cytotoxic challenge, time-series impedance fluctuations of cell-covered electrodes were monitored at 1 Hz sampling rate by rapid time collect (RTC) measurement. Calculations of variance (Var32) and variance of increment (VOI32) were used to detect the effects of H7 and CdCl₂ at concentrations as low as 10 microM and 25 microM respectively [1,2]. Data were also analyzed using discrete wavelet transform (DWT), a multi-scale method which has been broadly used in ECG, EMG, and EEG feature extraction and been recently used in ECIS time-series analysis [3,4]. By calculating the variance, energy, entropy, and signal magnitude area (SMA) from level 1 to level 5 DWT detail coefficients, we are able to distinguish cytotoxic levels of H7 and CdCl₂ as sensitive as Var32 and VOI32 methods have accomplished [5]. The frequency range of level 1, 1/4 ~ 1/2 Hz, is 16 times larger than that of level 5, 1/64 ~ 1/32 Hz, indicating the possible use of multi-well electrode arrays for high throughput screening using ECIS single frequency time-course (SFT) measurement. Our multi-scale analysis of impedance time series by down sampling data, by a factor of 2, 4, 8, and 16, also demonstrates that the sequential quantification of DWT detail coefficients is an effective method for determining sampling rate in ECIS cytotoxicity assay. Moreover, DWT analysis can determine affected frequency range associated with cellular micromotion in response to each cytotoxicity assay.

References:

- [1] D. Opp; B. Wafula; J. Lim; E. Huang; J.C. Lo and C.M. Lo, *Biosensors and Bioelectronics* 24/8 (2009) 2625–2629.
- [2] D.C. Lovelady; J. Friedman; S. Patel; D.A. Rabson and C.M. Lo, *Biosensors and Bioelectronics* 24/7 (2009) 2250–2254.
- [3] S.W. Chen; J.M. Yang; J.H. Yang; S.J. Yang and J.S. Wang, *Biosensors and Bioelectronics* 33/1 (2012) 196-203.
- [4] J.M. Yang; S.W. Chen; J.H. Yang; C.C. Hsu; and J.S. Wang, *Computers in Biology and Medicine* 69 (2016) 134-143.
- [5] C.V. Bouten; K.R. Westerterp; M.Verduin and J.D. Janssen, *Medicine and Science in Sports and Exercise* 26/12 (1994) 1516-1523.

Automated monitoring of impedance and extracellular acidification of fibroblasts in an eye irritation toxicity assay

J. Wiest

cellasys GmbH – R&D, Munich (Germany)

wiest@cellasys.com

The IMOLA-IVD technology was developed to monitor extracellular acidification, cellular respiration and changes in impedance of living cells. The system is computer controlled and incorporates an automated fluidic system to add and remove chemicals from the cells [1]. Due to the modular approach it is possible to mimic different protocols. In the presented work we set up a version similar to the cytosensor microphysiometer test for eye irritation [2].

This extracellular acidification test method (EATM) is capable of delivering the same data as the – no longer commercially available – cytosensor microphysiometer [3]. Furthermore the test results can be improved by additional analysis of the impedance of the living cells. The setup incorporating six IMOLA-IVD modules and the adaption of the INVITTOX #130 protocol [4] is described. First results of simultaneous measurement of extracellular acidification rate and changes in bioimpedance are presented (Figure 1).

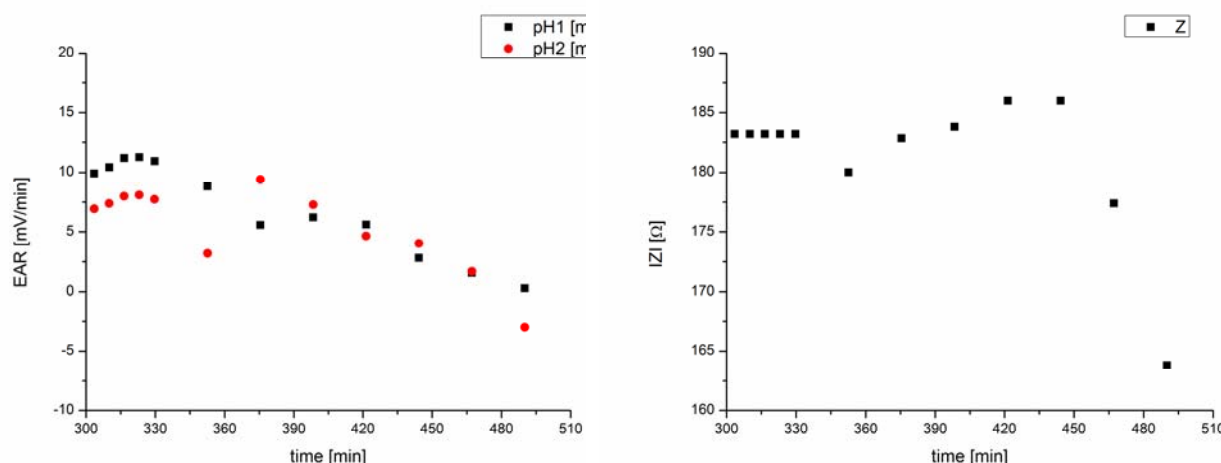


Figure 1: Changes in extracellular acidification rate (left) and magnitude of impedance (right) of L929 fibroblasts under the influence of an increasing concentration of sodium dodecyl sulfate.

References:

- [1] D. Weiss, M. Brischwein, H. Grothe, B. Wolf, J. Wiest, 35th Annual International Conference of the IEEE EMBS, Osaka, Japan, 3 – 7 July, 2013, 1607-1610.
- [2] S. Eggert, F.A. Alexander, J. Wiest, 37th Annual International Conference of the IEEE EMBS, Milano, Italy, 25 – 29 August, 2015, 2175-2178.
- [3] T. Hartung, L. Bruner, R. Curren, C. Eskes, A. Goldberg, P. McNamee, L. Scott, V. Zuang, ALTEX, 27, 1/10, 43-51, 2010.
- [4] www.ecvam-dbalm.jrc.ec.europa.eu.

Impedance based Analysis of Adherent Cells using interdigitated electrodes with subcellular dimensions

M. Zinkl^{1,2}; C. Götz^{1,2} and J. Wegener¹

¹Universität Regensburg, Regensburg (Germany)

²Fraunhofer EMFT, Business Area Sensor Materials, Munich, Germany

Maria.Zinkl@chemie.uni-regensburg.de

When cells attach and spread upon planar film electrodes, they change the electrode's AC impedance. This impedance change provides an experimental tool to analyze and characterize cell-cell or cell-substrate contacts.

It became apparent that size and geometry of the electrodes may have a big influence on the sensitivity of the impedance measurement. A pair of interdigitated electrodes (IDEs) is one of the most commonly used electrode layouts. Normally, the lateral dimensions of the electrodes (several hundred μm) are significantly bigger than those of the cells under study [1]. In this project we studied the use of interdigitated electrodes (IDEs) with subcellular, lateral dimension. The IDEs were prepared as 100 nm thick gold layers patterned in a finger-like arrangement (fig. 1). The fingers have a width of 6 μm and a pitch of 5 μm . To compare the impedimetric readout with conventional electrodes, we used commercially available 8W10E ECIS arrays (Applied BioPhysics Inc.) as a reference (fig.1). The ECIS arrays consist of eight independent measurement chambers each of which holds ten working electrodes (arranged in parallel) with an overall electrode area of $5 \cdot 10^{-3} \text{ cm}^2$ plus a common counter electrode with an area of $5 \cdot 10^{-1} \text{ cm}^2$.

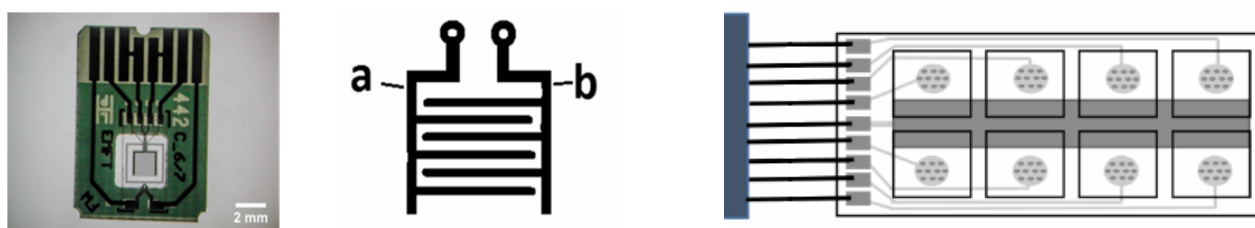


Figure 1: Photograph and schematic of the interdigitated electrode layout used in this study (left), schematic of the 8W10E ECIS array with eight independent measurement chambers each with ten working electrodes and a common counter electrode (right).

It was the objective of this project to characterize adherent, mammalian cells grown upon IDEs with subcellular lateral dimensions and to compare the results with those obtained from the established 8W10E arrays. With respect to the subcellular electrode dimensions of the IDEs, we expected high sensitivity and a strong signal contribution from the electrolyte filled space underneath the cells. As cellular model systems we used widely applied NRK (normal rat kidney), MDCK I and MDCK II (Madin Darby Canine Kidney) cells. The different impedimetric characteristics of the cells and their response to various stimuli on the IDEs and on 8W10E ECIS 'reference arrays' were recorded by repeatedly scanning the impedance spectrum from 1 Hz to 1 MHz.

References:

- [1] O. Lazcka; E. Baldrich; F.X. Campo, *Anal. Chem.* 80 (2008) 7239-7247.

A versatile lab-on-a-chip system for optical and impedance based cytotoxicity assays

B. Böttcher; M. Büttner; S. Eisenhuth; B. Büttner and K.-H. Feller

Ernst-Abbe University of Applied Sciences Jena, Jena (Germany)

Bastian.Boettcher@eah-jena.de

Due to the rapid development of novel substances as nanoparticles as drug delivery cargos or drugs itself efficient and sensitive systems to test possible cytotoxic side effects are required. A solution for this problem is the application of lab-on-a-chip-systems since they can integrate many functional fluidic structures and analytical methods.

Therefore a lab-on-a-chip system was developed that combines optical and impedimetric measurement methods to determine cytotoxicity of substances with human cells. The chip consists of three layers, each made of TOPAS® COC 6015 which are bonded via hot-melt foils. This allows the generation of complex fluidic structures e.g. micromixers. For cultivation of cells, nine reservoirs are integrated whereat three of these wells each are connected via microfluidic channels forming one lane. Two lanes are connected with to micromixers each allowing the addition of the test or a reference substance. For impedance measurement, gold electrodes are deposited on the bottom of each well. The optical assay is based on cell stress induced expression of a heat shock protein (HSP72/70b) coupled with green fluorescent protein (GFP) via genetic modification. The high sensitivity of the method was proven by the measurement of dose-dependent increase of HSP-GFP-expression in modified HaCaT cells at sub lethal concentrations of CdCl_2 . Although this range of concentrations is interesting, it is also limited [1].

To detect cellular reactions at higher toxin concentration, ECIS measurement is applied within the chip system. With this extension of possible measurement concentration, also chemosensitivity tests are possible. As an example, chemotherapeutic agent paclitaxel and doxorubicin were added to unmodified A549 cells showing different cytotoxic effects. Adapting this chemosensitivity test to primary cells from cancer patients, a possibility for tumor therapy optimization is given.

The measurements showed that the developed lab-on-a-chip system is well suited for determination of the cytotoxicity of substances in a wide range of concentrations and offers also applications with unmodified cells due to the non-invasive character of ECIS.

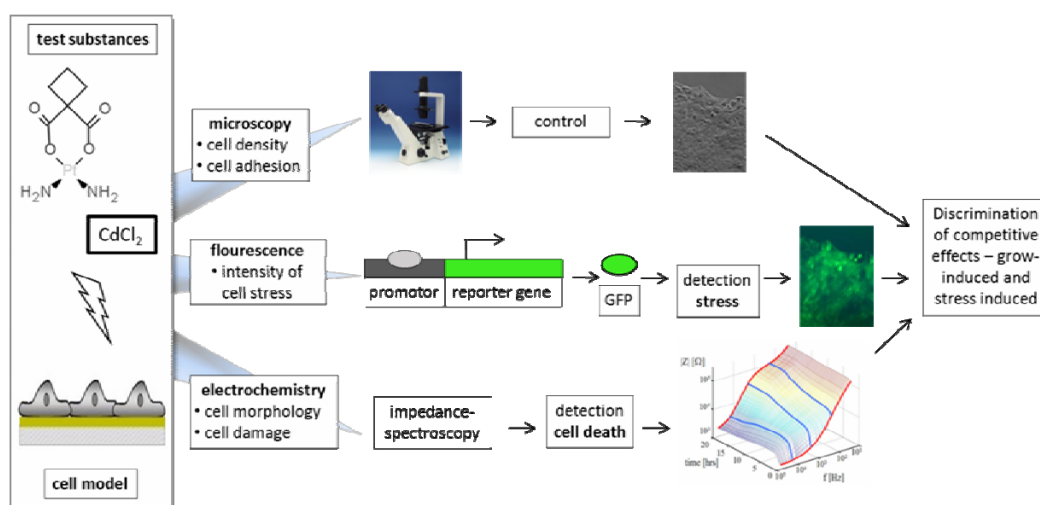


Figure: Overview of the analysis methods in the lab-on-a-chip system for cytotoxicity determination

References:

- [1] U. Hofmann; S. Michaelis; T. Winckler; J. Wegener and K.-H. Feller, *Biosensors and Bioelectronics* 39 (2013) 156-162.

PEDOT/PSS as transparent electrode material for impedance-based cell analysis

C. Götz^{1,2}, S. Trupp², and J. Wegener²

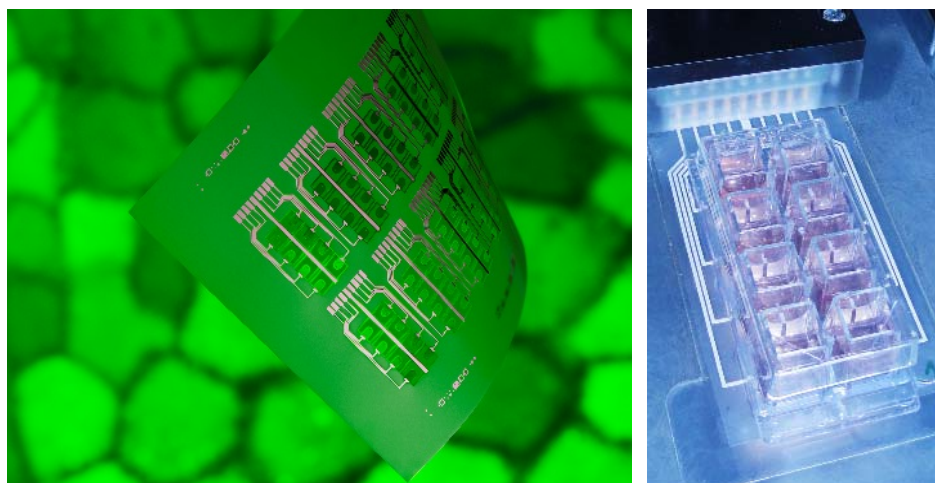
¹Fraunhofer EMFT, Business Area Sensor Materials, Munich, Germany

²Universität Regensburg, Regensburg, Germany

christian.goetz@chemie.uni-regensburg.de

Conducting polymers have increasingly found their way into products of everyday life. Established materials like gold and indium tin oxide are being replaced as conducting polymers stand out not only because of their low cost, but also some particular material characteristics like mechanical elasticity, optical transparency and their dielectric properties. Among all conducting polymers PEDOT/PSS shows the most prominent application potential. It can be dispersed in aqueous solutions and hence makes it accessible for large scale manufacturing techniques like screen printing. Layers of PEDOT/PSS show swelling in aqueous solutions, thus increase the interface capacitance between electrode and electrolyte, rendering it interesting for its application as a transparent transducer in impedimetric biosensors [1]. This research is focused on the use of PEDOT/PSS as electrode material in impedimetric whole-cell biosensors (Electric Cell-substrate Impedance Sensing, ECIS). 8-well electrode arrays have been fabricated by screen printing, showing great potential for commercial application. A comparison between screen printed PEDOT/PSS and the commonly used gold electrodes shows possibilities and limitations of this electrode material. Different applications are being investigated.

The distinct dielectric properties of PEDOT/PSS make investigation of the cell-cell and cell-substrate contacts highly sensitive and may even enable impedimetric measurements of cell lines formerly not accessible. Low cost and easy processing allow for electrode fabrication in industrial scales.



References:

- [1] A.S. Karimullah, D.R.S. Cumming, M. Riehle, N. Gadegaard, *Sensors and Actuators B* 176 (2013) 667– 674.

Impedance-based Analysis and Holographic Phase Imaging of the GnRH-III-based Drug-targeting in Melanoma Cells

E. Lajkó¹, S. Spring^{1,2}, O. Láng¹, S. Ingebrandt², G. Mező³ and L. Kőhidai¹

¹ Impedimetry MERILL Core Facility/Chemotaxis Research Group, Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest (Hungary)

² Department of Informatics and Microsystem Technology, University of Applied Sciences Kaiserslautern, Zweibrücken (Germany)

³ MTA-ELTE Research Group of Peptide Chemistry, Eötvös Loránd University, Budapest

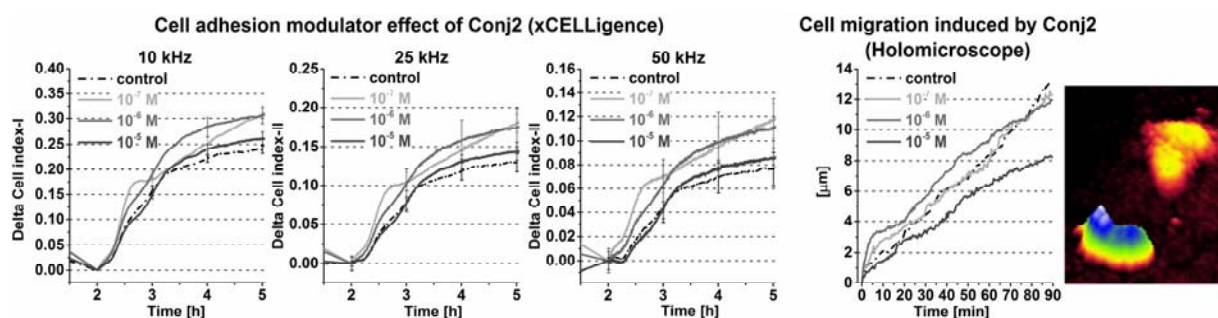
lajesz@gmail.com

Peptide hormone based targeted tumor therapy is an approved strategy to selectively block the tumor growth and spreading. The gonadotropin-releasing hormone receptors (GnRH-R) over-expressed on different tumors (e.g. melanoma) could be utilized for drug-targeting by application of a GnRH analog as a carrier to deliver a covalently linked chemotherapeutic drug directly to the tumor cells [1]. In this study our aim was (i) to analyze the effects of GnRH-drug conjugates on melanoma cell proliferation, adhesion and migration; (ii) to study the mechanisms of the tumor cell responses, and (iii) to compare the activities of the conjugates with the free drug.

In the conjugates, the daunorubicin (Dau) was coupled to GnRH-III (conj3) or its derivatives modified in position 4 with Lys(Ac) (conj1) or Lys(nBu) (conj2) [2]. The adhesion and proliferation of A2058 melanoma model cell was detected by xCELLigence SP. The holographic microscope, HoloMonitor M4 was used for the morphometry and migration studies. To investigate molecular biological background of the melanoma responses, flow cytometry, quantitative PCR and inhibition assay with PI3K inhibitors were employed.

All conjugates elicited irreversible, tumor growth inhibitory effect (IC₅₀: conj1: 6.4×10^{-6} M, conj2: 5.4×10^{-6} M, conj3: 1.45×10^{-5} M) mediated via the PI3K sensible pathway. The conj1 and 3 proved to be cytostatic (G2/M arrest), while a stronger, cytotoxic effect of conj2 was verified by apoptosis measurement after annexin staining and morphometry analysis. In short term, the melanoma cell adhesion was increased by conj1 (10^{-6} – 10^{-5} M: 122.8–131%) and conj2 (10^{-8} M– 10^{-6} M: 131.7–121.9%). The uptake of conjugates by A2058 cells proved to be time dependent. The modification of the GnRH-III in position 4 was accompanied with an increased cellular uptake, higher cytotoxic and cell adhesion inducer activity, as well. In general, the migratory behavior of the melanoma cells was increased by conj1, while the conj2 decreased this activity. Expression study of different apoptosis markers also referred to structure-dependent mechanisms induced by the conjugates.

The internalization, the toxic and apoptotic activities of the conjugates show that the GnRH-III peptides could guard the Dau to melanoma cells and promote antitumor activity. The model system was shown to be sensitive enough to test small structural differences (e.g. length of the side chain in ⁴Lys). Conj2 proved to be the best candidate for targeted tumor therapy due to its cytotoxicity and immobilizing effect on tumor cell spreading.



References:

- [1] P. Limonta, M.M. Montagnani, S. Mai et al. *Endocr. Rev.* 33 (2012) 784-811.
- [2] R. Hegedűs, M. Manea, E. Orbán et al. *Eur. J. Med. Chem.* 56 (2012) 155-65.

Impedimetric microtomography in rolled-up tubes

S.M. Weiz; M. Medina-Sánchez and O.G. Schmidt

Institute for Integrative Nanosciences, IFW Dresden (Germany)
Chemnitz University of Technology, Chemnitz (Dresden)

s.m.weiz@ifw-dresden.de

m.medina.sanchez@ifw-dresden.de

Electrical impedance tomography (EIT) is a label-free and non-invasive imaging technique that is suitable to study the conductivity distribution of an object. Recently, millimeter-sized EIT setups have been reported [1,2]. Smaller devices with sizes of few tens of microns would be desirable to study, for instance, cell-cell, cell-material or cell-drug interactions.

Hence, microtubular EIT (μ EIT) systems were fabricated by rolled-up technology [3]. Using sequential lithography and electron beam deposition steps, an array of 12 electrodes (Cr/Au) was patterned on top of a TiO_2 strained layer and a Ge sacrificial layer. Upon selective removal of the sacrificial layer, the TiO_2 thin films rolled up to form tubes with diameters of ca. 30 μm (Fig. 1a). Tube characteristics like diameter and number of windings can easily be defined by tuning the fabrication parameters.

For characterization measurements, a partially Au-coated SiO_2 microparticle was introduced into the μ EIT system as a phantom. Cyclic voltammetry and impedance measurements were conducted and revealed clear differences with and without the introduced particle (Fig. 1b, c), showing that the presence of a structure inside the tube can be detected. Furthermore, different results were obtained for electrodes in proximity to the dielectric or the conductive part of the Janus particle, respectively. In order to obtain cross-sectional tomography images, strategies for appropriate data acquisition and plotting are being developed. First cell insertion tests were carried out with a HeLa cell, demonstrating the feasibility of the introduction and removal of cells into the μ EIT structure. Future experiments will be conducted with different types of cells, e.g. neuronal stem cells.

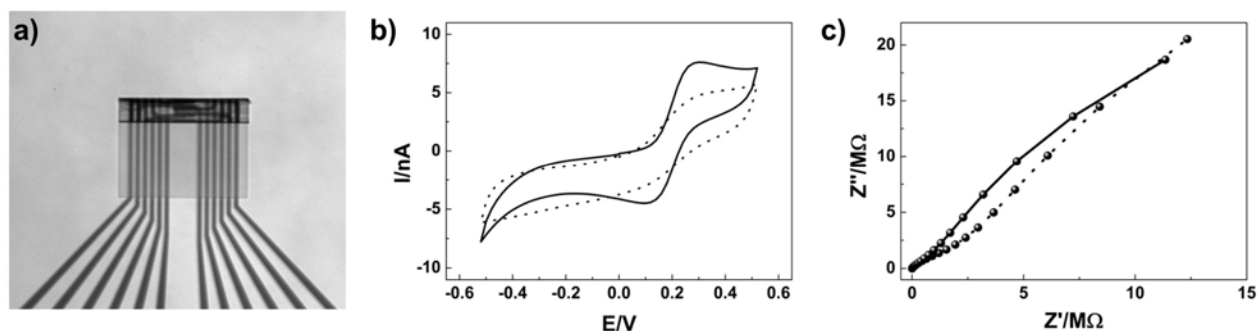


Figure 1: a) Tubular μ EIT structure. b) Cyclic voltammetry and c) Nyquist plot obtained with (dotted lines) and without (solid lines) particle inside the μ EIT device.

References:

- [1] T. Sun, S. Tsuda, K.-P. Zauner and H. Morgan, *Biosens. Bioelectron.* 25 (2010) 1109–1115.
- [2] T.A. York, T.N. Phua, L. Reichelt, A. Pawlowski and R. Kneer, *Meas. Sci. Technol.* 17 (2006) 2119–2129.
- [3] Y. Mei, G. Huang, A.A. Solovev, E.B. Ureña, I. Mönch, F. Ding, T. Reindl, R.K.Y. Fu, P.K. Chu and O.G. Schmidt, *Adv. Mater.* 20 (2008) 4085–4090.

Impedimetric monitoring of neural stem cell differentiation

H.-G. Jahnke, D. Seidel and A.A. Robitzki

Center for Biotechnology and Biomedicine (BBZ), Universität Leipzig, Germany

heinz-georg.jahnke@bbz.uni-leipzig.de

According to the needs of assays and screenings, human pluripotent stem cells are a unique source for functional human cell types. Especially human neural stem cell-derived neuronal networks represent the only accessible in vitro model that resembles a functional primary human phenotype. However, differentiation and maturation of these cells are a very complex and time-consuming process. In this context, non-invasive label-free techniques to monitor the neuronal differentiation and maturation of human stem cells are highly demanded. Our aim was to demonstrate the successful use of impedance spectroscopy for the sensitive label-free monitoring of the complete differentiation process from progenitor cells to neuronal networks.

Therefore, we used optimized high-dense microelectrode arrays (MEAs) in combination with high precision impedance analyzers to identify specific impedimetric characteristics for the neuronal differentiation process [1]. Moreover, we performed detailed correlative molecularbiological analyses to validate the identified impedimetric parameters. First, we identified the optimum MEA to analyze the differentiation of reference human neural stem/progenitor cell models by impedance spectroscopy. In this context, we needed to optimize the surface of the MEAs for achieving a long-term stable cell-electrode interface. Afterwards, we identified distinct cell dependent impedimetric parameters that could specifically be associated with the progress and quality of neuronal differentiation. Applying an adapted equivalent circuit model, we were able to understand the identified empiric impedimetric parameters. For validation, we analyzed the expression of progenitor versus mature neural marker and typical structural changes. The correlation analysis revealed a strong predictive power of the identified impedimetric parameters. More strikingly, we could demonstrate the capability of our impedimetric differentiation monitoring system to identify neuronal differentiation accelerating compounds.

For the first time, we proved that non-invasive impedance spectroscopy can be used for the specific quantitative monitoring of neuronal differentiation processes. Therefore, this technique will be a useful tool for quality control of neuronal differentiation, neurogenic compound identification and moreover, high-content screening demands in the field of safety assessment as well as drug development.

References:

- [1] D. Seidel, J. Obendorf, B. Englich, H.-G. Jahnke, V. Semkova, S. Haupt., M. Girard, M. Peschanski., O. Brüstle, A.A. Robitzki, *Biosens. & Bioelec.*, doi:10.1016/j.bios.2016.06.056

Impedance Analysis of TGF- β induced Fibroblast-Myofibroblast Transition

K. Hofmann¹; W. Zwickenspflug¹, Th. Gudermann¹, M. Königshoff² and A. Dietrich¹

¹Walther-Straub-Institut für Pharmakologie und Toxikologie, Mitglied des Deutschen Zentrums für Lungenforschung (DZL), Ludwig-Maximilians-Universität München (Germany)

²Comprehensive Pulmonology Center (CPC), Member of the DZL, München (Germany)

Alexander.Dietrich@lrz.uni-muenchen.de

Pulmonary fibrosis (PF) is a progressive lung disease ultimately leading to death with no effective therapeutic options. Next to other unknown factors PF may be induced by chronic exposure to toxic drugs like the cytostatic bleomycin during tumor chemotherapy. One of the pathological features of PF is the formation of myofibroblast foci and the deposition of extracellular matrix (ECM) e.g. collagen secretion. Many different cell types are suggested to transdifferentiate into these activated myofibroblasts e.g. alveolar epithelial cells type I and II (AEC type I and II), resident fibroblasts and peripheral fibrocytes [1]. Although the mechanism of PF is not fully understood, the profibrotic transforming growth factor β (TGF- β) is considered to play a crucial role. TRPC6 is an unselective cation channel highly expressed in different lung tissues which might contribute to pulmonary fibrosis because the protein plays an important role in myofibroblast transdifferentiation and wound healing in cardiac and dermal fibroblasts [2]. Moreover, TRPC6 is responsible for increased vascular permeability in lungs [3] which might help circulating fibrocytes to migrate to the injured areas during the development of the fibroproliferative foci. To study a potential role of TRPC6 in PF we analyzed life span, lung function and collagen expression in wild-type (WT) and TRPC6-deficient (*Trpc6*^{-/-}) lungs utilizing a bleomycin-induced PF-model. WT mice died more frequently than TRPC6-deficient mice during the fibrotic phase from day 7 after application of bleomycin, most probably due to severe changes in lung function which was similar to control mice in *Trpc6*^{-/-}-mice. Moreover, collagen accumulation in bleomycin-treated TRPC6-deficient lungs was indistinguishable to PBS-treated control animals, while WT mice treated with bleomycin showed increased collagen levels. To analyze TRPC6 function on a cellular level, we isolated **primary murine lung fibroblasts (PMLFs)** from mice of both genotypes and incubated them with TGF- β . Only WT cells were able to increase TRPC6 expression after TGF- β treatment. Most interestingly, TGF β -induced fibroblast-myofibroblast transition detected by electrical cell impedance sensing (ECIS) and contraction of a collagen matrix were significantly reduced in TRPC6-deficient PMLFs compared to WT cells. Therefore, defining TRPC6 function might help to identify pharmacological targets for new therapeutic options in PF.

References:

- [1] King Jr., et al. *Lancet* (2001)
- [2] Davis, et al. *Dev. Cell* 23 (2012) 705-715.
- [3] N. Weissmann, et al. *Nat. Commun.* 3 (2012) 649.

Use of cross-linked polypeptide multilayer-coated electrodes to monitor cardiac differentiation of human mesenchymal stem cells

S. Gomez, Y.-R. Chen, C.-C. Hsu and C.-M. Lo

Department of Biomedical Engineering, National Yang-Ming University, Taipei (Taiwan)

sofiag594@hotmail.com

Polyelectrolyte multilayer film is an emerging method for substrate coating to control stem cell proliferation and differentiation [1]. This material allows for modulation of thickness, stiffness, and adhesiveness [2,3]. In this study, human mesenchymal stem cells (hMSCs) were cultured on polypeptide multilayer films fabricated by the alternate deposition of cationic poly-L-lysine and anionic poly-L-glutamic acid, cross-linked with EDC/sulfo-NHS, and coated with gelatin. The characteristics of film thickness, roughness, Young's modulus, and adsorbed film mass were measured by AFM and QCM. Cardiac differentiation was evaluated by RT-PCR analysis and immunostaining of cardiac markers, and monitored by cross-linked polypeptide multilayer-coated electrodes using electric cell substrate impedance sensing (ECIS). The results showed that thicker native films exhibited lower Young's moduli than thinner native films, whereas the Young's moduli of cross-linked films was much larger than native films. Furthermore, hMSCs demonstrated better cell attachment and spreading on even number layer of films, where the top layers are poly-L-glutamic acid films. The cardiac differentiation of hMSCs showed a significantly higher differentiation rate on cross-linked films than on culture dishes or cover glasses. Our observations indicate a possible way to facilitate hMSCs toward cardiac differentiation using cross-linked polypeptide multilayer films.

In the ECIS monitoring of stem cell differentiation, hMSCs were seeded into electrode wells and allowed to form confluent cell layers, and the impedance time courses of the cell-covered electrodes throughout the induction towards cardiomyocytes were measured up to 10 days at 11 frequencies ranging from 62.5 Hz to 64 kHz [4]. Compared with the data of undifferentiated hMSCs, significantly lower impedance time courses of hMSCs treated with cardiac differentiation medium were observed. In addition, these two distinct time course profiles were detected as early as a few hours after induction and lasted for 10 days. To detect subtle changes in cell morphology in the early stage of cardiac differentiation, the frequency-dependent impedance data were analyzed with a theoretical cell-electrode model [5]. The results showed that the decrease of measured impedance of differentiated cells was mainly due to a decrease of the junctional resistance between cells (R_b) and an increase of the cell-substrate separation (h). We also analyzed the impedance changes between the differentiated groups with the cross-linked polypeptide multilayer film and without it. The results showed that hMSC differentiation towards cardiomyocytes is augmented when cross-linked polypeptide multilayer films were used.

References:

- [1] G. Decher, *Science* 277 (1997) 1232-1237.
- [2] L. Richert, A. J. Engler, D. E. Discher and C. Picart, *Biomacromolecules* 5 (2004) 1908-1916.
- [3] T. Boudou, T. Crouzier, K. Ren, G. Blin and C. Picart, *Advanced Materials* 22 (2010) 441-467.
- [4] P.O. Bagnaninchi and N. Drummond, *Proc. Natl. Acad. Sci. USA* 108 (2011) 6462-6467.
- [5] I. Giaever and C.R. Keese, *Proc. Natl. Acad. Sci. USA* 88 (1991) 7896-7900.

Investigations addressing the phototoxicity of bioanalytically relevant fluorophores and fluorescent particles using impedance analysis

R. Freund and J. Wegener

Universität Regensburg, Regensburg (Germany)

romy.freund@chemie.uni-regensburg.de

Fluorescent indicator dyes are of outstanding importance in analytical studies in general and in biomedical research in particular. By means of appropriate fluorescent dyes it is possible to analyze cell vitality, intracellular calcium levels as well as the intracellular pH. If these fluorescence-based investigations on living cells are not performed in terms of an end-point assay, but in a time-resolved manner with several subsequent readouts, phototoxicity of the indicators must not be neglected.

The cell-permeable fluorescent dye calcein AM is one of these dyes which are commonly used as a *vital stain* to determine viability of eukaryotic cells. In 2003 Knight *et al.* reported substantial phototoxicity of intracellular, photo-excited calcein during the investigation of chondrocytes [1]. Upon illumination within the light path of an optical microscope calcein was found to instantly produce reactive oxygen species (ROS). These, however, had a noticeable impact on cell physiology [2] which leads to the conclusion that subsequent readouts are at least questionable.

In this work the phototoxicity of bioanalytically relevant fluorophores as well as of fluorescent particles was investigated using label-free, impedance-based cellular assays. Appropriate electrode layouts made from indium tin oxide (ITO) were developed in order to allow for a defined illumination of the cells on the electrodes using an inverse microscope in parallel to time-resolved impedance readings. Cells were pre-incubated with organic dyes (e.g. calcein), probes (e.g. mitotracker) or particles (e.g. fluorescently-labeled latex beads) before light exposure was started. Unstained cells served as controls. Phototoxicity was systematically studied by varying the excitation wavelength, the duration of excitation as well as different incubation conditions. The effect of this exposure was monitored continuously via impedance-based monitoring. Finally, cytochemical LIVE/DEAD assays were applied to validate the label-free impedance measurements. The ROS-level induced by the excited fluorophores upon illumination was determined by ROS-detection assays.

References:

- [1] M.M. Knight, S.R. Roberts, D.A. Lee, D.L. Bader, *Am. J. Physiol. Cell Physiol.* 284 (2003) C1083-C1089.
- [2] P. Mroz, A. Yaroslavsky, G.B. Kharkwal, M.R. Hamblin, *Cancers* 3/2 (2011) 2516-2539.

Madin-Darby Canine Kidney Epithelial Cell Barriers are regulated by Transient Receptor Potential A1 channels

S. Dembla, N. Hasan, A. Becker, A. Beck and S.E. Philipp

Experimentelle und Klinische Pharmakologie und Toxikologie, Universität des Saarlandes, D-66421 Homburg, Germany

stephan.philipp@uks.eu

TRPA1 proteins form Ca^{2+} permeable, non-selective cation channels, which act as chemical nociceptors. Recent studies provided strong evidence for non-neuronal functions of TRPA1 in endo- and epithelial cells. The aim of this study was to determine, whether TRPA1 plays a role in the modulation of epithelial cell barriers. For that purpose, we choose canine cells of the line MDCK II, which have been previously shown to respond to the TRPA1 agonist AITC and described as a model to study barrier functions. We analyzed the expression of TRPA1 in MDCK II cells, pharmacologically characterized TRPA1 mediated Ca^{2+} signals and ionic currents, and studied the contribution of TRPA1-promoted modulation of MDCK II cell monolayers. Using RT-PCR and northern blot analysis, we show that TRPA1 transcripts are expressed in MDCK II cells. Employing Ca^{2+} imaging, we demonstrate that TRPA1 channels are functionally active in MDCK II cells and contribute to Ca^{2+} influx after stimulation. MDCK II cells develop spontaneous, low amplitude currents with the typical current-voltage relationship of TRPA1. These currents were independent of basic intracellular Ca^{2+} , absent in the presence of the TRPA1 antagonist HC-030031 and could be further stimulated by AITC. Impedance spectroscopy revealed that AITC evoked an immediate reduction of the transepithelial resistance that was not detectable in the presence of HC-030031. Thus, we provide evidence that the tightness of MDCK II epithelial cell barriers is TRPA1-dependent and may be regulated via the opening of TRPA1 channels¹.

References:

- [1] S. Dembla; N. Hasan; A Becker; A. Beck and S.E. Philipp, *FEBS letters* 590 (2016) 1509-1520.

Impedimetric Detection of Transepithelial Water Transport

F. Urban, K. Hajek and J. Wegener

Universität Regensburg, Regensburg (Germany)

Florian.Urban@chemie.uni-regensburg.de

Transepithelial water flux is essential for water and energy homeostasis. Water can cross cell membranes by two pathways: (I) diffusion of water across the hydrophobic phospholipid membrane, (II) water transport through particular protein channels known as aquaporins [1]. Aquaporins play important roles in brain water balance, cell migration, cell proliferation, neural function, epidermal hydration and ocular function. Defective water transport across epithelial barriers is associated with several severe diseases [2].

Therefore, monitoring of transepithelial water flux and exploring potential pharmacological interference are crucial. Experimental assays to address and study transepithelial water transport *in vitro* are typically based on epithelial cell lines that are grown to confluence upon highly porous polycarbonate membranes (e.g. Transwell) defining an apical and basal compartment. A transepithelial water flux is then induced by adding a membrane impermeable solute such as sucrose to one compartment. The osmotically induced water flux is then quantified by capillary tubes connected to the apical compartment that measure changes in fluid height [3] or by means of fluorophore dilution [4].

A novel approach of detecting transepithelial water transport based on impedance measurements is described here. It also relies on porous culture substrates covered with a confluent monolayer of epithelial cells. The bottom compartment contains an electrode layout consisting of two coplanar gold-film electrodes of 500 μm diameter each that are spaced just 800 μm apart from each other. The cell-covered filter is placed directly on top of this electrode arrangement. A glass ring clued onto the electrode substrate defines the electrode well. These gold electrodes were fabricated by spin-coating and photolithography.

By applying an iso-resistive but hyperosmotic solution to the apical compartment, epithelial cells such as MDCK (Madin-Darby Canine Kidney) cells are exposed to a transepithelial osmotic pressure inducing transepithelial water flux from the basal to the apical compartment. As a consequence of water transport the ion concentration underneath the filter membrane increases. This loss of water from the basal compartment leads to a time-dependent decrease of the medium resistance R_{med} that mirrors the transport of water. The changes of R_{med} can be monitored at a frequency of 100 kHz with a time resolution better than 1 sec. This label-free impedimetric approach of detecting transepithelial water fluxes is easily coupled to regular TEER readings reporting on epithelial barrier function.

References:

- [1] A.S. Verkman, *J. Membr. Biol.* 173 (2000) 73-87.
- [2] A. de Almeida, G. Soveral and A. Casini, *Med. Chem. Commun.* 5 (2014) 1444-1453.
- [3] R.A. Dorr, A. Kierbel and J. Vera, *Comput. Methods Programs Biomed.* 53 (1997) 9-14.
- [4] M.H. Levin, S. Sullivan and D. Nielson, *J. Biol. Chem.* 281 (2006) 25803-25812.

Role of claudins in Tumor Necrosis Factor-induced permeability and migration changes in tubular epithelial cells

Y. Amoozadeh, S. Anwer, E. Branchard, Q. Dan and K. Szaszi

Keenan Research Center, St. Michael's Hospital and University of Toronto

szaszik@smh.ca

Tumor Necrosis Factor- α (TNF) is a key pathogenic factor in kidney disease. TNF alters tubular epithelial permeability and promotes migration but the underlying mechanisms are not well known. Using filter-based Electric Cell-substrate Impedance Sensing (ECIS) we demonstrate that TNF causes a biphasic transepithelial resistance (TER) change in LLC-PK1 tubular cells. This effect involves an early drop in TER followed by recovery (1-3h), and a late increase (>8h) [1]. In search for the underlying mechanisms, we found that TNF α alters the expression of several claudin (Cldn) family members. Claudins are tight junction proteins with unique properties. The combination of various claudin isoform expressed in a given cell determines paracellular permeability. Interestingly, TNF causes a biphasic change in Cldn-2 and 3 expression: an initial transient increase due to reduced degradation (1-3h) is followed by a decrease in mRNA and protein levels (>8h). Prolonged TNF treatment also increases claudin-1, 4 and 7 protein and mRNA levels. The initial increase in Cldn-2 levels is mediated by the RhoA, ERK and JNK pathways. ERK and JNK are also required for the late expression changes of Cldn-1, 4 and 7, and have a key role in the TNF-induced late TER increase. To correlate TNF-induced claudin expression and TER changes, we silenced each claudin and monitored TER using ECIS. Cldn-1 is necessary for the early TNF-induced TER change. In contrast, Cldn-2 decrease appears to be the main contributor to the late TER increase, with only minor roles for Cldn-1, 4 and 7.

Claudins have also been implicated in cell migration, and TNF also enhances epithelial cell migration. Therefore, we used the ECIS wound healing assay to assess the role of the various claudins in the TNF-induced increased migration. Silencing Cldn-1, 2 or 3 significantly slowed wound healing. Our ongoing studies explore the role of these claudins in migration-associated cytoskeleton remodeling. Thus, TNF-induced altered claudin expression may have consequences beyond permeability changes. By affecting epithelial regeneration following injury, altered claudin expression could contribute to the pathogenesis of kidney disease.

References:

- [1] Y. Amoozadeh, Q. Dan, J. Xiao, F. Waheed, K. Szászi, *Am. J. Physiol. Cell Physiol.* 309/1 (2015) C38-50 .

High-Throughput Single-Cell Identification and Monitoring of using CMOS MEA

C. Van Den Bulcke; A. Enciso Martinez and L. Chengxun

Imec, Belgium

Carl.VanDenBulcke@imec.be

The ability to analyze single cells is crucial for the advancement of cancer biology research. Even within a single tumor lesion, cells differ in both genotype and phenotype. Current techniques that are often performed on a large assembly of cells are not well suited to study important features occurring in small subpopulations or at single-cell level. Among many cell features, single cell impedance is a promising cell property for non-invasive cell characterization. Since single cell impedance spectroscopy is very challenging because of the very high impedance magnitude, we designed and tested a CMOS multielectrode array (MEA) is presented that is able to accurately

The device has approximately 100×100 individually addressable pairs of microelectrodes for massive single cell measurements. It was designed with the mixed signal 1.8V/5V 0.18 μm CMOS technology. Figure 1 shows the microscopic view of a single measurement unit, where a MCF-7 cancer cell is covering one of the electrode pairs on the MEA surface. The EIS circuit was designed to measure the cell membrane capacitance, which characterizes biological features of the cell. The small electrode ($3 \times 3 \mu\text{m}$) allows full coverage of the electrode pair by the same single cell despite the cell morphology. Accurate measurements on small capacitance ($\sim 10 \text{ fF}/\mu\text{m}^2$) were performed using the integrated transistors surrounding the electrodes. EIS on multiple cell lines showed distinct areal membrane capacitances for different cell types in agreement with values reported in research literature based on established techniques such as patch clamping.

We believe this integrated MEA device is an ideal platform for high throughput non-invasive single cell analysis. Using this device our research group was already able to distinguish different cell types based on their unique impedance fingerprint. Current research interest goes towards migration assays of different melanoma cells lines that show different phenotypes depending on their epigenetic state.

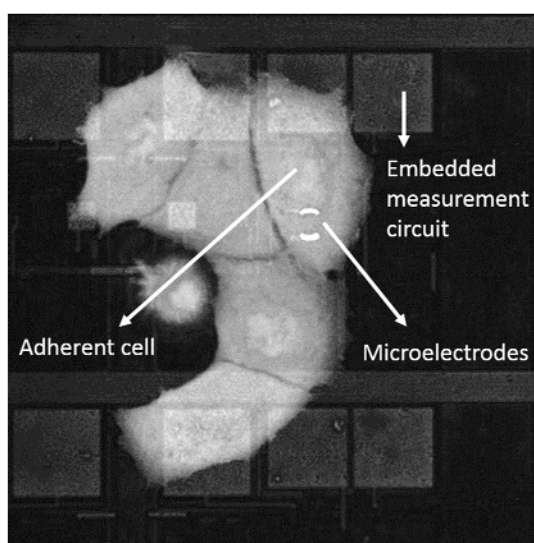


Figure 1: Cell covering microelectrodes on MEA

Impedance-Based Analysis of 3D Tissue Models

C. Hupf and J. Wegener

Universität Regensburg, Regensburg (Germany)

christina.hupf@chemie.uni-regensburg.de

Multicellular spheroids (MCS) are well-established non-vascularized tissue models in basic research, drug screening and as building blocks in tissue engineering. This is due to their biochemical, physicochemical and morphological characteristics that much more resemble the architecture of *in vivo* tissues.

Monitoring MCS commonly relies on endpoint assays, which are mostly invasive or microscopy techniques with the need for fluorescent labels. Impedance spectroscopy (IS) provides an alternative which allows for a non-invasive, label-free and time-resolved online monitoring of the spheroid response to certain stimuli.

For an impedimetric analysis of spheroids a novel setup was designed and tested in proof-of-concept studies. The setup features a flow channel with a central aperture (smaller than MCS diameter) which is mounted onto a planar indium-tin-oxide (ITO) electrode layout prepared from commercial ITO-coated PET foils using photolithography. Upon insertion of a spheroid into the flow channel it is held back at the central restriction and seals the channel like a cork on a bottle. Consequently, the electric sealing forces the current to go through the spheroid inducing a significant increase in impedance that is used for analysis.

The setup was tested by conducting measurements at multiple frequencies and at a single frequency with higher time resolution treating spheroids with substances whose effect on cells is well-known. The spheroidal response was then compared to the expectations. Since the flow channel as well as the electrode layout are transparent, the IS measurements were supported by information from digital and fluorescence microscopy. The preparation of the flow channel is cost-efficient and the channel can be cleaned easily and re-used several times. Furthermore, the chip design holds the potential for higher throughput, i.e. monitoring several spheroids in parallel. The flow-channel based setup shows an improved sensitivity compared to other devices for impedimetric MCS monitoring and can perform effect-based analysis of 3D tissue models.

Smart multimodal optoelectronic sensors for multiparametric tissue based real time impedimetric monitoring

A.A. Robitzki, H.-G. Jahnke, S. Erdmann, D. Seidel and D. Krinke

Universität Leipzig, Biotechnologisch-Biomedizinisches Zentrum, Leipzig (Germany)

andrea.robitzki@bbz.uni-leipzig.de

In the recent past three-dimensional organotypic cell aggregates are more and more of interest for toxicity and repeated dose toxicity assays or drug screening. The most advantage of organotypic tissues is the complexity of signaling as well as of cell-cell contacts and cell-extracellular matrix communication. Aspects like drug targeting, penetration, transport, internalization, and related changed physiology or morphology of the tissue are actually in the focus, and demand on fast and sensitive non-invasive, label-free recording and monitoring systems. Measuring cardiotoxicity [1], neurotoxicity/neurodegeneration [2] or chemo-sensitivity of tumors using 3D tissue models or biopsies [3] might be a challenge but is also a benefit because of a more physiological and comprehensive information concerning drug efficacy and efficiency.

This report will address the use of smart multimodal optoelectronic screening systems based on viable organotypic tissues for a non-invasive, real-time and online monitoring. The main topics address (i) the chemo-sensitivity of tumor biopsies, (ii) the metastasis of tumors, and (iii) the ability to distinguish different tumor types according their “*tumor fingerprint*”.

Electrochemical impedance spectroscopy (EIS) enables us to measure the changed physiology, morphology, and subcellular structure in real time in a frequency range of kHz to MHz on microcavity-electrode arrays. The frequency dependent complex resistance correlates directly with physiological, structural properties of viable cells and tissues. Herewith, multiparametric optoelectronic high content screenings of active chemo-pharmaceutical agents and biologics will be demonstrated e.g. concerning a fast, comprehensive, sensitive recording of drug efficacy in correlation with tumor drug sensitivity and/or resistance [3]. On the same technology platform impedimetric monitoring and *super resolution microscopy* can be carried out because of the use of optical transparent semi-conductive indium tin oxide electrodes on microarrays. Actually all of our developed and designed microelectrode arrays are extended to microtiterplate-electrode arrays [4] for industrial high content drug screening.

References:

- [1] H.-G. Jahnke, D. Steel, S. Fleischer, D. Seidel, R. Kurz, S. Vinz, K. Dahlenborg, P. Sartipy and A.A. Robitzki, *PLOS ONE* 8/7 (2013) e68971.
- [2] H.-G. Jahnke, A. Braesigk, T.G.A. Mack, S. Poenick, F. Striggow and A.A. Robitzki, *Biosens. Bioelectron.* 32 (2012) 250-258.
- [3] H.-G. Jahnke, S. Poenick, J. Maschke, M. Kendler, J.C. Simon and A.A. Robitzki, *Canc. Res.* 74/22 (2014) 6408-6418.
- [4] M. Eichler, H.-G. Jahnke, D. Krinke, A. Müller, S. Schmidt, R. Azendorf and A.A. Robitzki, *Biosens. Bioelectron.* 67 (2015) 582–589.

Impedance Analysis of Cells using Organic Electronics; focus on 3D cellular models

S. Inal, M. Ferro, A. Hama, V. Curto and R.M. Owens

Dept. of Bioelectronics, Ecole des Mines de St. Etienne, Gardanne (France)

owens@emse.fr

The development of electrical techniques for monitoring of biological phenomena is a field that is fast gathering pace. Advantages of electrical techniques are manifold, including the fact that they are label-free, and have the potential to be very efficient transducers, since the signal measured is already in an electrical readout format.

Electronic methods for live-cell sensing can be applied to applications involving extracellular recording of electrical activity from electrically active cells (neurons/myocytes), but also for monitoring of non-electrically active cells and tissue assemblies. Electrical impedance sensing (EIS) has emerged as a dynamic method, with demonstrated potential for use in monitoring barrier function, cellular adhesion, proliferation, and micro motion [1]. Until recently however, EIS has been restricted to monitoring of 2D cultures. We have successfully demonstrated the use of conducting polymer devices for monitoring a variety of *in vitro* models with the goal of developing physiologically relevant systems with integrated monitoring for use in diagnostics, toxicology or drug development [2,3,4]. CPs are 'smart materials' that have gained considerable attention of late for their use in interfacing with tissues *in vivo* and *in vitro* [3]. Mixed ionic/ electronic conduction, along with an ideal biocompatible surface and soft tissue-like mechanical properties, have contributed to the successful use of this material for integration with biological components. One key advantage of these materials is their amenability for processing in a variety of formats, including in 3D scaffolds [5]. A second major advantage of conducting polymers is their compatibility with high resolution imaging, allowing correlation of electrical data with time lapse imaging of the cells in real time [6]. In this presentation I will show recent work on the integration of 3D tissue models with conducting polymers to allow real time monitoring of cellular function.

References:

- [1] I. Giaever, C.R. Keese, *Proc. Natl. Acad. Sci. USA* 81 (1984) 3761.
- [2] M. Huerta, J. Rivnay, M. Ramuz, A. Hama, R.M. Owens, *Appl. Vitro Toxicol.* 2 (2016) 17.
- [3] X. Strakosas, M. Bongo, R. M. Owens, *J. Appl. Polym. Sci.* 132 (2015) 41735.
- [4] M. Huerta, J. Rivnay, M. Ramuz, A. Hama, R.M. Owens, *APL Mater.* 3 (2015) 30701.
- [5] A.M.-D. Wan, S. Inal, T. Williams, K. Wang, P. Leleux, L. Estevez, E.P. Giannelis, C. Fischbach, G.G. Malliaras, D. Gourdon, *J. Mater. Chem. B* 3 (2015) 5040.
- [6] M. Ramuz, A. Hama, J. Rivnay, P. Leleux, R.M. Owens, *J. Mater. Chem. B* 3 (2015) 5971.

Impedance Spectroscopy: A new tool to characterize reconstructed human epidermis

M. Schweinlin, L. Engelhardt, R. Seliger, F. Schmied, H. Walles, F. Groeber

GRK 2157, University of Würzburg, Würzburg (Germany)

Department of Tissue Engineering and Regenerative Medicine, University Hospital Würzburg, Würzburg (Germany)

Fraunhofer Translational Center Würzburg 'Regenerative Therapies in Oncology and Musculoskeletal Disease', Würzburg (Germany)

matthias.schweinlin@uni-wuerzburg.de

Despite advances in the development of *in-vitro*-tissue-models such as reconstructed human epidermis (RHE), the number of endpoints in toxicity-testing, which can be addressed with these models are limited. The analysis of the models are still dependent on invasive methods such as histological processing or 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) staining.

As an alternative for destructive methods, we established a non-destructive technology to analyze the integrity of the epidermal barrier based on impedance spectroscopy. RHE typically exhibits characteristic impedance spectra in a frequency ranging between 1 Hz and 100 kHz, which is comparable to the spectra of freshly isolated human epidermal biopsies. From these spectra, we extracted electrical parameters of the RHE such as the capacitance and the ohmic resistance. These parameters change significantly during epidermal differentiation and were used to quantify the effects of mechanical and chemical disruption of the epidermal integrity. Most relevant, impedance spectroscopy shows a sufficient sensitivity to detect a transient decreased ohmic resistance caused by 2-propanol, which is classified as a non-irritant by MTT assays. This result indicates that impedance spectroscopy can be employed as an additional method to assess mild irritative effects.

In our work we developed a non-invasive assay for analysis of tissue-models which is a vital requirement to increase the success of *in-vitro*-test-methods.

References:

- [1] F. Groeber, L. Engelhardt, J. Lange, S. Kurdyn, F.F. Schmid, C. Rücker, S. Mielke, H. Walles, J. Hansmann, ALTEX. 2016

Monitoring the Phototoxicity of Carbon Dots in 2D and 3D using Impedance-Based Cellular Assays

P.D. Pütz, R. Freund, C. Hupf and J. Wegener

Universität Regensburg, Regensburg (Germany)

pierre.puetz@chemie.uni-regensburg.de

Carbon Dots (Cdots) represent a relatively new allotropic form of carbon with interesting material properties such as photo-luminescence with high quantum yield and long-term photostability [1]. Easy to synthesize and moderately toxic up to concentrations in the mg/ml range [2], Cdots are enroute to their application in bioanalytical fields and even constitute a possible candidate for applications in photodynamic therapy.

One of the most interesting bioanalytical applications is their potential to serve as a viability reagent for animal cells. The nanoparticles do not penetrate the nuclear membrane in viable cells but reside in cytoplasmic vesicles. When cells are exposed to cytotoxic stressors, the particles accumulate in the nucleus. This re-localization is accompanied by a wavelength shift from blue to green fluorescence emission, rendering Cdots an interesting alternative for established live-dead cytotoxicity kit.

Another possible future application is photodynamic therapy (PDT), which has experienced a significant upturn since the nineties [3]. The underlying concept is the production of reactive oxygen species by photosensitizing chemical substances induced by radiation. The concept includes targeting of the tumor tissue and uptake of the photosensitizers before toxicity is triggered by light exposure. During the course of this work it was discovered that Cdots exert phototoxic effects not only on cell monolayers of different cell-lines but also on multicellular tumor spheroids (MCTS) when excited by near-ultraviolet light. The phototoxicity studies on MCTSs thereby bridge the gap between in-vitro studies on two-dimensional cell monolayers and animal testing.

In this work cell monolayers and multicellular tumor spheroids were exposed to Cdots and monitored by impedance analysis in the dark and after light exposure. The response of the cells in 2D and 3D models was recorded *in situ* immediately after exposure using specially designed electrode layouts. Impedance readings report on the toxicity profiles of the particles themselves and after photo-excitation.

References:

- [1] S. Sahu, B. Behera, T.K. Maiti, S. Mohapatra, *Chem. Commun.* 48 (2012) 8835–8837.
- [2] S.C. Ray, A. Saha, N.R. Jana, R. Sarkar, *J. Phys. Chem. C*, 113 (2009) 18546–18551.
- [3] T.J. Dougherty, C.J. Gomer, B.W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan, Q. Peng, *J. Natl. Cancer Inst.*, 90 (1998) 889–905.

Brain Endothelial Activation and Neuronal Damage

M.F. Stins^{1,2}, A. Tripathi², M. Motari¹, and D. Sullivan²

¹ Department of Neurology, Johns Hopkins School of Medicine

² Malaria Research Institute, Bloomberg School of Public Health, Baltimore MD, U.S.A.

mstins@jhmi.edu

Cerebral malaria (CM) is a serious complication of *Plasmodium falciparum* infection, especially in children, exposed non immune travelers and military personnel. Clinically, CM includes seizures, reversible coma and often death. Upon clearance of the infection, patients are often left with neurologic sequelae, such as seizures, learning and behavioral disorders. Recent data also show that ADHD can be linked to CM. Post malaria syndrome can also include psychotic or acute confusional episodes and tremor.

CM pathology is characterized by sequestration of Plasmodium infected erythrocytes (PRBC) to human blood brain barrier (BBB) endothelium without invasion into the brain. It is unclear as to how these PRBC that are confined to the brain vasculature induce neurological dysfunction. In CM, the BBB lies at the interface of the events occurring in blood and brain. The BBB is part of the neurovascular unit (NVU), a concept that emphasizes homeostatic interactions between its components to ensure optimal functioning of the central nervous system. It is hypothesized that activation of the BBB endothelium disturbs the homeostasis between the astro-glial and neuronal components of the NVU leading to neurological dysfunction.

Using an *in vitro* model for the human BBB, PRBC exposure increased ICAM-1 expression on the cells and decreased the integrity (barrier function) of monolayers in a dose dependent manner. Microarray and Gene Ontology (GO) analysis indicated a predominance of the NFκB mediated proinflammatory responses among the host signaling pathways. RT-PCR and protein analysis confirmed the increase in transcripts and release of cytokines and chemokines.

To assess whether BBB secretions could affect the brain, BBB models were constructed on TW inserts. Upon confluence, TW-BBB models were placed in the Cellscope and exposed to PRBC. Media was collected from the basal side of BBB models and added to astroneuronal cultures. These basal secretions caused dose-dependent abnormal astroneuronal morphology and cell death. Determination of the underlying pathogenesis of observed BBB activation and astroneuronal effects may lead to development of adjunctive neurotherapeutics to ameliorate neurologic sequelae.

References:

- [1] A.K. Tripathi, D.J. Sullivan and M.F. Stins *Infection and Immunity* 74/6 (2006) 3260-3270.
- [2] A.K. Tripathi, D.J. Sullivan and M.F. Stins, *Journal of Infectious Disease* 195/7 (2007) 942-950. Editorial commentary, JID Vol 195 (7)
- [3] A.K. Tripathi, W. Sha, V. Shulev, M.F. Stins, D.J. Sullivan, *Blood* 114/19 (2009) 4243-4252

Nanoparticle toxicity: cytotoxic and sub-cytotoxic measurements using xCELLigence real time cell analysis

M. McCorkel¹, C.L. Sherwood², N.G. Borrero^{2,3}, C. Zeng⁴, C.H. Nguyen⁴, J.A. Field⁴, R. Sierra-Alvarez⁴ and S. Boitano^{2,3}

¹Department of Biomedical Engineering, ²Asthma and Airway Disease Research Center,

³Department of Physiology, ⁴Department of Chemical and Environmental Engineering, University of Arizona (USA)

sboitano@email.arizona.edu

Despite the clear benefits of nanoparticles (NPs) in industry and everyday products, the understanding of nanoparticle toxicity has lagged, in part due to the sheer number of nanoparticles and their varied chemistries. We have used the impedance-based xCELLigence real time cellular analysis (RTCA) system as a high capacity screen to explore nanoparticle-induced cytotoxicity (i.e., cell death) on airway epithelial cells [1]. Because many of the compounds tested only showed cytotoxicity at relatively high concentrations, we used the RTCA to expand our nanotoxicity studies to explore the sub-cytotoxic effects of nanoparticles on airway epithelial cells. Following 24 hr exposure to hafnium oxide (HfO₂) concentrations that did not induce cytotoxic responses (i.e., < 2000 ppm), airway epithelial cells were exposed to ATP, a paracrine signaling molecule common to the airway, and their impedance response measured. We found a dose-dependent reduction in ATP signaling with HfO₂ concentrations as low as 50 ppm. Similar reductions in airway epithelial ATP signaling were observed in Ca²⁺ signaling experiments evaluated via digital imaging microscopy. Interestingly, cellular signaling toxicity experiments with non-nano sized HfO₂ suggested that the observed reduced signaling in airway epithelial cells was related to metals toxicity and not increased by nanomaterial chemistry.

In further experiments with RTCA we assayed synergistic toxicity effects of nanoparticles used in semi-conductor industries (e.g., gallium arsenide (GaAs) indium arsenide (InAs)) and associated nanomaterials used in chemical mechanical planarization (CMP) -- silica (SiO₂), ceria (CeO₂) and alumina (Al₂O₃). CMP processes, are characterized by a high water demand and thus can generate high volumes of effluents containing inorganic oxide NPs as well as nanoscale As particulates. Although GaAs and InAs NPs generated similar characteristic kinetic patterns, GaAs displayed much higher toxicity on airway epithelial cells (IC₅₀ ≈ 2.0 mg/L) than InAs (IC₅₀ ≈ 30 mg/L). We found that much of the cytotoxicity from these NPs could be explained by the dissolution of highly toxic arsenic. Similar to the HfO₂ studies above, sub-cytotoxic concentrations reduced cellular response to ATP. These results indicate that the release nanoscale GaAs, InAs and associated particulates in CMP effluents should be controlled due to the hazardous potential to humans and the environment.

References:

- [1] L. Otero-González, R. Sierra-Alvarez, S. Boitano and J.A. Field, *Environmental Science & Technology* 46 (2012) 10271-10278.

Combining ECIS with SPR for Multiparametric Cell Profiling

S. Michaelis, R. Robelek and J. Wegener

Universität Regensburg, Regensburg (Germany)

Stefanie.Michaelis@chemie.uni-regensburg.de

Non-invasive and label-free sensor technologies to monitor cell-based assays are receiving considerable interest in drug development and toxicity screening as these techniques provide an entirely unbiased, time-resolved detection of the holistic response of mammalian cells to chemical, biological or physical stimuli.

Two well-established techniques in this field are based on electrical impedance analysis (electric cell-substrate impedance sensing, ECIS) and optical sensing using evanescent waves, like surface plasmon resonance (SPR). ECIS and SPR have been successfully applied individually to study living cells [1, 2]. The SPR technique is based on the phenomenon of total internal reflection of an incident laser beam and the generation of an evanescent electric field along the growth substrate, which is affected by the refractive index within the first 100 – 200 nm perpendicular from the surface. Whenever the refractive index changes, the readout signal (reflectivity) changes. For cells grown on the SPR sensor surface, recorded changes in reflectivity and, thus, the refractive index, originate from dynamic mass redistribution induced inside the cell body or outside [3].

Combining ECIS and SPR technique on a single sensor chip provides a new means to monitor living animal cells simultaneously for electrical and optical changes in real time, label-free and non-invasively. This combination is advantageous since for both techniques cells are usually grown on thin gold-films which can serve as electrode and plasmonic substrate at the same time. The ECIS-SPR dual sensor platform is based on commercial gold-coated SPR chips which are processed by photolithography to provide a pair of electrodes for impedance readings (ECIS) and locally distinct gold areas for plasmon excitation and SPR detection on the same substrate.

This study shows proof-of-concept experiments using the dual ECIS-SPR sensor platform for different cell-based assays: (i) cell attachment and spreading onto the sensor surface as well as cell differentiation of initially suspended epithelial cells, (ii) manipulation of the mature confluent cell layer by pharmacological substances and (iii) stimulation of G-protein coupled receptors (GPCRs).

Simultaneous recordings do not interfere with each other but provide complementary, time-resolved information on cell shape changes and the dielectric structure of the entire cell body (ECIS) as well as dynamic mass redistribution close to the basal membrane within the sensitive layer 100 – 200 nm from the substrate surface (SPR) from one and the same cell population [3]. The ECIS-SPR dual sensor allows for a detailed, spatially-resolved, multiparametric cell profiling in a label-free manner, thus increasing the information depth of one-dimensional cell-based assays.

References:

- [1] J. Wegener, C.R. Keese and I. Giaever, *Experimental Cell Research* 259 (2000) 158-166.
- [2] R. Robelek and J. Wegener, *Biosensors and Bioelectronics* 25 (2010) 1221-1224.
- [3] S. Michaelis, J. Wegener and R. Robelek, *Biosensors and Bioelectronics* 49 (2013) 63-70.

Comparative study on the effectiveness of bortezomib in combination with alpha lipoic acid and thiamine in melanoma and myeloma cell lines

A. Takács¹, E. Lajkó¹, I. Istenes², L. Köhidai¹, O. Láng¹

¹ Impedimetry MERILL Core Facility/Chemotaxis Research Group, Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest (Hungary)

² 1st Department of Internal Medicine, Semmelweis University, Budapest, Hungary

angela.takacs1@gmail.com

Bortezomib is a proteasome inhibitor chemotherapeutic agent. So far it was utilized in the treatment of multiple myeloma; however, recent studies offer it as a new, promising drug also to treat melanoma [1]. Bortezomib-induced peripheral neuropathy (BIPN) is one of the most common and dose-limiting side-effects of bortezomib. The first line agents of BIPN treatment are antioxidants, e.g. alpha lipoic acid and thiamine [2].

The objective of our experiments was to verify the effects of bortezomib in adherent melanoma and in suspension growing myeloma cell lines. Furthermore, the influence and concentration course effects of the alpha lipoic acid and thiamine on the tumor growth inhibitory effect of bortezomib were also tested.

The model cells were WM35 primary, A2058 metastatic melanoma and U266 myeloma cell lines. Based on the therapeutic serum levels the tested concentrations were: (i) bortezomib: 20, 100 and 300 ng/mL; (ii) alpha lipoic acid: 10 and 100 µg/mL (iii) thiamine: 150 and 300 nM. A real time system of impedimetry (xCELLigence SP) was used to evaluate the cytotoxic effects elicited by bortezomib and the cell viability modulated by the antioxidants. Apoptotic effects were analyzed by flow cytometry after annexin V and phospho-p53 immunostaining. Propidium iodide was applied to perform the cell cycle analysis by flow cytometry.

Our results show that bortezomib acts as a cytotoxic substance both in myeloma cells growing in suspension and in adherent melanoma cell lines (IC₅₀: WM35 – 2.1×10^{-7} M, A2058 – 1.6×10^{-7} M). The significant diversity was recorded in WM35 cells where the lowest concentration of bortezomib killed the highest ratio of the cells. The bortezomib proved to be apoptosis inducer in case of all model cells and arrested the cell cycle of melanoma cells in G2/M phase.

Thiamine was barely cytotoxic only on myeloma cell line; nevertheless, in combination with bortezomib (100 and 300 ng/mL) both concentrations of thiamine reversed the cytotoxic effect. However, on A2058 cells thiamine was an enhancer of the cytotoxic effect of 20 and 300 ng/mL bortezomib.

Alpha lipoic acid did not reduce significantly the cell number of melanoma cell lines, but it had an effective cytotoxic effect on myeloma cells. Alpha lipoic acid had a restrictive effect on the anti-tumor actions of bortezomib both on melanoma and myeloma cells. This character of alpha lipoic acid was more pronounced in case of higher concentrations of bortezomib.

None of the tested antioxidants had apoptotic effect in all model cells and decreased the number of the annexin positive cells induced by 300 ng/mL bortezomib.

In conclusion, decreasing effects of alpha lipoic acid and thiamine on cytotoxicity of bortezomib were proved to depend on the concentrations and the cell type. Based on our results the combination therapy of bortezomib and low dose of antioxidants could be offered for the treatment of melanoma, while in case of multiple myeloma it appeared to be avoided.

References:

- [1] M.A. Shahshahan, M.N. Beckley, A.R. Jazirehi. *Am. J. Cancer Res.* 1 (2011) 913-24.
- [2] B. Mohty, J. El-Cheikh, I. Yakoub-Agha et al. *Haematologica*, 95 (2010) 311-19.

Sponsors' Presentations

Impedance Toxicity Screening of Cardiomyocytes and Hepatocytes

K. Juhasz^{1,2}, S. Quixtner¹, S. Stölzle-Feix¹, C. Bot³, N. Becker¹, U. Thomas¹,
L. Doerr¹, M. Beckler¹, M. George¹, N. Fertig¹

¹Nanon Technologies, Munich, Germany, ²Technical University of Munich, Munich (Germany),

³Nanon Technologies Inc., Livingston, NJ, 07039 (USA)

Gesa.Rascher-Eggstein@nanion.de

The CardioExcyte 96 system provides a non-invasive, label-free, high temporal resolution approach for safety screening on iPSCMs [1]. It is a hybrid screening instrument that combines impedance with MEA-like extracellular field potential (EFP) recordings [2,3]. It can be either used in an incubator or directly on a lab bench. The system is capable of electrically pacing the cells, allowing screening of cells which beat with individual frequencies and investigations of frequency dependent compound inhibition.

We describe the development and optimization of cell-based assays that is sensitive and provides reproducible results for safety pharmacology, toxicity screens of proliferating cells and hepatotoxicity. Changes in the impedance signal indicate effects on cell contractility, cell shape and proliferation, whereas the field potential parameters provide information about the electrophysiological activity of the beating network of cells.

We have shown combined impedance and EFP measurements using the commercially available platform CardioExcyte 96 can be performed reliably from a number of different hiPSC-Cardiomyocytes including Cor.4U (Axiogenesis AG), iCell Cardiomyocytes2 (Cellular Dynamics International), Pluricytes (Pluriomics) and Cellartis® hiPS-CM (Takara Bio Europe AB). These cells are becoming increasingly important for cardiac safety testing. The CardioExcyte 96 is, so far, the only platform on the market capable of recording in combination impedance and EFP measurements from same cell. This provides a unique opportunity to detect changes in both contractility and ion channel function at a high throughput which may prove crucial for cardiac safety screening particularly in the light of the new CiPA guidelines.

Table 1: Changes of human-induced pluripotent stem cell-derived cardiomyocytes (Cor.4U) beating pattern measured by impedance and extracellular field potential methods.

Compound		Impedance				EFP				Effect
		Amp	Rate	PW50	BRRI	Amp	Rate	FPD	BRRI	
Dofetilide	10 nM									hERG blocker
Cisapride	1 µM									hERG blocker
E-4031	30 nM									hERG blocker
Sotalol	1 µM									hERG blocker
Astemizole	1 nM									hERG blocker
Quinidine	1 µM									mixed blocker
Terfenadine	1 µM									hERG, Nav blocker
Isoproterenol	1 µM									β-adrenergic agonist
Nifedipine	300 nM									Cav blocker
BAYK8644	1 nM									Cav activator

decrease
 increase

References:

- [1] L. Doerr, U. Thomas, D.R. Guinot, C.T. Bot, S. Stoelzle-Feix, M. Beckler, M. George, N. Fertig, *J. Lab. Autom.* 20/2 (2015) 175–188.
- [2] I. Giaever, C.R. Keese, *Proc. Natl. Acad. Sci. U.S.A.* 88/17 (1991) 7896–7900.
- [3] M. Clements, N. Thomas, *Toxicol. Sci.* 140/2 (2014) 445–461.

List of Posters

P01	Kozłowska, A.	Investigation of the Effects of the Cholestatic Drug Chlorpromazine in Human HepaRG cells using ECIS
P02	Bryans, A.	AMAP, the putative 'non-toxic' isomer of acetaminophen, is shown to be toxic in primary human hepatocytes using ECIS
P03	Lieb, S.	Label-Free Analysis of GPCR-Stimulation: The Critical Impact of Cell Adhesion
P04	Michaelis, S.	Impedance-Based Theranostics in Tumor Research
P05	Selmi, A.	Measurement of Endothelial Permeability based on the Electric Cell-substrate Impedance Sensing (ECIS) method
P06	Thoms, S.	Impedance Sensing of HUVECs after Tacrolimus Treatment
P07	Küppers, V.	Using a TEER-based screen to find novel mechanisms that regulate retinal endothelial barrier function
P08	Hajek, K.	Independent observation of two co-cultured cell layers grown on filter supports
P09	Alexander, F.A.	An Automated Perfusion System for Monitoring Complex Transepithelial Electrical Resistance (TEER) of Membrane-bound Cultures
P10	Nielsen, D.S.G.	Administration of protein kinase d1 induce migration of intestinal epithelial cells <i>in vitro</i>
P11	Sauer, L.	Impedance Analysis of pH-dependent cell migration
P12	Ruckdäschel, S.	Quartz Crystal Microbalance as a Transducer in Cell-Based Assays
P13	Schmittlein, C	Combination of Impedimetric and Luminescent Readout for online Analysis of Adherent Cells
P14	Lai, Y-T.	Use of Hilbert-Huang Transform to Characterize Impedance Time Series of TGF- β Induced Cellular Activity of MDA-MB-231 Cells
P15	Tung, T.H.	Discrete Wavelet Transform Algorithms for Multi-Scale Analysis of Impedance Fluctuation Obtained from Cellular Micromotion
P16	Wiest, J.	Automated monitoring of impedance and extracellular acidification of fibroblasts in an eye irritation toxicity assay
P17	Zinkl, M.	Impedance based Analysis of Adherent Cells with interdigitated electrodes with subcellular dimension
P18	Böttcher, B.	A versatile lab-on-a-chip system for optical and impedance based cytotoxicity assays
P19	Götz, C.	PEDOT/PSS as transparent electrode material for impedance-based cell analysis

P20	Lajko, E.	Impedance-based Analysis and Holographic Phase Imaging of the GnRH-III-based Drug-targeting in Melanoma Cells
P21	Weiz, S.M.	Impedimetric microtomography in rolled-up tubes
P22	Janke, H.-G.	Impedimetric monitoring of neural stem cell differentiation
P23	Hofmann, K.	Impedance Analysis of TGF- β induced Fibroblast-Myofibroblast Transition
P24	Gomez, S.	Use of cross-linked polypeptide multilayer-coated electrodes to monitor cardiac differentiation of human mesenchymal stem cells
P25	Freund, R.	Investigations on phototoxicity of bioanalytically relevant fluorophores and fluorescent particles using label-free Whole-Cell Biosensors
P26	Dembla, S.	Madin-Darby Canine Kidney Epithelial Cell Barriers are regulated by Transient Receptor Potential A1 channels
P27	Urban, F.	Impedimetric Detection of Transepithelial Water Transport
P28	Amoozadeh, Y.	Role of claudins in Tumor Necrosis Factor-induced permeability and migration changes in tubular epithelial cells
P29	van den Bulcke, C.	High-Throughput Single-Cell Identification and Monitoring of using CMOS MEA
P30	Hupf, C.	Impedance-Based Analysis of 3D Tissue Models
P31	Robitzki, A.	Smart multimodal optoelectronic sensors for multiparametric tissue based real time impedimetric monitoring
P32	Inal, S.	Impedance Analysis of Cells using Organic Electronics; focus on 3D cellular models
P33	Schweinlin, M.	Impedance Spectroscopy: A new tool to characterize reconstructed human epidermis
P34	Pütz, P.	Monitoring the Phytotoxicity of Carbon Dots in 2D and 3D using Impedance-Based Cellular Assays
P35	Stins, M.F.	Brain Endothelial Activation and Neuronal Damage
P36	McCorkel, M.	Nanoparticle toxicity: cytotoxic and sub-cytotoxic measurements using xCELLigence real time cell analysis
P37	Michaelis, S.	Combining ECIS with SPR for Multiparametric Cell Profiling
P38	Takacs, A.	Comparative study on the effectiveness of bortezomib in combination with alpha lipoic acid and thiamine in melanoma and myeloma cell lines

Information for Participants

(1) Internet Access

A **guest account** for internet use is available.

Please use the following data for your login:

- ID: **conference**
- Passwort: **unirconf**

Moreover, **eduroam** is available in all university buildings

(2) Time table for bus Nr.6 to the center and from the main station to the university campus:

Please see the following pages

(3) Campus Map

see last page



Linie 6 **Wernerwerkstraße - Westbad - Bismarckplatz -** **HBF/Albertstraße - Otto-Hahn-Straße - Klinikum**

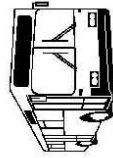
gültig von 01.08. bis vrsl. 26.08.2016

Umleitung über Am BioPark wegen Sperrung der Universitätsstraße stadtauswärts (01.08. bis vrsl. 26.08.2016)

Die Haltestellen Universität und An der Kreuzbreite können nicht angefahren werden.

Umleitung über Altstadtumdümmung wegen Sperrung des St.-Georgen-Platzes (01.08. bis 30.09.2016)

Montag - Freitag	5	6	7-18	19	20-22	23	0
Wernerwerkstraße	17 37 56	16 31 46	01 16 31 46	01 16 39	09 39	09 39	
Westbad	18 38 57	17 32 47	02 17 32 47	02 17 40	10 40	10 40	
Westheim	19 39 58	18 33 48	03 18 33 48	03 18 41	11 41	11 41	
Weinweg	20 40 59	19 34 49	04 19 34 49	04 19 42	12 42	12 42	
Theresienweg	21 41	00 20 35 50	05 20 35 50	05 20 43	13 43	13 43	
Boessnerstraße	22 42	01 21 36 51	06 21 36 51	06 21 44	14 44	14 44	
Josef-Adler-Straße	23 43	02 22 37 52	07 22 37 52	07 22 45	15 45	15 45	
Albertus-Magnus-Gym.	24 44	03 23 38 53	08 23 38 53	08 23 46	16 46	16 46	
Gumpelzheimerstraße	25 45	04 24 39 54	09 24 39 54	09 24 47	17 47	17 47	
Ostdeutsche Galerie	27 47	06 26 41 56	11 26 41 56	11 26 49	19 49	19 49	
Bismarckplatz	29 49	09 29 44 59	14 29 44 59	14 29 51	21 51	21 51	
Justizgebäude	30 50	11 31 46	01 16 31 46	01 16 31 52	22 52	22 52	
Bahnhofstraße	31 51	13 33 48	03 18 33 48	03 18 33 53	23 53	23 53	
Hauptbahnhof	32 52	14 34 49	04 19 34 49	04 19 34 54	24 54	24 54	
HBF/Albertstraße an	35 55	17 37 52	07 22 37 52	07 22 37 57	27 57	27 57	
HBF/Albertstraße ab	38 58	18 38 53	08 23 38 53	08 23 38 00	30 00	30 00	
HBF Süd/Arcaden	39 59	19 39 54	09 24 39 54	09 24 39 01	31 01	31 01	
Haydnstraße	40 00	20 40 55	10 25 40 55	10 25 40 02	32 02	32 02	
TechCampus/OTH	41 01	21 41 56	11 26 41 56	11 26 41 03	33 03	33 03	
Otto-Hahn-Straße	42 02	22 42 57	12 27 42 57	12 27 42 04	34 04	34 04	
Neuprüll	46 06	27 47	02 17 32 47	02 17 32 47 08	38 08	38 08	
Klinikum West	48 08	29 49	04 19 34 49	04 19 34 49 10	40 10	40 10	
Klinikum	50 10	31 51	06 21 36 51	06 21 36 51 12	42 12	42 12	



Linie 6 **Klinikum - Universität - HBF/Albertstraße - Bismarckplatz - Westbad - Wernerwerkstraße**

Umleitung über Altstadt südumgehung wegen Sperrung des St.-Georgen-Platzes (01.08. bis 30.09.2016)

Montag - Freitag	5	6	7-18	19	20	21-23	0
Klinikum	37 57	15 30 45	00 15 30 45	00 17 32 47	17 47	17 47	
Neuprüll	38 58	16 31 46	01 16 31 46	01 18 33 48	18 48	18 48	
An der Kreuzbreite	39 59	17 32 47	02 17 32 47	02 19 34 49	19 49	19 49	
Universität	40 00	19 34 49	04 19 34 49	04 21 36 51	21 50	20 50	
Otto-Hahn-Straße	42 02	21 36 51	06 21 36 51	06 23 38 53	23 52	22 52	
TechCampus/OTH	43 03	22 37 52	07 22 37 52	07 24 39 54	24 53	23 53	
Haydnstraße	44 04	23 38 53	08 23 38 53	08 25 40 55	25 54	24 54	
HBF Süd/Arcaden	45 05	25 40 55	10 25 40 55	10 27 42 57	27 55	25 55	
HBF/Albertstraße an	47 07	27 42 57	12 27 42 57	12 29 44 59	29 57	27 57	
HBF/Albertstraße ab	48 08	28 43 58	13 28 43 58	13 30 45 00	30 00	30 00	
Justizgebäude	50 10	30 45	00 15 30 45	00 15 32 47	02 32	02 32	
Bismarckplatz	52 12	32 47	02 17 32 47	02 17 34 49	04 34	04 34	
Ostdeutsche Galerie	54 14	35 50	05 20 35 50	05 20 37 52	07 37	05 35	
Gumpelzhaimerstraße	55 15	36 51	06 21 36 51	06 21 38 53	08 38	06 36	
Albertus-Magnus-Gym.	56 16	37 52	07 22 37 52	07 22 39 54	09 39	07 37	
Josef-Adler-Straße	57 17	38 53	08 23 38 53	08 23 40 55	10 40	08 38	
Boessnerstraße	58 18	40 55	10 25 40 55	10 25 42 57	12 42	09 39	
Weinweg	59 19	41 56	11 26 41 56	11 26 43 58	13 43	10 40	
Westheim	00 20	42 57	12 27 42 57	12 27 44 59	14 44	11 41	
Westbad	02 22	44 59	14 29 44 59	14 29 46 01	16 46	13 43	
Wernerwerkstraße	04 24	46 01	16 31 46 01	16 31 48 03	18 48	15 45	

Universität Regensburg General Map

Sources: Digital Data of
State Office for
Building and Construction
Design: Referat V/4, February 2016

