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Electric field simulation assisted electrostatic fibre formation and characterisation of the fibre matrices

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Viktor Gergő Szilágyi

Medical Biotechnology MSc.

Supervisors: Dr. Ákos György Juhász, Dr. Angéla Jedlovszky-Hajdú

Semmelweis University

Department of Biophysics and Radiation Biology

Laboratory of Nanochemistry

Internal consultant: Dr. Kristóf Iván

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Abstract

Electrospinning is a widely investigated and used non-mechanical technology that can be used to produce nano and microfibres. These fibre structures have wide applicability in medicine, pharmaceutical and in engineering. For medical purposes, these structures are widely researched because they mimic the extracellular matrix thus making them an excellent candidate to be used in tissue engineering applications. Outside of the applications in tissue engineering, these fibre matrices are also excellent drug delivery systems because of the known dissolution rate, therefore the dissolution rate of their delivered pharmacological substance. In medicinal applications, they are also excellent candidates for antibacterial wound dressing where they deliver metal nanoparticles, antibiotics, or other antimicrobial materials. The nanofibre matrices also have applications outside of medicine, especially in filtration systems, new generational energy storage devices and in different biosensor systems.

The applications of the fibre structures are dependent on different physical and chemical properties, the chemical applications are dependent on the solution conditions especially the solvent and the polymer that is used for the electrospinning. The physical properties are dependent on the machine and on the ambient parameters during the electrospinning. The focus of my presentation will be the mesh modelling of the electric field during the electrospinning and the effect of the added CaCl_2 on the created polymer fibre matrix.

The electric field modelling can give us a better understanding of the whole electrospinning process especially of polymer deposition on the different collector geometries. These simulations can also lead to create better collector or needle geometries that can help to

The physical and chemical parameters of the fibre structure also depend on the chemical components of the electrospinning solution. During my research I investigated the effect of different CaCl_2 concentrations in the polysuccinimide polymer solution. The inorganic salt content can affect the diameter of the fibre strands and can influence the cytotoxicity of the fibre structure.

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Abbreviations

PSI - polysuccinimide

PASP - Poly(aspartic acid)

DMF - dimethylformamide

SEM - Scanning electron microscopy

FT-IR – Fourier transformation infrared spectroscopy

Electrospinning - electrostatic fibre formation

DC – Direct current

3D – Three-dimensional

PLA – Poly (lactic acid)

1 Introduction

Electrospinning is the most versatile method to create micro- and nanoscale fibres. These continuous fibre structures have potential applications in medicine, in pharmaceuticals and in engineering. My main interest in this technology is the applications in tissue engineering where these fibre structures have the potential to act a scaffolding for cells therefore mimicking the extracellular matrix.

1.1 Regenerative medicine

Regenerative medicine is relatively new interdisciplinary approach in medicine that focuses on the replacement of damaged or diseased tissue in organs. To reestablish the function of the damaged organs or tissues regenerative medicine utilizes technologies like cellular therapies, tissue engineering strategies and artificial or biohybrid devices. The mentioned techniques typically rely on combinations of biological building blocks like cells or genes combined with bioengineered materials and technologies to heal the defects of the damaged organ or tissue. These techniques are usually used when the natural regenerative processes of the body are incapable to repair the damage and restore the function of the damaged tissue or organ[1], [2].

Regenerative medicine includes nuclear cloning, gene therapy, cell and stem cell therapy and tissue engineering as its main research and therapeutic routes. The main focus and purpose of these therapeutic treatment is either to introduce new function to the cells or to restore the function of a damaged cells, using different medical and engineering technologies.[3].

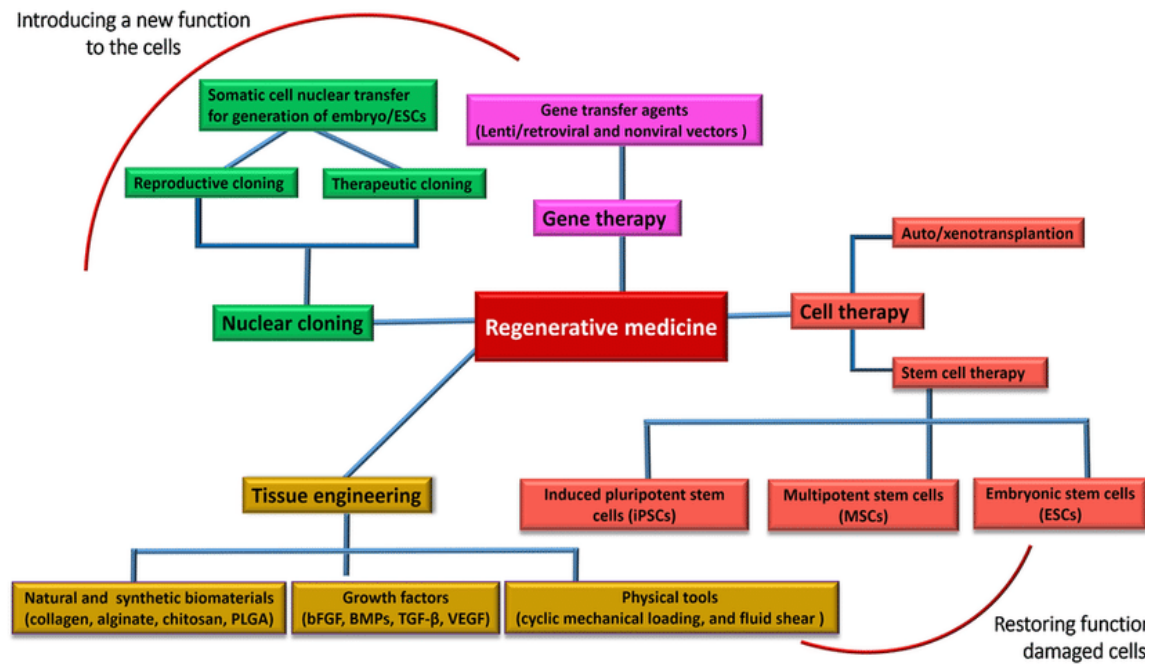


Figure 1: The research fields in Regenerative medicine [3]

1.1.1 Tissue engineering

Tissue engineering is a multidisciplinary approach in regenerative medicine that combines the principles of engineering and life sciences in order to develop biological substitutes to repair and restore the function of diseased or damaged organs and tissues. The main focus of tissue engineering is to use living cells, biomaterials, biochemical or physical factors and their combinations in order to create biomimetic tissue like structures. In tissue engineering applications the creation of the cellular microenvironment is essential to allow seed cells to be able to act their role thus ensuring the natural and effective regulation of cellular behaviour. [4].

Biomimetics is the field of tissue engineering in which the biological structures and events are deconstructed and then transferred and reconstructed to technological solutions. [5]. Biomimetics as a term was first introduced in 1957 and since it found its way not only to biomedical sciences but also to art, architecture, material and structural science and engineering. [6].

1.2 Polymers

Polymers are chain-structured macromolecules composed of repeating structural monomers held together with covalent bonds between each structure [7]. Polymers can have chain or branching structure depending on the monomer placements in the molecule. There

are two types of polymers depending on the source synthetic polymers and natural polymers. With synthetic polymers the most commonly used polymers are the petroleum-based polymers. These polymers can be tailored for various applications. These materials are non-biodegradable polymers which means they are resistant to aging and to biological degradation therefore they pose a serious environmental problem in terms of pollution after disposal, and most of these monomers are synthesised from fossil hydrocarbons therefore accelerating climate change [8].

Opposed to petroleum-based polymers biopolymers are extracted from naturally renewable resources including plants, arthropod shell, and silkworm cocoons from bioprocessing and chemical modifications. Most natural biopolymers are built from protein or polysaccharide monomers in chain structures. These natural biopolymers have characteristics such as renewable supply, low cost, biodegradability, antimicrobial activity, biocompatibility, renewability, nontoxicity, and absorption properties, these characteristics make them excellent materials for various applications [9] The most abundant and researched biopolymers are the cellulose from plants, chitosan from arthropod shells, alginate from algae, collagen and gelatine [10].

Polysuccinimide is a biopolymer synthesised from L-aspartic-acid with thermal polycondensation. PSI is the anhydrous form of PASP poly(aspartic acid), which is a biodegradable hydrophilic poly(amino acid). In biological environment PSI hydrolysis to PASP and then the dissolution of the amino acid form is relatively fast, therefore its use in the PSI form is limited without treatment the polymer to achieve cross-linking [11].

1.3 Nanotechnology in regenerative medicine

Nanotechnology is the science behind the design, synthesis, and applications of devices and materials that has the smallest organisation on at least 1 dimension is in the nanometre scale. Most of the nanoengineered materials and substrates are designed and manufactured specific physical and chemical properties. These specificities allow these materials and devices to interact with cells and tissues in a subcellular level thus allowing integration of these technologies with medicine and physiology [12]. With the recent developments in nanotechnology, it acquired a huge potential in regenerative medicine, since cell functions at the nanometre scale, nanotechnology can alter and influence of cellular behaviour. The traditional approaches of nanotechnologies in regenerative

medicine are related to: nanoparticles, scaffolds with nanofibres, drug and gene delivery, extracellular matrix patterning[13].

Nanoparticles are material particles that has the diameters in the nanoscale dimension that causes certain physical and chemical properties that makes them beneficial for a wide range of applications. In the medical field these nanoparticles are used as: controlled drug delivery systems, probing of DNA structures, gene delivery, biomolecular sensing and in tissue engineering. In addition to these applications the nanoparticles are also used in different the treatments of cancer, diabetes, allergy, infection and inflammation [14].

1.3.1 Electrostatic fibre formation

Electrostatic fibre formation or electrospinning is a technology that is widely used in tissue engineering which is part of regenerative medicine. This technique can be used to create fibres in nanometre or micrometre scale from polymer either dissolved in a solvent or in a molten state and applying high voltage. The fabricated nano- and microfibres have unique properties such as determined porosity and surface area [15].

As it is shown in *Figure 2*. the high voltage is applied to the capillary tube or needle holding the polymer solution and thereby applying charge to the surface of the polymer droplet, while the collector object is grounded. When the electric field overcomes the surface tension of the droplet the Taylor-cone is formed, and a charged jet of polymer is ejected towards the collector. While the polymer is jetted to the collector the polymer is solidified by the evaporation of the solvent or by cooling down, this process is subject to different forces that can stretch, direct, and speed up solidifying fibres [16], [17].

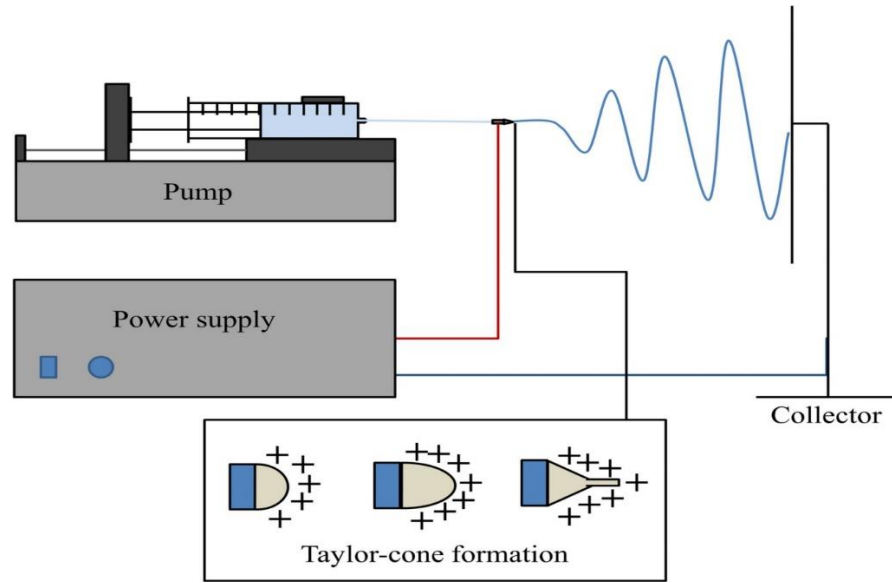


Figure 2: Schematic drawing of the single-needle electrospinning and of the Taylor-cone formation [18]

The physical and mechanical properties of these fibres can be influenced by several factors which are divided into three categories: machine parameters, material parameters and ambient parameters. Machine parameters are controlled before the fibre formation and are limited by the electrospinning apparatus such as the applied electric field, distance between needle and collector, flow rate and needle diameter. Polymer, polymer concentration, solvent type, viscosity and conductivity are the solution parameters. The ambient parameters are the relative humidity and the temperature. All these parameters have a huge impact on the final quality of the produced fibre structure [19], [20].

The effect of the machine parameters is critical for producing beadless and uniform fibre system. The machine parameters, depending on the polymer system that is used in the fibre formation, has an optimal range where the produced fibres are uniform and beadless. If these parameters are below the optimal range of the employed polymer system, the fibre formation will not occur especially it is true to the applied electric voltage because the Taylor-cone will not appear. If the machine parameters are above the optimal range the fibre formation will create ununiform and beaded fibres, the evaporation rate and deposition time will be insufficient, or in the case of flow rate there will be droplets that detach from the Taylor-cone therefore losing efficiency and depositable fibre mass.[21], [22].

The polymer parameters during electrospinning can highly affect the parameters of the produced fibre matrices. The electrospinning process is relying on the phenomenon of uniaxial stretching of the charged jet. The concentration of the polymer solution affects the

stretching of the charged jet, for example if the concentration is too low than the applied electric field and the surface tension will cause the polymer chain to fragment before fibre deposition on the collector, causing non-continuous or beaded fibre matrices [23]. The higher concentration beyond the optimal threshold of the polymer solution will increase the viscosity of the polymer solution and cause problems with the flow of the solution through the needle thus causing ununiform fibre formation [24]. The conductivity of the polymer solution also affects the characteristics of the produced fibre matrices. The increased conductivity of the solution tends to decrease the produced fibre diameter due to the increased carried electric charge in the jet. The increased electric charge increases the tensile strength in the electric field which affects the uniaxial charge of the polymer jet [25]. The most commonly used method to increase the conductivity of the polymer solution is the addition of inorganic salts. The increased conductivity and surface charge can also cause the formation of 3-dimensional fibre deposition on flat collector geometries [26].

The humidity as ambient parameter during the fibre formation can affect the diameter of the fibres and it will increase the standard deviation of the fibre diameters meaning the higher the humidity during the spinning the less uniform diameter of fibres will be created thus being an important parameter in the process [27]. The temperature plays an important role as it will typically decrease the viscosity of the polymer solution this affecting the stability and behaviour of the created jet thus affecting the diameter of the created fibres and as humidity also affects the variation in diameters [28].

1.3.2 Electric field modelling

The electric field plays a crucial role in the process of fibre production during electrospinning. The propagation of the electric field effects the jet trajectory, which determines the fibre alignment, morphology, location, and size of special fibre deposition, therefore the production of fibre matrices with desired macroscale properties can be achieved with the manipulation of this electric field between the needle and collector geometry [29]. The jet formed during the electrospinning follows the electric field current density lines thus the manipulation of the electric field is an effective method to control the jet path, and the fibre deposition are. The two common methods are the modification of the collector geometry for controlled fibre deposition, and the use of auxiliary electrodes between the needle and the collector. The modification of the collector geometries include cylinder, funnel, conveyor belt, ring collectors and collectors with parallel electrodes and

membranes. The manipulation of the electric field with auxiliary electrodes is an inexpensive and effective method. The use of these electrodes between the needle and collector geometry results in a more uniform electric field distribution and also increases the electric field density which results in an increased fibre strength and decreased average fibre diameter [30], [31].

1.3.3 Three-dimensional fibre structures

In general electrospinning produces two-dimensional fibre matrices which limits their applications in tissue engineering due to their small pore size. The main obstacle in engineering three-dimensional tissues is hypoxia caused by oxygen and nutrient diffusion limitations in thick scaffold materials. The diffusion limitations can be mitigated with three-dimensional fibre scaffolds due to their nano- and microscale fibre size and micro- to milli-scaled pore sizes [32]. The production approaches to produce these three-dimensional fibre structures which has at least centimetre in each direction, can be divided into two main approaches, direct preparation and post processing of the fibre meshes. The direct preparation approaches include multilayered electrospinning, the use of sacrificial agents, wet electrospinning and three-dimensional collector designs. In these direct preparation approaches the control of the electrospinning parameters especially the relative humidity, polymer solution additives like inorganic salts are crucial to successfully produce the three-dimensional fibre macrostructures. The post processing approaches include gas foaming, ultrasonication and short fibre assembly [26].

1.4 Biomedical applications of the nanofibre matrix

In medicine the nanofibre matrices produced by electrospinning are used as materials for cellular scaffolds, antibacterial wound dressing, drug delivery system, and in different biosensor applications.

1.4.1 Cellular scaffolds

The cellular scaffold in tissue engineering plays a crucial in the process of cell adhesion, growth and cellular proliferation. The effective scaffolds need to have the following characteristics: biocompatibility, successful cell adhesion, appropriate mechanical strength, ability mimic of extracellular matrix, appropriate functionality at the implantation site, sufficient durability for their applications [33]. These cellular scaffolds can be produced by numerous technologies that are constantly developed, the main

technologies are: melt-blowing, phase-separation and electrospinning. Out of these technologies electrospinning is one of the leading methods as it offers the ability to produce micro- to nanoscale fibres with broad surface area. The high surface area of these electrospun fibre scaffolds provide ample space for protein absorption and also promote binding sites for the cell adhesion which is one of the most crucial factor for successful tissue engineering application [34].

1.4.2 Antibacterial wound dressing

The electrospun nanofibre matrices has applications in the medical field beside the cellular scaffold application in tissue engineering. One of the most researched use cases is to use them as wound dressing material. The main purposes of these wound dresses are to accelerate the wound healing process and simultaneously to prevent and inhibit microbial infection and its spreading. The polymer matrixes that are used as wound dressing are embedded with materials that has the antimicrobial properties. These antimicrobial materials can be antibiotics, nanoparticles, cationic chemical compounds, biomaterials [35]. The other important property is the acceleration of the wound healing, this can be achieved with synthetic drugs, natural plant-based alternatives, and metal nanoparticles. Synthetic drugs in the acceleration of wound healing and also in antimicrobial effect are associated with drug resistance and adverse effects, therefore their long-term effectiveness might be limited. The use of natural plant-based compounds can be an excellent possibility however further investigations are necessary to identify the bioactive compounds in these extracts [36], [37].

2 Objectives

The aim of the work was to produce nano scale fibre matrices with electrospinning technology with simulate the electric field propagation during the production for adjusting the parameters of the electrospinning.

My project work can be divided into two main parts. The first part was the simulation work where I used QuickField computer program to simulate and visualise the electric field propagation during the electrostatic fibre formation. My second part was the laboratory work where I produced and characterised the polysuccinimide nanofibre matrices with the aim to determine whether the CaCl_2 containing PSI fibre matrices can be used as material for the development for electrospun antibacterial wound dressing. The main parts of my project were the following:

- Simulation work
 - Simulation of electric field propagation in base geometries for literature comparison
 - Simulation of the electric field during electrostatic fibre formation with conventional collector geometries
 - Design and 3D printing of non-conventional collector geometries and fibre production on them
 - Simulation of the electric field during electrostatic fibre formation using non-conventional 3D printed collectors
 - Comparison of the fibre deposition characteristic of the non-conventional collector geometries with the simulation data
- Experimental work:
 - Synthesis of Polysuccinimide
 - Production of Polysuccinimide nanofibre matrices
 - Characterisation of the produced matrices with Scanning electron microscopy and FT IR spectroscopy
 - Indirect cytotoxicity assay on 155 BR fibroblast cells

3 Materials and methods

3.1 Electric field modelling during electrostatic fibre formation

During electrospinning there is a potential difference between the needle or capillary tube where the high voltage is applied, and the grounded collector. During the project I used the QuickField computer program to visualize the electric field in the electrospinning apparatus due to the program being the student version it had limited mesh size: for 2-dimensional simulation it has a maximum of 255 mesh count and in 3 dimensional simulations it has a maximum mesh count of 4095. The program uses approximation by creating a finite number of meshes to calculate the functions in the meshes. For the three-dimensional simulation I used three-dimensional extrusion method to create the desired geometries.

In the program I used the DC Conduction problem solving, the needle has 17 kV voltage, and the collector is grounded, between the collector and the needle the air gap is 14 cm, the air conductivity is 1×10^{-12} S/m.

3.2 Materials

L-Aspartic Acid (reagent grade $\geq 98\%$, Mw ~ 133 , Sigma Aldrich, USA), Orthophosphoric Acid (reagent grade $\geq 99\%$, Mw ~ 98 , Sigma Aldrich, USA), N,N-Dimethylformamide (DMF) (reagent grade $\geq 99.8\%$, AnalaR NORMAPUR®, VWR Chemicals BDH®, VWR International, USA), , CaCl_2 ($\geq 99\%$, Reanal).

3.3 3D printed collector

During the project I designed custom collectors using Autodesk Fusion 360 computer program. These designs are based on the collector geometries that are available from different companies or were investigated in the literature connected to electrospinning [38]. The collectors are 3D printed using a Creality Ender 3 printer using PLA filament. After the 3D printing the objects were coated with aluminium spray that made their surface electrically conductive, it is a crucial step because it is necessary for the target object to be conductive and grounded.

3.4 Preparation of polymer solutions

The polysuccinimide was synthesised from L-aspartic-acid and 99% n-phosphoric-acid by thermal polycondensation. The synthesis occurred using an IKA RV21 rotary evaporator with an IKA HB 21 digital heat tank and a Vacuubrand PC3001 VAEIO vacuum generator. After mixing 20g of L-aspartic acid and 20 g crystalline phosphoric acid. This mixture was added to a pear shape 1 litre flask which is submerged to the silicon oil which is heated to 180 °C and the inside of the flask is under 3 mbar. The synthesis is 7 hours long and by the end a yellowish foam like structure can be observed as the product of the synthesis. The product was then solved in 200 mL of dimethylformamide. For the removal of the unreacted materials the product then was washed and filtrated 3-4 times until it reached neutral pH. The washing was done with 1600 mL distilled water and stirring for 10 minutes, and the vacuum filtration was done with G3 type glass filter. The filtered and washed product was dried for three days at 40 °C in a dehydrator. The final PSI powder was white in colour.

The electrospinning solutions were prepared in a 30 mL vial, and 5 mL solutions were prepared as it is shown in *Table 1*. After weighting out the CaCl₂ salt the DMF solvent were added and magnetically stirred until full solvation. The PSI were added to this salt-DMF solution and stirred until complete dissolution. The PSI solutions have a golden colour and has a honey like viscosity.

Table 1: Components in the PSI solutions by weight

Components by weight	PSI (g)	DMF (g)	CaCl ₂ (g)
20 m/m % PSI	1	4	-
22,5 m/m % PSI	1,125	3.875	-
25 m/m % PSI	1.25	3.75	-
20 m/m % PSI with 5 m/m % CaCl₂	1	3.75	0,25
22,5 m/m % PSI with 3 m/m % CaCl₂	1,125	3.725	0.15

25 m/m % PSI with 1 m/m % CaCl₂	1.25	3.7	0.05
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3.5 Electrostatic fibre formation

The Polysuccinimide polymer fibres were produced using a home-made electrospinning device. The walls of the device were constructed from fabric bakelite and aluminium railing to create a box which is separating enough for the spinning to be safe. The doors of the device were constructed from clear acrylic sheets. The whole box is grounded on 2 sides so that any excess charge is dissipated on the ground wires. The dimensions of the electrospinning apparatus are the following: 115 cm x 76 cm x 56 cm. The device contains 2 KD Scientific 100 single syringe pump and a GENVOLT high voltage power supply. The apparatus can go up to 20 kilovolts during electrospinning, at higher voltages there is a possibility of arc production which is disadvantageous to the fibre formation.

For the electrostatic fibre formation, the Polysuccinimide solution was loaded into a 1 mL syringe. The syringe with an 8-gauge needle was placed into the syringe pump and a crocodile clamp was placed on the needle as it would supply the electric voltage to the needle and the solution. The rotating drum collector or the designed collectors were placed in 14 ± 0.5 cm distance from the point of the needle and grounded with crocodile clamp, this collecting distance is optimal for the PSI electrospinning. The applied electric voltage was 17.5 ± 0.5 kV and the flow rate of the polymer solution was 1 mL/h. The temperature during the fibre collection was 24.5 ± 1 °C and the relative humidity was at 52 ± 3 %. The parameters of the electrostatic fibre formation were in the optimal range for the PSI polymer solution.

3.6 Characterisation of the fibre structures

3.6.1 ATR-FT-IR spectroscopic characterisation

The infrared spectroscopy measurements were made with a JASCO 4700A instrument with ATR head. Each spectrum was measured between 400-4000 cm^{-1} wavenumber range, 64 measurements were made on every wavenumber step and the data

was averaged. The resolution was 4 cm^{-1} . The spectra were visualised with the SpectraGryph 1.2 computer program.

3.6.2 Average fibre diameter characterisation

The average fibre diameter of the fibre matrices was defined with the help of scanning electron microscopy. The samples were cut out and glued to the sample tray of the scanning electron microscope. The samples than sputter coated with a conductive layer of gold thus preventing the charge of the samples from the electron beam. The coated samples than inserted to the microscope and photographed at 1000x, 2500x, 5000x times magnification.

The fibre diameters were defined using the Fiji computer program which can calculate the width by counting the pixel length of a line that is fitted to the edges of the individual fibres. I defined 100 of these fitted lines for each fibre sample in three different magnifications and then calculated the average diameter and the standard deviation using excel. For the average diameter analyses Welch's statistical test was performed on the cell viability values using excels data analysis tool comparing the diameters of the fibres containing CaCl_2 with their polymer concentration pair without the CaCl_2

3.7 Indirect cytotoxicity assay

The in vitro indirect cytotoxicity assay was made by using 155 BR fibroblast cell culture. Cells were grown in vitro, increasing their number until sufficient cell number were available to measure cytotoxicity. During the cytotoxicity assay I measured the cell viability of the cells with WST-1 proliferating reagent. The WST-1 reagent was diluted to 5% with colourless culturing medium. The WST-1 cell proliferation reagent was used for the colorimetric quantification of cell viability in each well before treatment and after 24 or 72 h treatment. Everything was done under a sterile hood preventing the infection of the cell cultures.

The cytotoxicity assay took 5 days to measure the cell viability of the cells. Before the beginning of the cytotoxicity assay, I cut $0.50\text{ mg} \pm 0.1\text{mg}$ samples from the electrospun nanofibre. With the known weight of the samples I calculated and measured out the CaCl_2 salt quantity in the electrospun samples that contained the salt for 100x concentration solution.

On the first day of the measurement the nanofibre samples were submerged in culturing medium for 24 hours and the measured salt quantities were also solved in the culturing

medium. The cell quantity was counted using burker chamber. After counting the cells, they were seeded to two 96 well plates at a concentration of 10000 cells in 100 μ l and maintained in the culturing box for 24 hours to provide the possibility to for attachment to the surface and formation of a semi-confluent monolayer.

On the second day the nanofibre containing mediums were filtered and the salt containing concentrated mediums were diluted and filtered. The wells containing the cell cultures were photographed under a microscope. The cell culturing medium was than replaced with 200 μ l of polymer and salt containing mediums, for the control samples the culturing medium was replaced with fresh medium and in the death cell (DC) negative control samples the cells were treated with 10% dimethylsulfoxide that will be cytotoxic. The cells were than incubated for 24 hours on 37°C.

On the third day the 24-hour plate wells were photographed under microscope. The cells were first washed 2 times with PBS solution then 100 μ l of 5% WST1-proliferating agent was added to the cell containing wells and to 6 empty wells for blank solution. The cells were incubated for 4 hours. After the 4 hours of incubation 90 μ l of supernatant reagent were pipetted to an empty 96 well plate. The absorbance of the supernatant was measured at 450 nm with a reference wavelength of 655 nm using a microplate reader.

On the fifth day the protocol of the third day was repeated on the 72-hour plate.

For the data analysis, Welch's statistical test was performed on the cell viability values using excels data analysis tool.

4 Results

4.1 Electric field simulations

4.1.1 Electric propagation between base geometries

Simulating the electric field propagation between base geometries helps to get a better understanding on the process of Electrostatic fibre formation. The polymer jet's path is following the electric field through the air onto the grounded collector geometries; therefore, the fibre deposition behaviour can be controlled by the changes in the electric field characteristics during electrospinning,

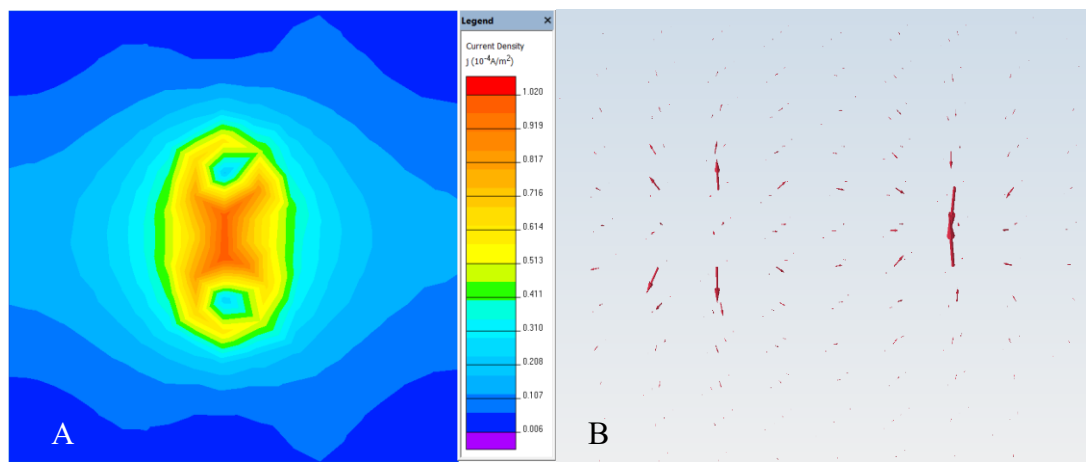


Figure 3: Electric field propagation between two points in two-dimension (A) and three-dimensions (B)

In *Figure 3* the simulation shows that the electric field is not propagating linearly the highest current density is between the two points, but the electric field does propagate much further in space and it is propagating in 360 degrees.

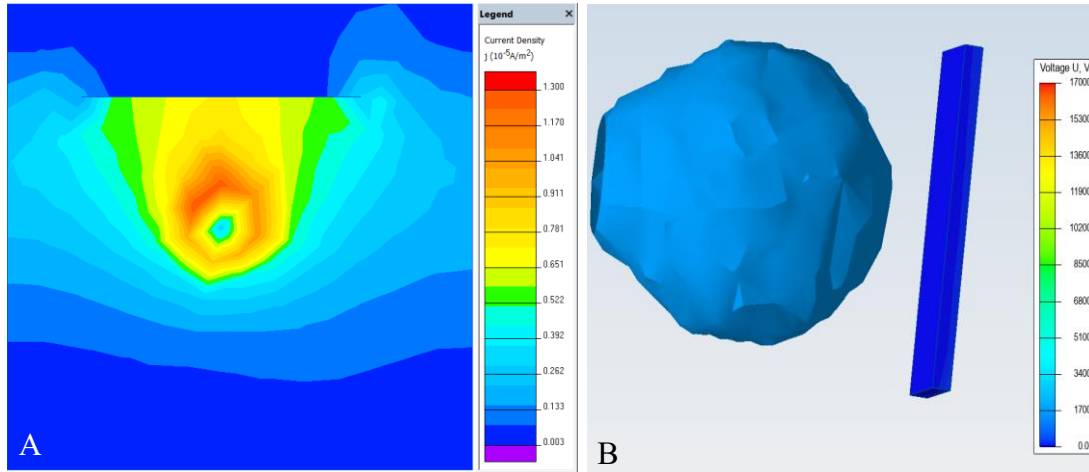


Figure 4: Electric field propagation between a point and a flat surface in in two-dimensions (A) and in three-dimensions (B)

In Figure 4 the simulation shows that the electric field between a grounded flat surface and a point where the charge is placed, it will propagate to the grounded surface and even go around the surface to the back side of the ground surface. This behaviour is also observed during electrostatic fibre formation when the collector is too small or the collected polymer acts as an insulator on the collector, the biopolymer fibre structure also appears on the backside of the collector.

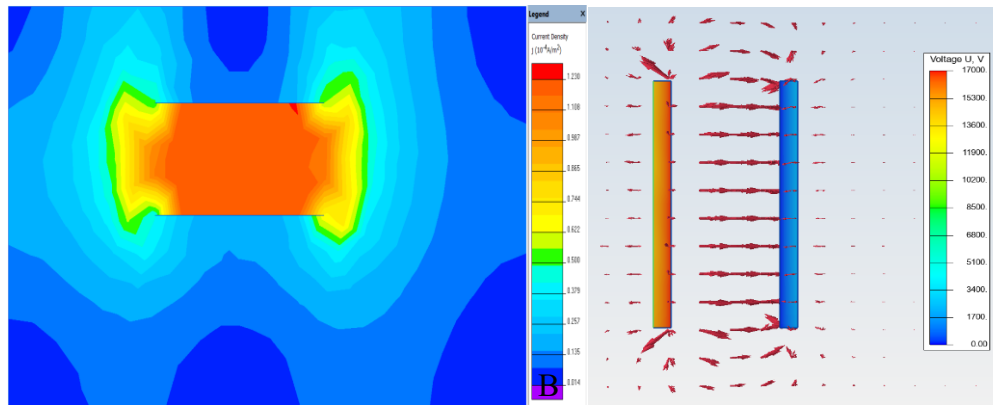


Figure 5: Electric field propagation between 2 flat surfaces in two-dimensions (A) and in three-dimensions (B)

In Figure 5 the electric field between two flat surfaces propagates evenly but at the edges it widens out and propagates outside of the length of the two flat surfaces.

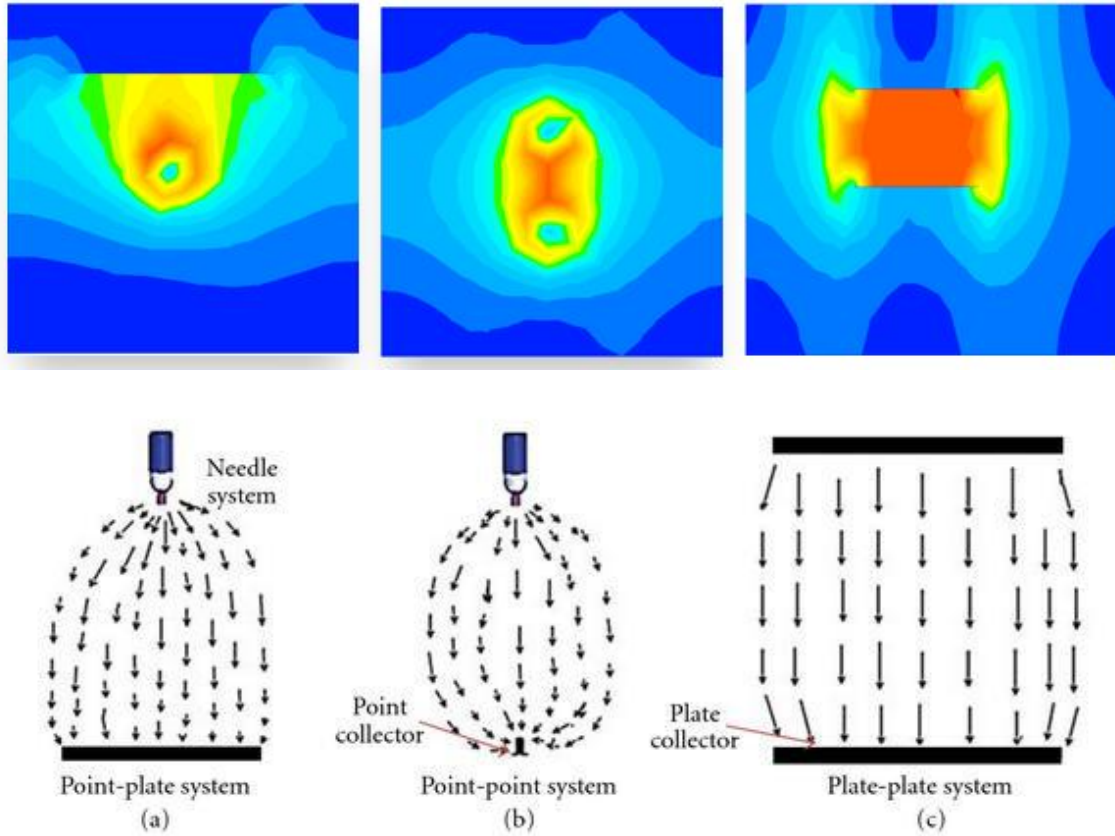


Figure 6: Comparison of the base geometries with literature data [39]

Comparing the results of the electric field propagation simulations with the results in the work of Sahay 2011, we can see that the simulations between the basic geometries that are relevant for electrospinning show the same electric field propagation. This comparison shows us that the result from the simulations is successful and can give a better understanding to the operation of this technology and can be used to design new collecting geometries that can benefit the fibre collection.

4.1.2 Electric propagation during electrostatic fibre formation

During electrostatic fibre formation the needle is under high voltage which is delivered by a crocodile clip. In *Figure 7 (A)* the electric field is propagating is not only in the entire length of the collector surface but also behind the collector and on the sides. In *Figure 7 (B)* the electric field is simulated in a 50 mm radius around the needle, due to the constraints of the student version of the program larger than the current simulation contains too many meshes and the program does not simulate it. As it is shown in these simulations the electric field is homogenous at the collector which means the polymer. Deposition will occur on the surface of the whole collector.

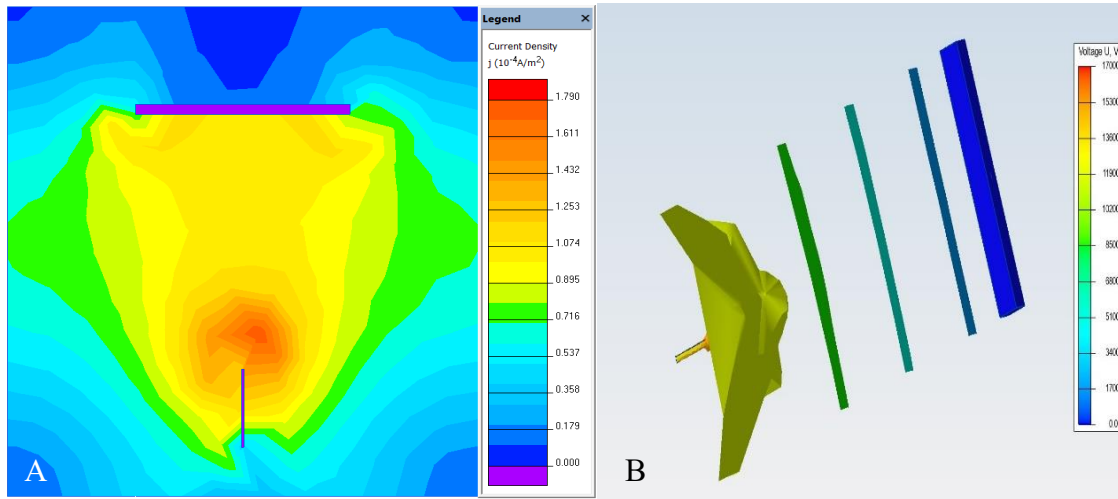


Figure 7: Electric field propagation during electrostatic fibre formation with flat surface collector geometry in two-dimensions (A) and in three-dimensions visualized as an isosurface plot(B)

Figure 8 shows the electric field propagation with a rotating drum collector. These collectors are used when the goal is to produce fibre matrices with either homogenous fibre coating on slow rotation or to produce directional matrices on high rotation speed.

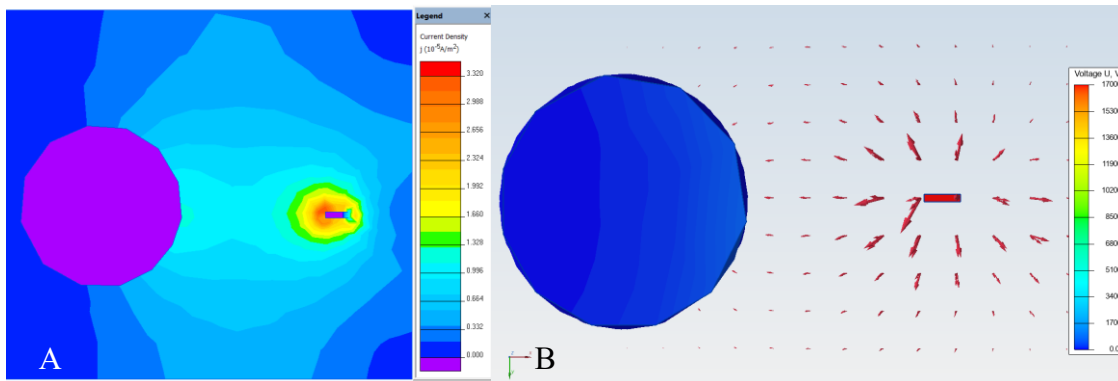


Figure 8: Electric field propagation during electrostatic fibre formation with rotating drum collector in two-dimensions (A) and in three-dimensions

4.2 Collector designs

The collector designs shown in Figure 9 have a similar parameter that the gaps in the collector are in 6 mm distance. The purpose of these collector designs was two-fold the first was to collect the fibres in the gaps of the collector therefore collect directional fibre matrices without using high speed rotating drum collector. The second was to simulate the electric field using these collectors and compare the simulation data to the fibre deposition behaviour.

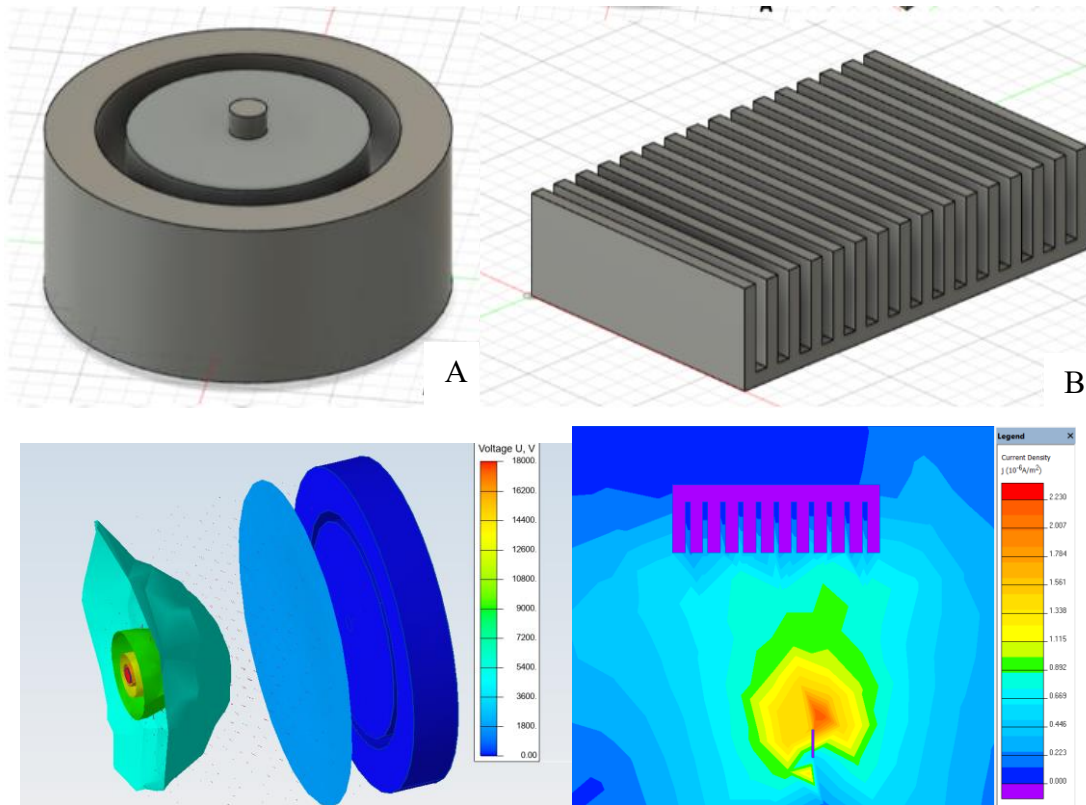


Figure 9: Collector designs and their electric field simulations

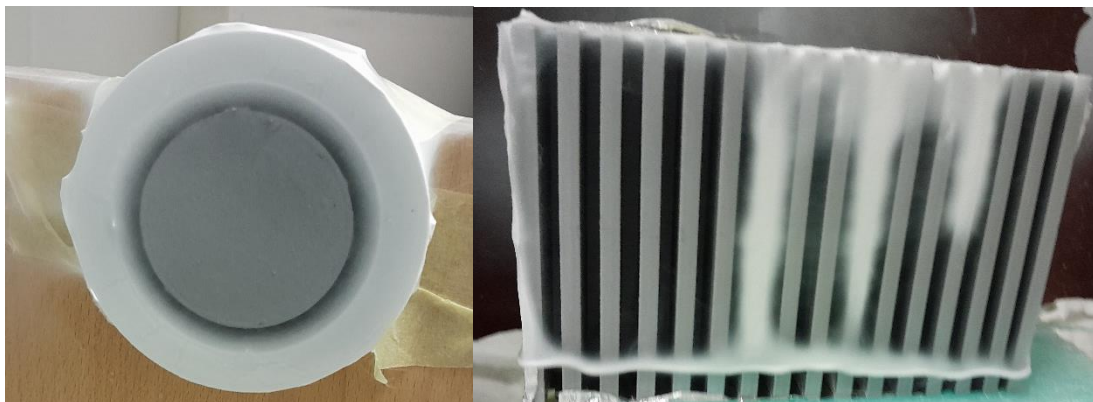


Figure 10: Fibre deposition behaviour on the designed collector geometries

On *Figure 10* the fibre deposition shows that fibre deposition did not occur in the collector gaps which would cause the fibre matrices to be directional. The fibre collection only occurred on the outside surfaces of the collectors. The explanation to this behaviour is given by the simulation data on these collectors. The simulations show that the electric field does not penetrate into the gaps on the collector geometries, these gaps are shielded from the electric field. To combat this behaviour we would need to use auxiliary electrodes with

bigger gap sized collectors to guide the polymer jets from the surface of the collector and into the gaps.

4.3 Fibre structures

In the scanning electron microscopic pictures on *Figure 11*, we can see that in the 20% PSI sample not only fibres were produced but also polymer beads in the fibre matrix. These beaded fibre matrices are widely observed, and their formation is related to the instability of the fibre solution jet during electrostatic fibre formation. This instability is related to specific characteristics of the polymer solution namely: viscoelasticity, carried charge density by the jet and surface tension. To combat this bead forming in the fibre matrices I added CaCl_2 in 5 m/m% concentration to the 20% PSI solution that increases the viscosity and improves the electric conductivity of the polymer solution.

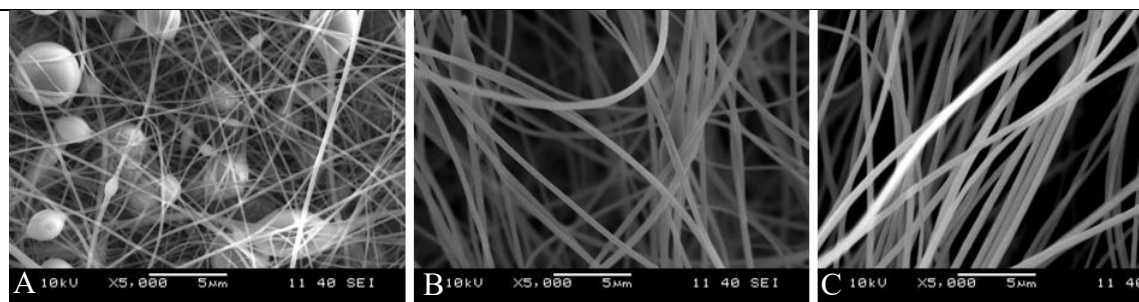


Figure 11: Scanning electron microscopic pictures of the polysuccinimide fibre matrices A: 20% PSI sample, B: 22.5% PSI sample, C: 25% PSI sample

The 22.5% and 25% PSI samples show the regular polymer fibre matrices although on the 25% sample we can observe a high degree of alignment between the fibres.

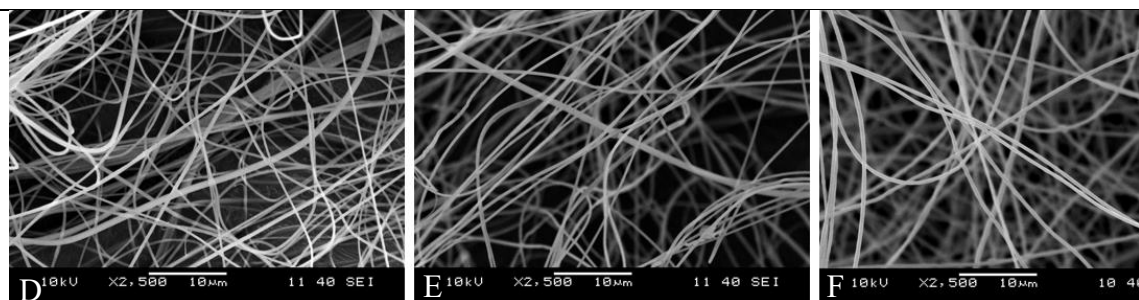


Figure 12: Scanning electron microscopic pictures of the Polysuccinimide and CaCl_2 containing fibre matrices D: 20% PSI +5% CaCl_2 sample, E: 22.5% PSI+3% CaCl_2 sample, F: 25%+1% CaCl_2 PSI sample

We can conclude from *Figure 12* that the addition of CaCl_2 to the polymer solutions prevented the formation of beaded fibre matrices.

Table 2: Parameters of the produced fibre structures

Mark in Figure 11 and 12	Fibre matrix sample	Average width of fibres (nm)	SD of fibre diameter	p-value comparing the PSI concentration pairs
A	20 wt% PSI	263	122	
B	22.5 wt% PSI	553	166	
C	25 wt% PSI	503	170	
D	20 wt% PSI + 5m/m% CaCl ₂	542	201	1.66E-68
E	22.5 wt% PSI + 3m/m% CaCl ₂	492	145	2.22E-06
F	25 wt% PSI + 1m/m% CaCl ₂	666	195	1.91E-25

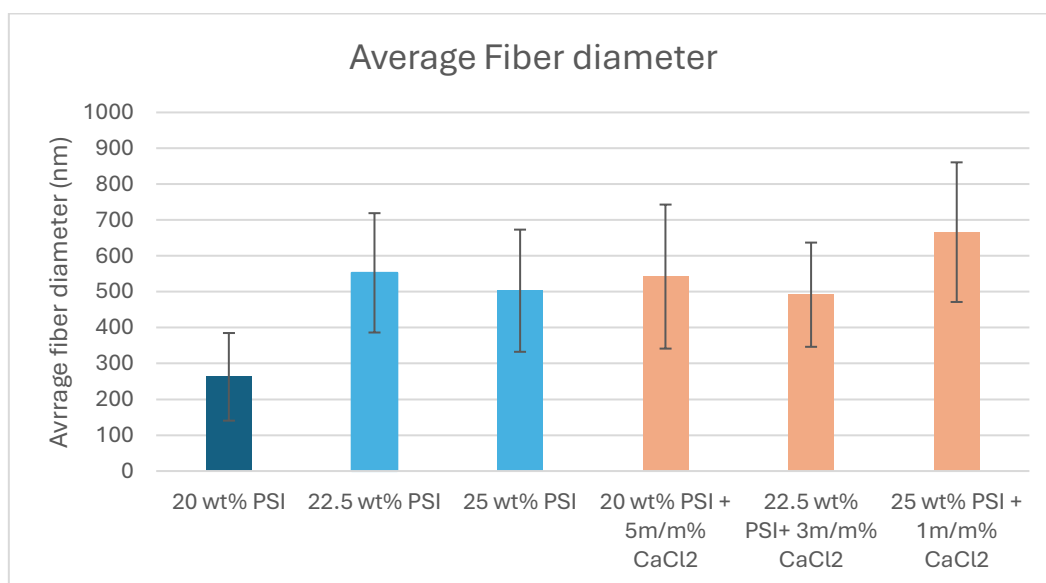


Figure 13: Average fibre diameter in the Polysuccinimide fibre matrices

The average fibre diameter in the produced matrices is in the nanoscale because they are below 1 μm in this regard I successfully produced nanofibre matrices.

The added CaCl_2 salt to the polymer solutions not only prevented the bead formation in the fibre matrices but also significantly changed the average fibre diameters in the fibre matrices.

4.4 FT-IR Characterisation

The FT-IR spectrums on *Figure 14* of the matrices containing CaCl_2 show peaks at 3400 cm^{-1} and at 1600 cm^{-1} these peaks are marked with the blue arrow in *Figure 14* The marked peaks show the presence of water in these matrices. This water content can have two explanations, the first is that these matrices absorb the water from the air humidity during storage, and the second is that the Dimethyl formamide solvent introduces a small amount of water during the PSI solvation. This water content does not cause the precipitation of PSI polymer.

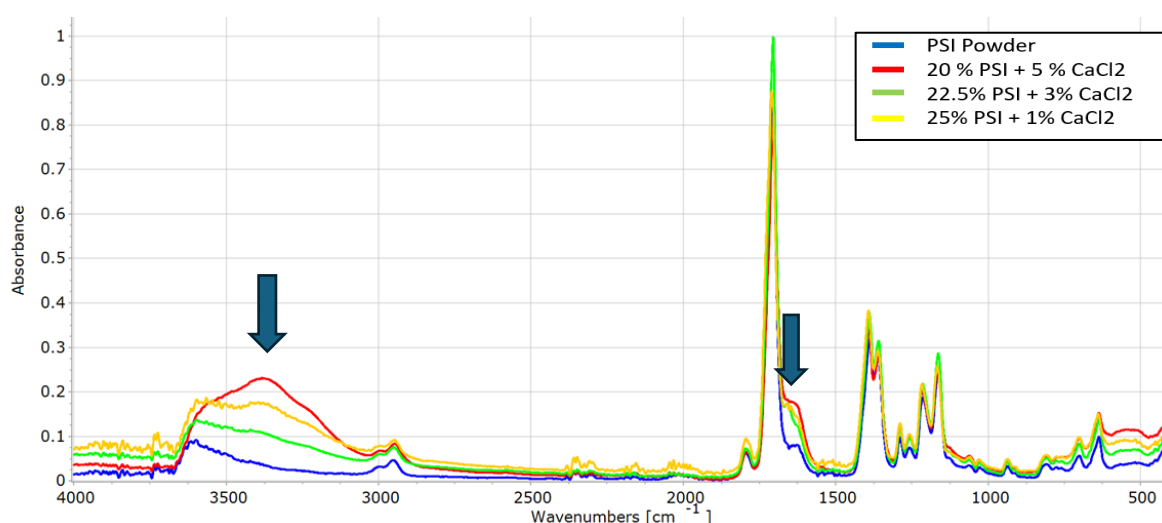


Figure 14: FT-IR spectrum of the matrices containing CaCl_2

4.5 Indirect cytotoxicity assay

In *Figure 15* we can conclude that the cell proliferation was not inhibited during the cytotoxicity assay because the cell coverage in the wells show an increase.

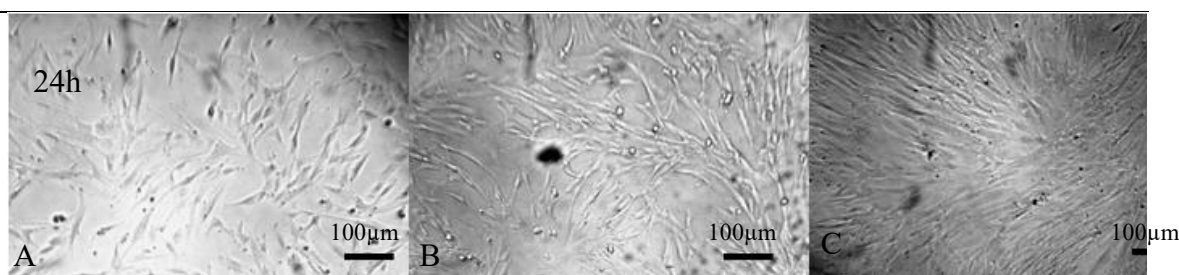


Figure 15: 155BR cells during the cytotoxicity assay: Picture A: Day 0 after seeding the cells to the 96 well plate, Picture B: Cells after 24h incubation in the treated culturing medium, Picture C: cells after 72h incubation in the treated culturing medium

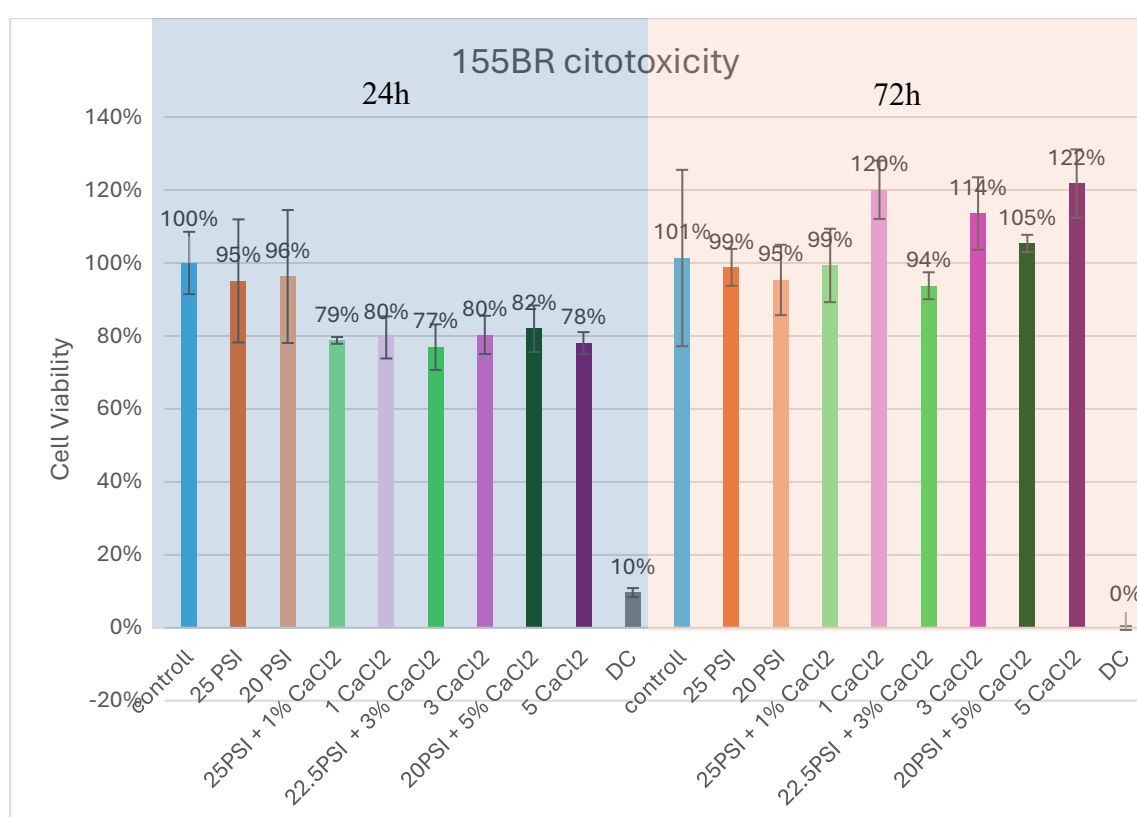


Figure 16: Cell viability results after 24 hours and 72 hours

Table 3: Cell viability and p-value from Welch's test compared to control results. The significance was marked with grey background

	24 hours	p-value	72 hours	p-value
control	100%		133%	
25 PSI	95%	0.626947	129%	0.849544
20 PSI	96%	0.728881	125%	0.671506

25PSI + 1% CaCl₂	79%	0.003548	130%	0.88396
1 CaCl₂	80%	0.003496	157%	0.215567
22.5PSI + 3% CaCl₂	77%	0.002025	123%	0.573725
3 CaCl₂	80%	0.003659	149%	0.403727
20PSI + 5% CaCl₂	82%	0.008302	138%	0.763835
5 CaCl₂	78%	0.002327	159%	0.194605
DC	10%	3.67E-06	0%	0.003563

From the data in *Table 3* we can conclude that the on the cell viability compared to the control sample did not change significantly when the cells were incubated in the culturing medium where the PSI fibres were submerged for 24 hours. In the culturing mediums which contained the CaCl₂ and where the CaCl₂ containing fibres where submerged the cell viability decreased significantly after 24 hours of incubation.

The data in *Table 3* shows that the cell viability after 72 hours in the control samples is at 133% This is due to the fact that all percentages were calculated using the data from the 24 hours control samples, this calculation method also shows us that the cells were proliferating at a normal rate because the cell viability results are also dependent on the number of cells in the measured samples. After 72 hours of incubation the cell viability did not change significantly from the 72 hours control sample.

From the data of the indirect cytotoxicity assay, we can conclude that the PSI fibres do not exhibit significant cytotoxic effect on the 155BR fibroblast cells.

5 Summary

Electrostatic fibre formation is a nonmechanical technology to create nanoscale fibre structure that has a wide application range in medicine as cellular scaffolds, antimicrobial wound dressing, drug delivery systems, bone grafting, and as artificial blood vessels. The electrospun nanofibres are produced from synthetic or natural polymers and their application is dependent on the properties of the employed polymer and on the properties of the produced fibre structure. My dissertation contains two main parts the first part was to simulate the electric field propagation during electrospinning and the second part was the laboratory work where I characterised the produced polymer fibres.

For the simulation of the electric field propagation, I firstly simulated base geometries that are relevant for the electrospinning technology and compared them to literature data to conclude that the simulated electric field show the same behaviour that is reported in the literature. The next part of my simulation work was to simulate the electric field during a conventional electrospinning, this gained us a better understanding on the process of fibre deposition on the collector geometries. The final part of the simulation tasks was to design collector geometries and compare the fibre deposition behaviour in the electrospinning with the simulation data on the same collectors.

The second part of this project was the production and characterisation of the electrospun Polysuccinimide fibre matrices, these matrices were collected on conventional rotating drum collectors. The Polysuccinimide fibre matrices were electrospun on the same parameters for the aim that the properties of the matrices only be changed by the characteristics of the polymer solution. The main changes in the polymer solutions were the concentration change of the polymer in the solution and the addition of CaCl_2 in different concentrations to the polymer solution. The determination of the average fibre diameter of the fibre matrices was done with scanning electron microscopy. The fibre matrices were also characterised with FT-IR spectroscopy. The last part of my project was to determine the cytotoxic effect of the produced fibre matrices in an indirect cytotoxicity assay on 155BR human fibroblast cells. From the result collected during the characterisation of the polymer matrices we can conclude that the CaCl_2 containing matrices can be used as base material for electrospun antibacterial wound dressing.

In summary with the electric field propagation simulation, I successfully simulated the electric field during the electrostatic fibre formation and successfully simulated the fibre

deposition characteristics on custom 3D printed collector geometries. During the characterisation of the produced fibre matrices, I found that the addition of CaCl_2 can prevent the formation of beaded fibre matrices, but the IR spectroscopy data shows that the CaCl_2 containing fibre matrices also contain water. The results from the indirect cytotoxicity assay show that the PSI fibre matrices does not decrease the cell viability significantly therefor they do not have cytotoxic effect on the cells. The CaCl_2 containing fibres lowered the cell viability significantly after 24 hours of incubation but after 72 hours of incubation the cell viability shows that there is no significant change compared to the control samples.

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