Use of polymers in tissue engineering

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Doctoral Thesis Summary

Tomas Bata University in Zlín

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Centre of Polymer Systems

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Využití polymerů pro tkáňové inženýrství

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ABSTRACT

Medicine and pharmacy have experienced significant advances in recent years, yet there are still unmet goals, such as complete tissue and organ construction. The successful artificial construction of any tissue is based on the design of the appropriate biomaterial and the culture conditions, both of which should mimic the tissues' natural environment. This thesis focuses on understanding the material-cell-environment interactions and in particular looks at the impact of conductive polymers, scaffold design, and advanced culturing methods on the engineering of not only electrically sensitive tissues. Through a review of current research and experimental studies, the aim of this work is to improve the understanding of this complex system and potentially contribute to techniques for the artifical formation of cardiac tissue *de novo*.

Key words: *Tissue engineering, conductive polymers, tissue scaffold design, cytocompatibility, cell-material interaction.*

ABSTRAKT

Medicína a farmacie prožívá za poslední roky významné pokroky, existují však stále nedosažené cíle, jako je například kompletní konstrukce tkání a orgánů. Úspěšné vytvoření jakékoli tkáně je založeno na návrhu vhodného biomateriálu a kultivačních podmínek, přičemž obojí by mělo napodobovat přirozené prostředí tkáně. Tato práce se zaměřuje na pochopení interakcí mezi materiálem, buňkou a prostředím a zejména se zabývá vlivem vodivých polymerů, konstrukcí scaffoldů a pokročilých metod kultivace na inženýrství nejen elektricky citlivých tkání. Cílem této práce je prostřednictvím přehledu současného výzkumu a experimentálních studií přispět k porozumění tohoto složitého systému a potenciálně přispět k technikám umělé tvorby srdeční tkáně *de novo*.

Klíčová slova: *Tkáňové inženýrství, vodivé polymery, návrh tkáňového nosiče, cytokompatibilita, interakce buňka-materiál.*

TABLE OF CONTENT

1. INTRODUCTION

The standards and efficiency of medicine and pharmacy have been steadily increasing in recent years; however, some long-term challenges still remain, albeit in the distant future. The complete regeneration of any damaged tissue or organ within a living organism, or the acquisition of reliable results in drug testing on reconstituted tissues in a laboratory, eliminating the need for animal and clinical testing – these are the dreams that motivate researchers around the world to progress in the field of tissue engineering (TE).

Central to this progress in TE is the development of biomaterials that can mimic the complex macro and microenvironment of native tissues. When a biomaterial is designed and fabricated appropriately, it promotes cell adherence, growth, migration, specific stem cell differentiation and expected phenotype manifestation. Among the diverse range of biomaterials, polymers have emerged as versatile candidates for TE applications mainly due to their highly tunable properties and biocompatibility.

A subclass of polymers with intrinsic conductivity, called conductive polymers, has gained significant attention in TE strategies for their unique combination of facile synthesis and precise control over electron-based and ion-based conductivity. These properties are essential aspects for the engineering of functional electrosensitive tissues, such as nervous or cardiac tissues.

It is also important to consider that cell behavior is influenced not only by the carefully prepared biomaterial, but also by the environmental conditions during cultivation, which can amplify or suppress each other's effects. Thus, to obtain objective results from TE experiments, it is considered necessary to create dynamic culture conditions in a laboratory that will impose the same effects on the cells as in a living organism; for example, flowing media, mechanical stresses or electric fields.

In this thesis, the milestones of TE, the role and use of various polymers in TE, and the factors that need to be considered in the design and biocompatibility testing of tissue scaffolds are discussed. The aim of experimental part is to uncover and improve the understanding of how biomaterials and cultivation conditions influence cell behavior. Through a comprehensive review of the current state of the art, experimental studies and innovative approaches, this work aims to contribute to the development of TE techniques that hold promise for repairing damaged cardiac tissues and improving the quality of life of countless individuals affected by heart-related disorders.

2. TISSUE ENGINEERING

The first attempts to reconstruct damaged tissues were carried out by transplanting natural materials such as bone or skin from another organism, often resulting in limited success due to immune rejection and donor scarcity. With the advancement of medicine and material science, synthetic materials like metals, ceramics, and glass progressively became commonly used for medical implants and devices. However, physico-chemical properties of these materials restricted their use primarily to hard tissues, such as bone and dental tissue. The development of polymers marked a significant shift in tissue engineering (TE).

2.1 Utilization of polymers

Polymers have one huge and important advantage over these traditional materials – the ability to easily and widely modify all the (chemical, physical, mechanical, and technological) properties they possess. This entails an enormous variability in their structures and shapes, as well as polarity, durability, flexibility, conductivity, manufacturability, and manipulability, also biocompatibility and biodegradability, and many others. Polymers and in particular polymer-based composites can develop combinations of properties that cannot be achieved using other materials, such as aforementioned metals or ceramics. Thus, polymers can be customized to the specific needs of an individual patient and his issues. In addition, polymers have relatively easy manufacturing and secondary processability with little material waste, plus they are usually reasonable priced.

Polymers vary widely in their structure and properties. Natural polymers, such as deoxyribonucleic acid (DNA), hyaluronic acid (HA), or cellulose, play essential roles in biological systems, providing structural support, signaling, or genetic information. In contrast, synthetic polymers, such as polyurethane (PU) or polyvinylidene fluoride (PVDF), and much more, are engineered and extensively used in medicine and TE mostly for their versatility and stability. Recently, attention has turned to conductive polymers.

2.1.1 Conductive polymers

Conductive polymers have electron-based and ionic-based conductivity and magnetic properties combined at the same time with low density and easy processability. Chemical structure of conductive polymers is specific by the conjugated π–bonds and binding sites for doping ions. They also can adopt various redox states and switch between them depending on environmental conditions. As a result, these polymers are preferably used in electrically

sensitive applications, which are in biomedicine: scaffolds for cardiac and neural TE and biosensors. To date, polyaniline (PANI) and polypyrrole (PPy) have been at the forefront of research on conductive polymers for the last decade. However, there are also other hitherto neglected conductive polymers and polyazulene (PAz) is one of them. Thin layers of these conductive polymers in tissue culture dishes (TCDs) are shown in Figure 2.1.

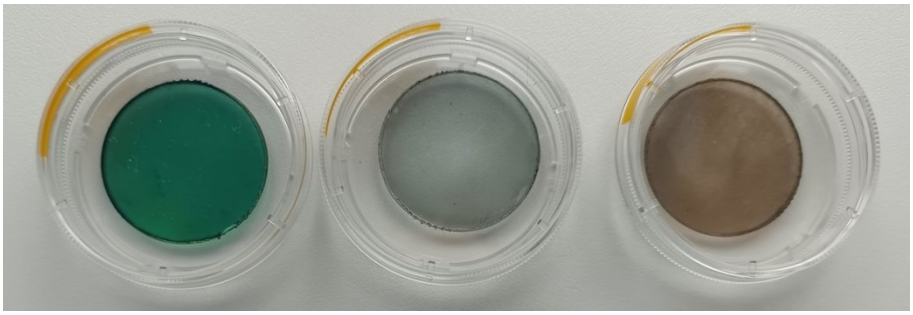

Figure 2.1 Concuctive polymer films: (left) PANI, (middle) PPy, (right) PAz films on TCDs all prepared by in situ chemical polymerization oxidized with APS. (Figure made by the author of this thesis).

Polyaniline

PANI has garnered significant attention due to its unique redox states, which include leucoemeraldine, emeraldine, and pernigraniline. The emeraldine salt form is highly conductive (up to 1.29×10^3 S/cm under optimal conditions and full protonation). (Venkatesh and Vishista, 2018). However, this same form also shows significant cytotoxicity compared to the less conductive emeraldine base. (Beygisangchin et al., 2021a; Borah et al., 2022) Toxicity depends on oxidation state, molecular weight, and type of dopant (Kašpárková et al., 2016) Moreover, PANI conductivity decreases in physiological environments due to pH shifts and ionic interactions, leading to deprotonation and performance loss. (Du et al., 2016)

Polypyrrole

Compared to PANI, PPy offers greater environmental and chemical stability, making it a strong candidate for biomedical applications. Although some studies confirm that PPy extracts are non-toxic and do not cause hemolysis or mutagenesis *in vivo* (Wang et al., 2004), other reports question its cytocompatibility with various cell types. (Ateh et al., 2006) Cytotoxicity appears to depend on whether PPy is in its salt or base form (Humpolicek et al., 2018a), as well as on the presence of low molecular weight byproducts, which can influence stem cell neurogenesis. (Skopalova et al., 2021)

Polyazulene

Azulene (see Figure 2.2) is a dark blue isomer of naphthalene, containing 5- and 7-membered combined rings, which can be oxidized to an azulenylium carbocation. This cation is more stable than azulene itself and has an electron-donating feature influencing electrochemical synthesis. (Neoh et al., 1988; Iwasaki et al., 1993; Latonen et al., 2009) It is not that often used but PAz can also be polymerized by a chemical reaction with brominated or jodinated azulen. (Neoh et al., 1988; Wang et al., 2003; Kaewchingduang et al., 2019) PAz polymerization also has been carried out by a direct chemical reaction of azulene with an oxidizing agent. (Grądzka et al., 2018)

In the synthesis of PAz, mostly 2 types of azulene monomer binding occur: 1,3 bonding and 1,5 bonding. The former leads to a planar configuration that forms a long conjugated chain favorable for π–electrons delocalization and high electric conductivity respectively. The second type of bonding, where the planar configuration is disrupted by steric crowding due to van der Waals radius of hydrogen atoms and the conductivity is thus reduced. (Gao et al., 2019; Hou et al., 2022; Iwasaki et al., 1993; Murai et al., 2012; Zhuang, 2020)

Still, the conductivity of non-substituted PAz is a very questionable issue. It may be apparently increased by protonation. (Wang et al., 2003) This is given by various redox states PAz can take on. (Latonen et al., 2009) These different redox states might also influence biocompatibility of PAz; however, to my knowledge, it has not been researched or at least published yet.

Suggesting that conventional PAz should be called polyazulenylene or dehydropolyazulene, Kihara et al., 1997 introduced a cationic polymerization method for "**true polyazulene**" (truePAz). The synthesis involved dissolving azulene in heated trifluoroacetic acid, followed by precipitation in methanol with triethylamine. The resulting brown powder contained 71% truePAz (see Figure 2.2) and 29% truePAz-acetate complex.

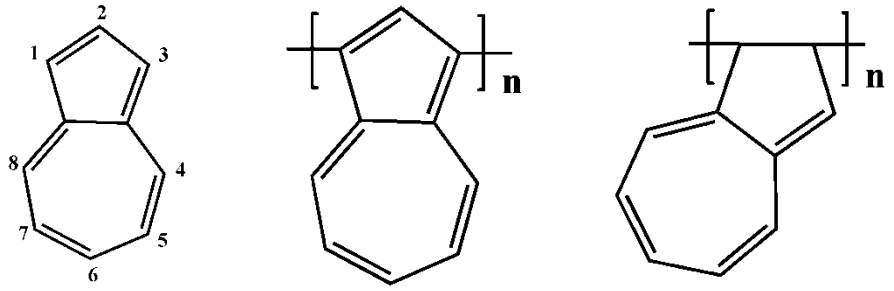

Figure 2.2 Scheme of (left) azulene, (middle) PAz, and (right) truePAz. (Figure made by the author of this thesis).

3. POLYMERIC SCAFFOLD DESIGN

When creating a potential biomaterial, the site of the intended application must first be considered. This is due to the vast differences in properties and functions across various tissues. Therefore, the successful assembly of scaffolds or biocomposites depends on understanding their interactions with cells and good biomimicry of the target tissue. Achieving suitable results thus relies on the design and fabrication of biomaterials with cell-specific properties. The proces of scaffold designing is schemed in Figure 3.1.

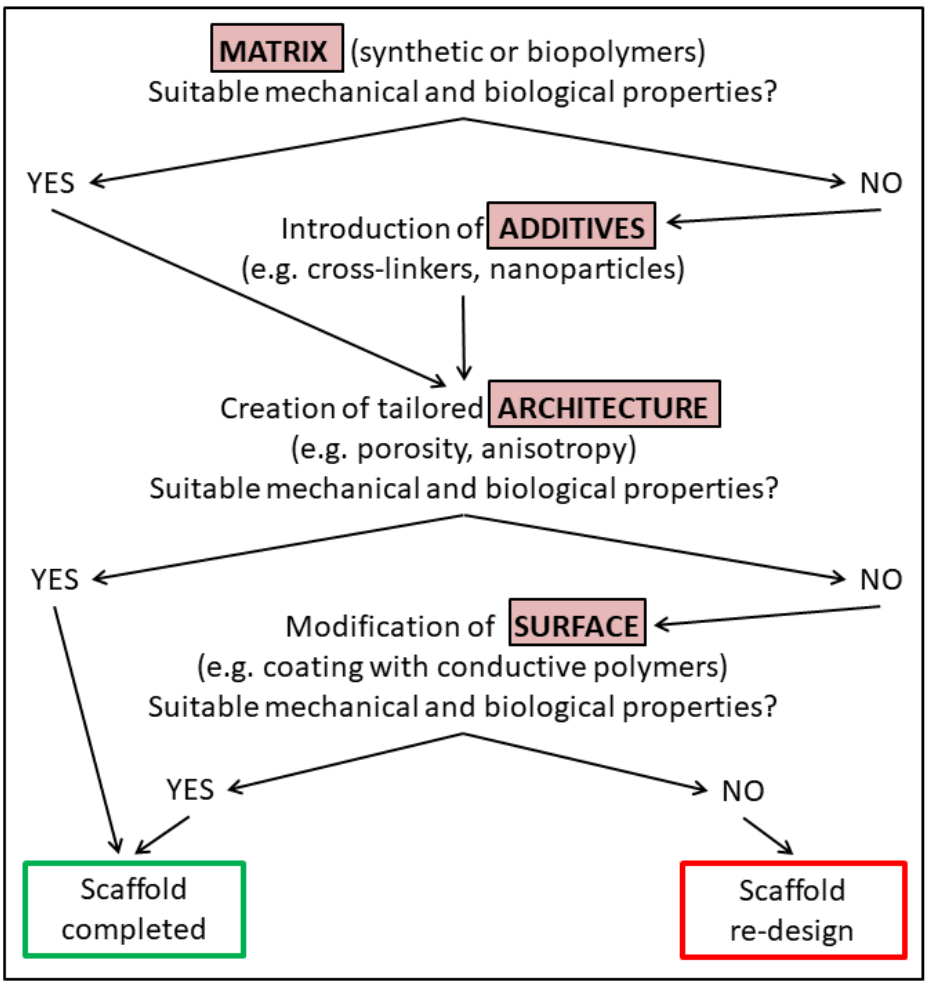

Figure 3.1 Simplified scheme of the main steps required for successful design of bioactive polymer scaffolds. (Figure made by the author of this thesis).

Scaffold design starts with choosing **matrix** that provides mechanical support and a 3D framework for cells. Natural polymers offer biocompatibility but often lack stability, while synthetic polymers provide strength but are less bioactive. The elasticity and mechanical strength of HA scaffolds can be finely tuned through chemical modifications and cross-linking techniques to develop hydrogels that mimic ECM and support cell adhesion, proliferation, and

differentiation. (Gorgol et al., 2024; Nimmo et al., 2011; Rezaeeyazdi et al., 2018; Vítková et al., 2022). The mechanical properties must match the target tissue – soft materials for brain, stiffer for bone. Mismatches can impair cell behavior. (Fu et al., 2010; Maity and Sarkar, 2017) Some polymers possess also other advantageous properties, such as PVDF, which is promising for neural and cardiovascular use thanks to its piezoelectricity. (Adadi et al., 2020; Kitsara et al., 2022; Li et al., 2019; Lins et al., 2017)

Various **additives** can be incorporated into the polymer matrix to modify different properties, including mechanical stability/degradation, bioactivity, as well as conductivity or stimuli responsiveness. The range of additives and their effects is as extensive as the range of polymers and their properties. Recent interest has focused on "smart scaffolds" that respond dynamically to environmental stimuli (e.g. magnetic fields), often created by embedding nanoscale active fillers into polymer matrices. (Bardajee and Hooshyar, 2014; Cvek et al., 2020; Tanasa et al., 2020; Vítková et al., 2022)

The next step is to process the matrix, with or without additives, into a scaffold with a suitable **architecture**. Creating a 3D shape makes the scaffold habitable for cells and can also generate specific cell-instructive signals. The main requirement for a 3D construct in TE is porosity. Pores must be interconnected to allow the migration of cells in the inner parts of a scaffold as well as the flow of the culture medium for nutrient supply and waste outflow. (Mukasheva et al., 2024) Technologies like electrospinning and 3D bioprinting enable the creation of aligned or complex, patient-specific scaffolds that impact cell morphology, mechanical response, and even support neural tissue formation *in vivo*. (Fee et al., 2016; Smith and Mele, 2021; Yin et al., 2010)

Last but not least, the **surface** of the designed and prepared scaffold is the site that directly interacts with cells and thus strongly affects the scaffold's success. Surface properties, such as chemical composition, physical properties, topography (Kulkarni et al., 2017; Nikkhah et al., 2012; Unadkat et al., 2011; Wrzecionko et al., 2017), or biological activity (Tallawi et al., 2015; Van Vlierberghe et al., 2011; Wu et al., 2020) strongly affect cell adhesion and response, and overall bioactivity. All these influencing factors can be modified all at once by surface coating. In addition, if the surface is coated with conductive polymers, another important cell-instructive cue occurs – **conductivity** (see Figure 3.2). (Grancarić et al., 2018; Hirata et al., 2010; Lee et al., 2022)

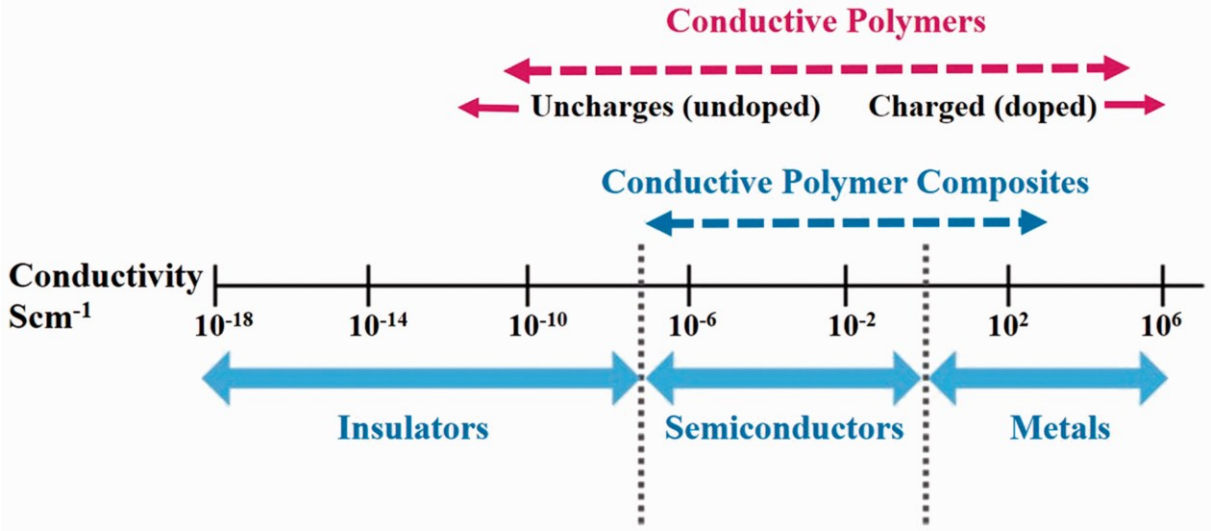

Figure 3.2 Scheme of the conductivity range of conductive polymers in compaison to other materials. (Grancarić et al., 2018)

4. BIOLOGICAL TESTING

4.1 Static versus dynamic cultivation conditions

Most *in vitro* studies on biomaterials are conducted under static conditions, which limit nutrient transport and poorly replicate the *in vivo* environment. Bioreactors address these issues by introducing dynamic stimuli improving cell behavior and tissue development. (Baker and Goodwin, 1997; Chen and Hu, 2006; Hammond and Hammond, 2001) All bioreactors apply one specific stimulus to the cells – hydrodynamic shear stress of the flowing medium. However, there are also other advanced types of bioreactors applying other external stimuli.

4.1.1 Mechanical forces

Mechanical stimuli are essential in native tissue environments and directly influence cell behavior through mechanosensitive structures. Even during static culture, cells experience hydrostatic pressure, substrate stiffness, and intercellular tension, but bioreactors introduce additional forces like hydrodynamic shear and specific cyclic loading. Such stimuli regulate physiology, including F-actin formation and vascular morphogenesis, and affect cell–cell interactions. (Citi, 2019; Joung et al., 2006; Le et al., 2016; Rodriguez-Boulan et al., 2005) However, inappropriate strain may cause issues in tissues as can be seen *in vivo*, such as chronic hypertension in blood vessels resulting in vascular endothelium dysfunction or chronic fast and shallow breathing that destructs the lung epithelium. (MacNee, 2006; Peng et al., 2019)

The foundational observation of load-dependent bone remodeling by Wolff (1892) initiated studies on how mechanical forces affect proliferation, migration, and stem cell differentiation. (Chen and Hu, 2006; Le et al., 2016; Lee et al., 2005; Nikkhah et al., 2012; Nokhbatolfoghahaei et al., 2020; Rosenfeld et al., 2016) Cellular responses vary based on origin – vascular endothelial cells endure 2D stretch from blood flow, while lung and heart cells face 3D cyclic strain. (Man et al., 2022)

4.1.2 Electrical field

Electrical stimulation plays a crucial role in TE, especially for tissues that are electrosensitive. Studies have shown that electric fields promote cell growth, migration, and differentiation, particularly into neural and cardiac lineages. (He et al., 2019; Nazari et al., 2020; Shrestha et al., 2019; Yan et al., 2020)

Conductive polymers, such as PPy and PANI, enhance these effects by providing bioactive, cell-instructive substrates. (Gajendiran et al., 2017) In nerve repair, chitosan/PPy scaffolds combined with electrical stimuli improved neurotrophin secretion and accelerated axonal regeneration *in vivo*. (Huang et al., 2012; Qi et al., 2013) Aligned conductive fibers also better mimic neural ECM, supporting regeneration after complex injuries. (Jin et al., 2022; Pourkhodadad et al., 2023) In cardiac applications, polyethersulfone/PANI scaffolds enhanced cardiomyogenesis and impulse conduction when exposed to uniaxial electrical fields. (Mohammadi Amirabad et al., 2017) Similarly, PPy/chitosan hydrogels synchronized contractions between isolated cardiomyocyte clusters and improved electrical propagation in scar tissue post-injection (Cui et al., 2018). Thus, conductive polymers paired with electrical stimulation offer promising strategies for functional tissue regeneration.

4.1.3 Magnetical field

Magnetic external stimuli can significantly affect cell behavior by altering membrane properties, activating biochemical pathways, and enhancing signaling. Static magnetic fields, for instance, influence membrane fluidity and ion channels, promoting differentiation in cells like osteoblasts. (Yang et al., 2018), while alternating fields can trigger calcium influx via mechanosensitive channels (Wong et al., 2018). Additionally, magnetic forces can guide cell alignment and migration, resulting in improved tissue organization and functionality. (Du et al., 2017) Overall, magnetic manipulations provide dynamic approach for controlling cellular behavior in various TE applications.

4.2 Cell image analysis

There are two main approaches to evaluating the effects of biomaterials or culture conditions on cell viability and behavior. The first involves assays like MTT or neutral red uptake, which use flow cytometry or spectrophotometry to detect viability markers in living or dead cells. These are suitable for high-throughput testing but may be limited by factors like material coloration or solvent interactions. The second approach uses fluorescence microscopy to visualize cell morphology and internal structures, though interpretation relies heavily on the researcher's expertise.

When standard methods aren't applicable, image analysis offers an alternative. Software CellProfiler (Carpenter et al., 2006) enable to quantify and qualify cellular features such as cell count, size, shape, and protein localization. (for example, Figure 4.1) This method supports large-scale data processing and enables evaluation of cell cycle, migration, or yeast growth.

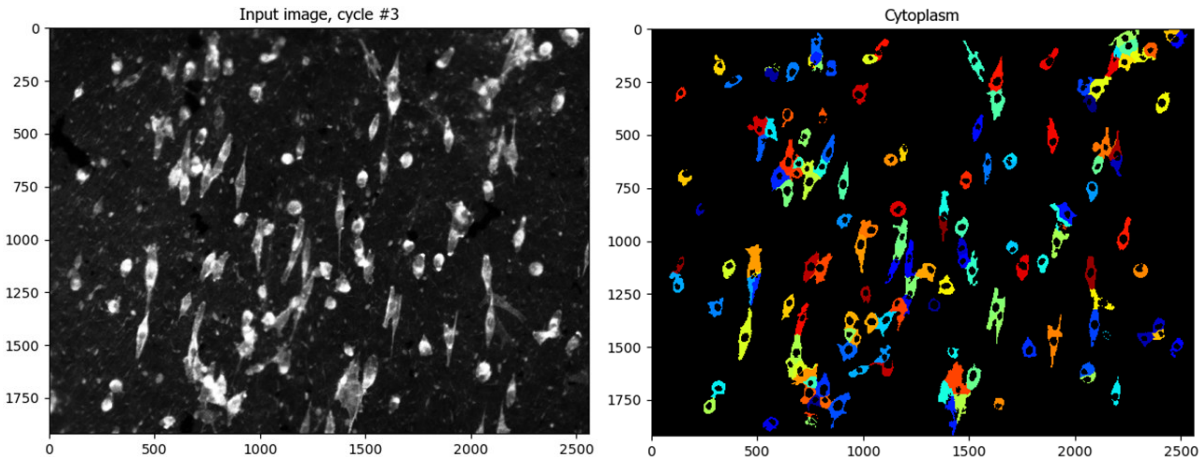

Figure 4.1 Example of the cell image analysis: On the left – fluorescence microphotograph of NIH/3T3 mouse fibroblasts grown on a tested material. On the right – the process of the image analysis focused on the cytoplasm size and shape. (Figure made by the author of this thesis).

5. OBJECTIVES OF THE THESIS

The main objective was to advance our understanding of how to coordinate the key components, such as biomaterial design, external stimuli, and selected cell lines, in order to achieve harmonious integration leading to the *de novo* formation of functional tissue, much like orchestrating individual instruments to create a cohesive and beautiful symphony.

To achieve this objective:

- Various thin films and scaffolds with potentially bioactive properties were designed and fabricated.
- Material properties of such films and scaffolds were characterized to reveal their compliance with requirements.
- Cytocompatibility and bioactivity of such films and scaffolds were determined using advanced *in vitro* biological evaluation to confirm the performance quality.

6. EXPERIMENTAL PART

6.1 Preparation of thin films

In the field of TE, conductive polymers have been very well known as highly promising materials, particularly for applications involving electrosensitive tissues, such as cardiac and neural systems. Their unique ability to conduct electrical signals makes them ideal for supporting the function and regeneration of cardiomyocytes, which rely heavily on synchronized electrical activity. Additionally, conductive polymers have shown potential in enhancing wound healing in skin tissue by promoting cell migration and proliferation under electrical stimulation.

During my research of conductive polymers, I focused on **PANI**, **PPy**, and **PAz**, each offering distinct advantages. PANI is known for its controllable conductivity and redox states, while PPy boasts more stabile electroactivity in biological environment. In contrast, PAz introduces a unique nonbenzenoid backbone that may open new avenues for functional design, but its practical advantages in TE remain unknown.

Conductive polymers were predominantly synthesized *via* **chemical oxidative polymerization**. With the aim of their application in TE, emphasis was placed not only on producing polymer powders, but especially on the fabrication of thin films. In the case of PAz, however, the results obtained through conventional chemical synthesis were questionable. Therefore, alternative approaches were employed: **electrochemical synthesis** of polyazulene and chemical synthesis of so-called '**true polyazulene**'.

Due to the high novelty of the research of PAz, the preparation procedure and results are not included in the dissertation. However, the committee will have access to the manuscript that has been submitted to a peer-reviewed journal.

The key material property investigated was the specific conductivity, determined using the four-point van der Pauw method. Furthermore, surface free energy by contact angle was analyzed, as it significantly influences cell and protein adhesion at the material interface. These and additional characterization techniques were conducted in collaboration with colleagues from the Centre of Polymer Systems and the Faculty of Technology, Tomas Bata University in Zlín.

6.2 Preparation of scaffolds

Another part of the research was the preparation and subsequent modification of various polymeric substrates in order to create a biocompatible scaffold with multiple cell-instructive cues. The choice of materials of interest must be in accordance with the intended application. For heart TE, thus, the scaffold must possess significant elasticity since cardiomyocytes are responsible for the contraction of the heart. Cohesion and rift resistance were also important properties necessary for reliability under further handling and stress.

The primary substrate I focused on was medical-grade **PU** electrospun into nanofibrous mats. After process and post-process optimization, scaffold with stable cytocompatibility was obtained. However, the product of electrospinning had randomly oriented fibers. To better mimic *in vivo* environments such as extracellular matrix or myocardium structure and thus provide cells with cues, **anisotropy** needed to be involved in the substrates. Therefore, the PU mats were modified to align their fibers in one direction. Whether the anisotropy was not only structural, but also mechanical, was determined by tensile testing.

Another material I investigated as a suitable alternative for the preparation of tissue scaffolds for electrosensitive tissues was **PVDF** and its copolymers. This material is particularly interesting due to its piezoelectric properties and, as demonstrated this work, its good cytocompatibility. With the use of a new electrospinning device, it was possible to fabricate anisotropic nanofibrous scaffolds directly during the spinning process, rather than through post-processing, as was the case with polyurethane.

The main functions of cardiomyocytes are induction, propagation, and reaction to electrical impulses. Thus, scaffold **conductivity**, which facilitates cell-to-cell communication, serves as a strong cellular cue possibly promoting stem cell cardiomyogenesis. For this purpose, conductive polymers were applied to substrate surfaces by *in situ* polymerization.

Characterization of the physicochemical properties of the prepared scaffolds was carried out in cooperation with my colleagues from the Center for Polymer Systems and the Faculty of Technology of Tomas Bata University in Zlín.

However, even the most sophisticated scaffold design is useless when cells do not adhere to the scaffold. This occurs for many reasons; for example, unsuitable functional groups on the surface and associated too low or too high free surface energy. These limitations in **cytocompatibility** were overridden by coating the scaffold's surface with serum albumin and bovine gelatin.

6.3 Cytocompatibility testing

The research was followed by a series of cytocompatibility tests, utilizing unipotent cells – mouse embryonic **fibroblast** cell line NIH/3T3, human **keratinocyte** cell line HaCaT, and totipotent cells – mouse embryonic **stem** cell line ES R1, including their formation of embryonic bodies that differentiated towards cardiomyocytes.

A series of standard experiments was implemented to determine the overall cytocompatibility of the prepared thin films and scaffolds. In addition to these biomaterials, the author cooperated with other colleagues and characterized the biological properties of **other scaffolds** with a potential for employment in TE of electrosensitive tissues: porous scaffolds made of CIPs, hyaluronan-based hydrogel scaffolds containing CIPs, and Pebax scaffolds.

Another essential component of my study was the **dynamic cell cultivation**. The cultivation of cells with various external stimuli is intended to mimic *in vivo* physiological conditions. Using three different bioreactors, as well as self made electrodes, I devoted myself to explore the use of electromagnetical filed, hydrodynamic shear stress, and tensile strain as such external stimuli to cells.

During my research activity, I encountered several approaches to evaluate cell viability, such as MTT, ATP, and Red uptake assays, and the use of UV-VIS. For cell morphology visualization, optical microscope, fluorescence inverted microscope, and confocal laser microscope were used. For the observation of cellular migration, a scratch assay was used.

Cell image analysis is an advanced evaluation technique that allowed to quatify viable cells which could not be quantified by other standard approaches. Moreover, it was used it to qualify cell morphology more accurately. Cell image analysis was performed in the open-access CellProfiler software.

7. SELECTED PROCESSING METHODS

7.1 Synthesis of conductive polymers

PANI powder was synthesized *via* chemical oxidative polymerization. A 0.2 M aqueous solution of aniline hydrochloride (Penta, Czech Republic) was mixed with a 0.25 M aqueous solution APS (Sigma-Aldrich, USA) at room temperature. After 24 h, the resulting green precipitate was vacuum filtered, thoroughly washed with 0.2 M hydrochloric acid (HCl, Penta, Czech Republic) and methanol (Penta, Czech Republic), and then air-dried.

PANI films were formed directly on TCDs, or indium tin oxide (ITO) glass, or pre-fabricated scaffolds by *in situ* polymerization that was carried out under the same conditions as the synthesis of PANI powder, except for earlier termination of the polymerization reaction, specifically after 1 h.

PPy powder was synthesized *via* chemical oxidative polymerization. A 0.2 M aqueous solution of pyrrole (Sigma-Aldrich, USA) was mixed with a 0.25 M aqueous solution of either APS or anhydrous $FeCl_3$ (IPL, Czech Republic) at room temperature for 24 h. The resulting black precipitate was vacuum filtered, thoroughly washed with 0.2 M HCl and methanol, and then air-dried.

PPy films were formed directly on TCDs, or ITO glass, or pre-fabricated scaffolds by *in situ* polymerization that was carried out under the same conditions as the synthesis of PPy powder except for earlier termination of the polymerization reaction, specifically after 60 s.

7.2 Preparation of scaffolds

PU Desmopan 385 S (Covestro AG, Germany) was electrospun into nanofibrous mats using the electrospinning device Nanospider (Elmarco, Czechia). The PU solution was extruded into 32 jets into a 75 kV electric field to the planar collector with a moving film. The alignment of fibers in one direction was introduced by hand stretching of the PU mats above a 150 °C heat source with subsequent securing to a microscope cover glass.

PVDF with various molecular weights (Sigma-Aldrich, USA) was dissolved in dimethylformamide (Sigma-Aldrich, USA) at 60 °C while stirring for at least 1 h to create a 10 wt. % solution. Electrospinning was then done on the device built by colleagues at the Faculty of Technology, Tomas Bata University in Zlin. The solutions were extruded from 1 jet into a 14–40 kV electric field to either a planar or a cylindrical collector. The higher the speed of cylinder rotation, the more aligned the fibres.

7.3 Material characterization

The electric conductivity of conductive films was determined by the four-point van der Pauw method. A system consisting of Keithley 6517B electrometer, Keithley 2410 source meter, and Keithley 7002 switch (Keithley Instruments, USA) was used at room temperature.

Measurements of contact angles on conductive films were done by a Theta Optical Tensiometer (Biolin Scientific, Finland) with demineralized water, ethylene glycol, and diiodomethane (all from Sigma-Aldrich, USA). Contact angles were measured at 10 μL droplets after (10 ± 2) s at room temperature with consequent ten times repetitions. The surface free energy was calculated using the "acid-base" method.

Tensile properties of PU mats were measured on MT350-5CT (Testometric, UK). The samples were cut in a rectangle shape with a length of 40 mm, width of 10 mm, and thickness of 0.015 mm. The elongation was carried out with a speed of 100 mm/min. and at the room temperature.

7.4 Cell lines

Mouse embryonic fibroblast cell line NIH/3T3 (ATCC CRL-1658 NIH/3T3, USA and ECACC 93061524, England) was cultivated in Dulbecco's modified eagle's medium (BioSera, France) + sodium hydrogen carbonate (Penta, Czech Republic) + calf serum (to 10 vol. %) (BioSera, France) + 100 μg/mL antibiotics penicillin/streptomycin (to 1 vol. %) (BioSera, France).

Human keratinocyte cell line HaCaT (Boukamp et al., 1988) was cultivated in RPMI medium 1640 with L-glutamin (Gibco™, USA) + sodium hydrogen carbonate + fetal bovine serum (to 10 vol. %) (Gibco™, USA) + 100 μg/mL antibiotics penicillin/streptomycin (to 1 vol. %).

Mouse embryonic stem cell line ES R1 was cultivated in Dulbecco's modified eagle's medium (Gibco™, USA) + fetal calf serum (to 16.5 vol. %) + 100 μg/mL antibiotics penicillin/streptomycin (to 1 vol. %) + 100 mM of non-essential amino acids (Gibco™, USA) + 0.05 mM β-mercaptoethanol (Sigma-Aldrich, USA) + 5 ng/mL leukemia inhibitory factor (LIF) (Chemicon, USA).

Mixed cell population was created by two approaches. First, fibroblasts and stem cells were seeded on scaffold surface at once. Second, the fibroblasts were seeded first and after 24 h of cultivation, stem cells were seeded on top of the fibroblasts. In both approaches, the stem cell line culture medium containg LIF was used.

7.5 Cultivation under static conditions

All cell lines cultivated under standard static conditions were maintained in a HERAcell 150i incubator (Thermo Scientific, USA) under standard culture conditions: 5% CO_2 atmosphere, a stable temperature of 37 °C, and consistent relative humidity.

Extracts of various tested materials were prepared according to ISO 10993-12 standards. The cytotoxicity testing was carried out with MTT assay, ATP assay, or Neutral red uptake assay conducted according to the ISO 10 993-5.

All tested materials were sterilized before coming in contact with cells. The method of sterilization was chosen in accordance to the material properties. Cells were seeded onto the surface of the tested materials and references at various concentrations. For stem cells, all tested substrates, including references, were coated with 0.1 % gelatin from bovine skin (Sigma-Aldrich, USA) in ultrapure water.

To evaluate the migratory capacity of cells, the scratch assay was carried out. In this method, a uniform straight scratch was manually introduced into a confluent monolayer of cells using a sterile pippet tip or a strip mask. The migration of cells into the wound area was monitored and documented using optical microscopy at defined time intervals.

7.6 Cultivation under dynamic conditions

Dynamic cultivation builds on standard static conditions by introducing external stimuli while keeping the same controlled environment within the incubator (37 °C, 5% CO_2, high humidity).

Direct electrical current stimulation was introduced to cells through selfmade electrodes. Cells were exposed to either constant or pulsed direct current electrical stimulation, with varying exposure/pause intervals and voltage levels ranging from tens of volts lasting minutes to tens of millivolts lasting for hours.

To apply hydrodynamic shear stress, the Rotary cell culture system (Synthecon, Inc., USA) and the Ibidi pump system (Ibidi, Germany) were used. For the former, cells were pre-adhered to a substrate that was then attached to a sel-made holder and placed in to the Rotary system. For the latter, the cells were seeded directly in to the testing chamber. Both systems allowed for the continuous circulation of culture medium over cell monolayers, creating controlled hydrodynamic shear stress conditions

The TC-3 bioreactor (Ebers Medical Technology, Spain) enables the application of multiple stimuli at ones: hydrodynamic shear stress from flowing media, tensile strain from stretching, and electrical pulses delivered by electrodes. Cell cultivation on electrospun PU mats was conducted with fibroblasts and embryonic stem cells. The cells were allowed to proliferate under static conditions for 3 days before being transferred to the bioreactor. The system was set to apply cyclic stretching (1 mm at 1 mm/sec speed) for various time periods (e.g. 10 min of stretching followed by 50 min pause) together with electrical field or pulses (0.1 V) over a 3-day period. Due to repeated infections, gentamycin (20 μg/mL) was added to the medium to prevent contamination.

7.7 Cell morphology visualization

For regular checks on cell behavior, a phase-contrast **optical** microscope (Olympus IX51, Japan) was employed. For a further determination of cellular morphology, the cells were fixed with 4% formaldehyde (Penta Chemicals, Czech Republic) in ultrapure water and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, USA) in phosphate-buffered saline. Fluorescent dyes ActinRed^{555} or ActinGreen^{488} (Life Technologies, USA) and Hoechst 33258 (Sigma-Aldrich, USA) were used for staining. ActinRed or ActinGreen binds to the proteins contained in the cytoskeleton of the cells and Hoechst penetrates the nuclei of the cells where it binds to the DNA. Then the stained cells were observed under a phase-contrast inverted **fluorescence** microscope (Olympus IX 81, Japan). In the case of 3D scaffolds, a **confocal** laser scanning microscope (Olympus FV3000, Japan) was used.

7.8 Image analysis

Cell image analysis is used to, among other things; more accurately quantify cells as well as qualify cell morphology. The image analyses were performed in the open-access CellProfiler software 4.0.7. (Carpenter et al., 2006)

For successful and more presice analysis, the fluorescent cell photographs were divided into separate images of nuclei and cytoskeletons, converted to grayscale, and slightly adjusted for higher contrast and less fluctuation in brightness, especialy those caused by substrate structural inequities.

Cell image analysis provides a variety of results, such as cell/cytoskeleton/ nucleus number, area, perimeter, major and minor axis length, eccentricity, orientation and much more. Therefore, only the most relevant features were always selected and discussed. Cellprofiler was also used to evaluate scratch test assays.

8. SUMMARY OF RESULTS

8.1 Polypyrrole surface coating within a minute

The author of this thesis researched, during her doctoral studies, several methods to enhance the overall performance of conductive polymers. However, her main attention was paid to the preparation of polymer conductive coatings that can be synthesized directly on various surfaces by a method as easy, fast, and versatile as possible. The fulfillment of these conditions is necessary for the successful application in practice.

Such synthesis of PPy can be achieved by chemical oxidative polymerization, enabling the formation of both powder and thin film forms suitable for biomedical integration. While effective, utilizing this method for PPy coating often requires electrical input, uncommon solvents or stabilizers, long reaction times, and/or low temperatures. (Golgovici et al., 2020; Maráková et al., 2017; Thunberg et al., 2015)

Through a series of chemical *in situ* oxidative polymerizations of PPy under different settings and conditions, a method was developed that allows the synthesis of a uniform conductive layer of PPy in aqueous solution without any additives at room temperature, all done within a minute. The description of this PPy coating method alongside its characterization, application on PU fibrous scaffolds, and cytocompatibility testing was published by **Mahelová, L.**, Slobodian, P., Kocourková, K., Minařík, A., Moučka, R., Trchová, M., Martínková, M., Skopalová, K., Víchová, Z., Kašpárková, V., Humpolíček, P., 2024. *Method for in situ polypyrrole coating, and the example of its use for functionalization of polyurethane anisotropic electrospun mats.* Heliyon 10. https://doi.org/10.1016/j.heliyon.2024.e27883**.**

The simplicity of this PPy thin layer synthesis method makes it well-suited for routine use or large-scale production. Scientific progress often depends not only on the publication of methods that can be reproduced by other researchers. The value of this article lies not only in the introduction of a novel procedure for the preparation of PPy films, which in itself represents a significant scientific contribution, but also in the clear documentation of the specific skills required for the successful fabrication of these films. In particular, it highlighted the necessity of maintaining a very short polymerization time, which is critical for achieving reproducible results. Also, due to the reaction's rapid pace, precise control of polymerization time is crucial. As shown in Article I, prolonged

reaction leads to the growth of PPy particles until they detach from the surface, compromising the coating continuity. Conversely, the earlier the polymerization procedure is terminated, which can be done by removing the reaction solution and rinsing the newly formed PPy with hydrochloric acid, the more uniform PPy coating layer is manufactured.

Additionally, PPy coatings synthesized within only 15 seconds demonstrated favorable conductivity levels, specifically in higher tens of S/cm. These are relatively good conductivity results since similar PPy products may occupy a range beginning at zero or approximately 10^{-10} S/cm, ending with less than 2×10^{3} S/cm. (Gh et al., 2017; Guimard et al., 2007; Mahmoodian et al., 2015; Navale et al., 2014; Pang et al., 2021; Sasso et al., 2011; Thunberg et al., 2015). To establish the conductivity of PPy thin layers, the four-point van der Pauw method was utilized as it is commonly used for measuring the resistivity of thin-shaped materials. (Kašpárková et al., 2017; Patois et al., 2010) However, the drawback of this method is that it is strongly dependent on the film thickness.

To measure the true thickness of PPy thin-layer coatings, the clear edge had to be created first. Figure 8.1 shows how this was achieved. A silicon mask was placed to the centre of a TCD and firmly clamped to its surface. Then, the *in situ* oxidative polymerization of PPy was carried out as usual. After the reaction termination, it was left to dry (still clamped), and only after that, the mask was removed, revealing the rectangular shape of the pristine TCD surface with clear edges, where the thickness of the PPy film was then determined by profilometry.

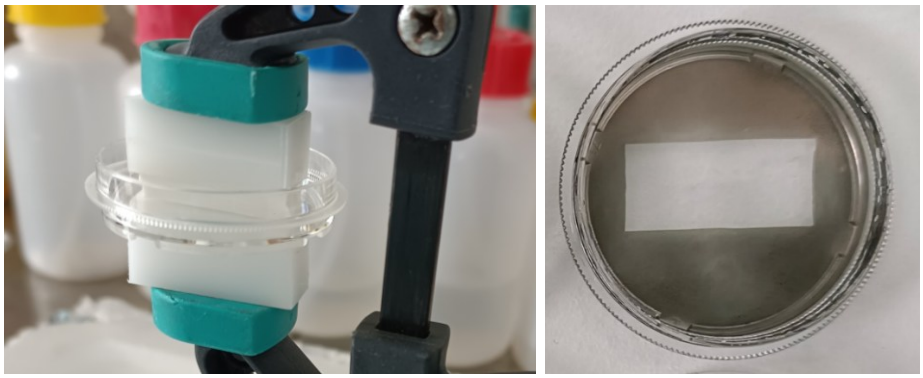

Figure 8.1 Creation of edges in PPy film: (left) the silicon mask placed and secured in the middle of a TCD, (right) the resulted PPy film after the mask removal. (Figure made by the author of this thesis).

8.2 Polyazulene holds great potential in tissue engineering

Polyazulene is particularly intriguing due to its unique nonbenzenoid structure, which imparts unconventional electronic properties along with potentially favorable mechanical and optoelectronic behavior; however, despite these promising characteristics, it has not yet been explored within the field of biomedicine, making it a novel and entirely untapped material in this context. An article addressing this issue was prepared and submitted for peer review. At the time of submitting this dissertation, the article had not been published yet. Therefore, the methodology and results are not presented here; however, the article will be available for inspection during the thesis defence: **Mahelová, L.,** Trchová, M., Kotowicz, S., Škoda, D., Kocourková, K., Víchová, Z., Vícha, J., Kašpárková, V., Humpolíček, P., 2025. *Is polyazulene cytocompatible? It depends.* Beyond the scope of the paper, I can present the following results.

In addition to chemical polymerization, PAz can be also prepared *via* electrochemical polymerization. Due to the lack of suitable equipment, it was not possible to perform this synthesis at the Centre of Polymer Systems. Therefore, the author attended an internship at the University of Silesia in Katowice, where the electrochemical synthesis of PAz was carried out. The most significant influence was observed with changing azulene concentration. The differences in the PAZ films are notable in Figure 8.2 and 8.3.

Figure 8.2 Electrochemicaly prepared PAz: (left) the standard three-electrode cell with coiled Pt counter electrode, ITO glass working electrode, and Ag reference electrode; (right) films on ITO glass prepared from azulene concentrations 5×10^{-4}, 1×10^{-3}, and 5×10^{-3} mol/dm^3 respectively. (Figure made by the author of this thesis).

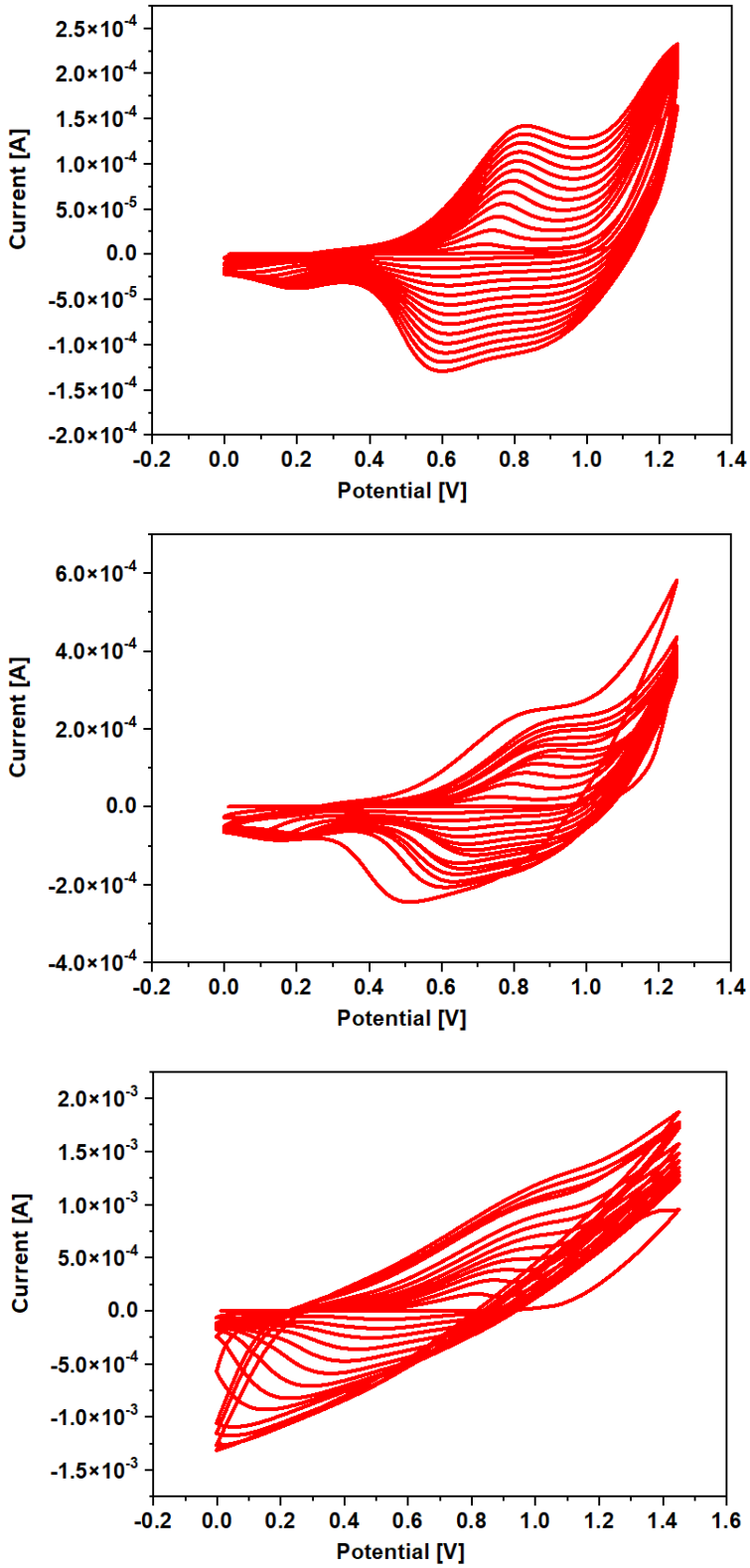

Figure 8.3 Electrochemicaly prepared PAz: Cyclic voltametry of azulene oxidation and electrochemical PAz polymerization on ITO glass in 13 cycles with 100 mV/s scan rate and different azulene concentrations: (up) 5×10^{-4} mol/dm^3, (middle) 1×10^{-3} mol/dm^3, (down) 5×10^{-3} mol/dm^3. (Figure made by the author of this thesis).

Figure 8.3 shows a voltammetric plot of the oxidation of azulene at various concentrations and the subsequent formation of PAz. It is evident that at the lowest concentration, the cyclic voltammetric curves showed low, well-defined peaks, indicating slow, controlled film growth. As the concentration increased, the peak currents increased and the voltammograms broadened, indicating faster polymerization and thicker film formation. At the highest concentration, the curves were distorted by high capacitive currents, indicating rapid, less controlled deposition and possible electrode passivation.

Electrochemical synthesis has proven effective for producing conductive PAz films; however, it also presents limitations – the requirement for conductive substrates such as platinum and other metals or ITO, FTO glasses, both of which are not suitable for TE applications since most tissues are soft tissues. However, a flexible alternative has emerged lately: ITO coated PET – transparent, conductive and flexible. (Krukiewicz et al., 2023; Nur Hidayah et al., 2024)

8.3 Polyurethane scaffolds benefit from anisotropic structure

Since the main aim of this thesis is to prepare and characterize biomaterials in respect to electrosensitive TE, elastic materials were sought first. A set of various PU fibrous scaffolds were electrospun on Nanospider device in cooperation with colleagues at the Centre of polymer systems. Primary cytotoxicity testing showed differences between various PUs used and also emphasized the importance of thorough washing (at least 10 days in ultrapure water with changes every second day). The reason behind this lies in removal of remaining solvents that may be cytotoxic, salts that may impare pH of the environment, and other potentionally impurities.

All PU scaffolds were originally isotropic, featuring randomly oriented fibers. However, the anisotropy of biomaterials plays a critical role, as the ECM *in vivo* is typically anisotropic. (Hoque, 2017) This property is particularly important especially when replicating myocardium architecture. Cardiomyocytes cultured in anisotropic environments develop aligned actin filaments, organized sarcomeres, and elongated nuclei (Bursac N. et al., 2002) and their contractile function is closely tied to cell alignment and elongation. (Nikkhah et al., 2012) To introduce anisotropy, PU scaffolds were manually stretched under heat.

Topographical anisotropy of the stretched PU scaffold was confirmed by SEM and AFM; however, what about mechanical anisotropy? The introduction of structural anisotropy into the PU scaffold presumably also evokes mechanical

anisotropy, which cells may sense and be affected by. To determine this, tensile testing was carried out. However, a few challenges arose in the process. First of all, cutting the PU scaffolds into a suitable and unite shape with clean edges was tried with several approaches: from laser cutting, over manual die cutting press, cutting with a heated scalpel, to regular cutting with scissors. None of these tactics worked perfectly; however, the use of scissors when handled diligently resulted in a sufficiently precise cut with minimum edge jams, which would act as a soft spot, causing a premature rupture. Another issue that complicated the tensile testing was the easy disruption of the PU scaffolds by the tensile device clamps and the constant slipping out of the clamps at the same time. This was overcome by taping the top and bottom edges with a regular tape. However, the taping had to be done carefully and precisely as well, because even small deviations were affecting the tensile testing results.

The tensile testing results are shown in Table 8.1 and Figure 8.4. Even though, there are indisputable deviations, the results showed mesurable differences. In comparison to the isotropic PU scaffold, anisotropic PU scaffold tested parallel to fibre orientation withstanded higher forces with elongation not significantly changed. On the other hand, the tensile test of anisotropic PU scaffold perpendicularly to fibre orientation led to notably higher elongation and lower stress build compared to isotropic PU scaffold. Therefore, the mechanical testing suggests mechanical anisotropy in the PU scaffolds with aligned fibres.

Table 8.1 Results of tensile testing (stress, force nad strain peaks) of isotropic and anisotropic PU scaffolds (tested parallel or perpendicular to fibre orientation). The values given are the mean with the standard deviation of the mean. (Table made by the author of this thesis).

	Stress peak [N/mm²]	**Force peak [N]**	**Strain peak [%]**
Isotropic PU scaffold	14	2,1	92
	± 4	± 0,6	± 18
Anisotropic PU scaffold **Parallel** to fibre orientation	19	2,9	100
	± 7	± 0,9	± 30
Anisotropic PU scaffold **Perpendicular** to fibre orientation	11	1,6	120
	± 3	± 0,4	± 30

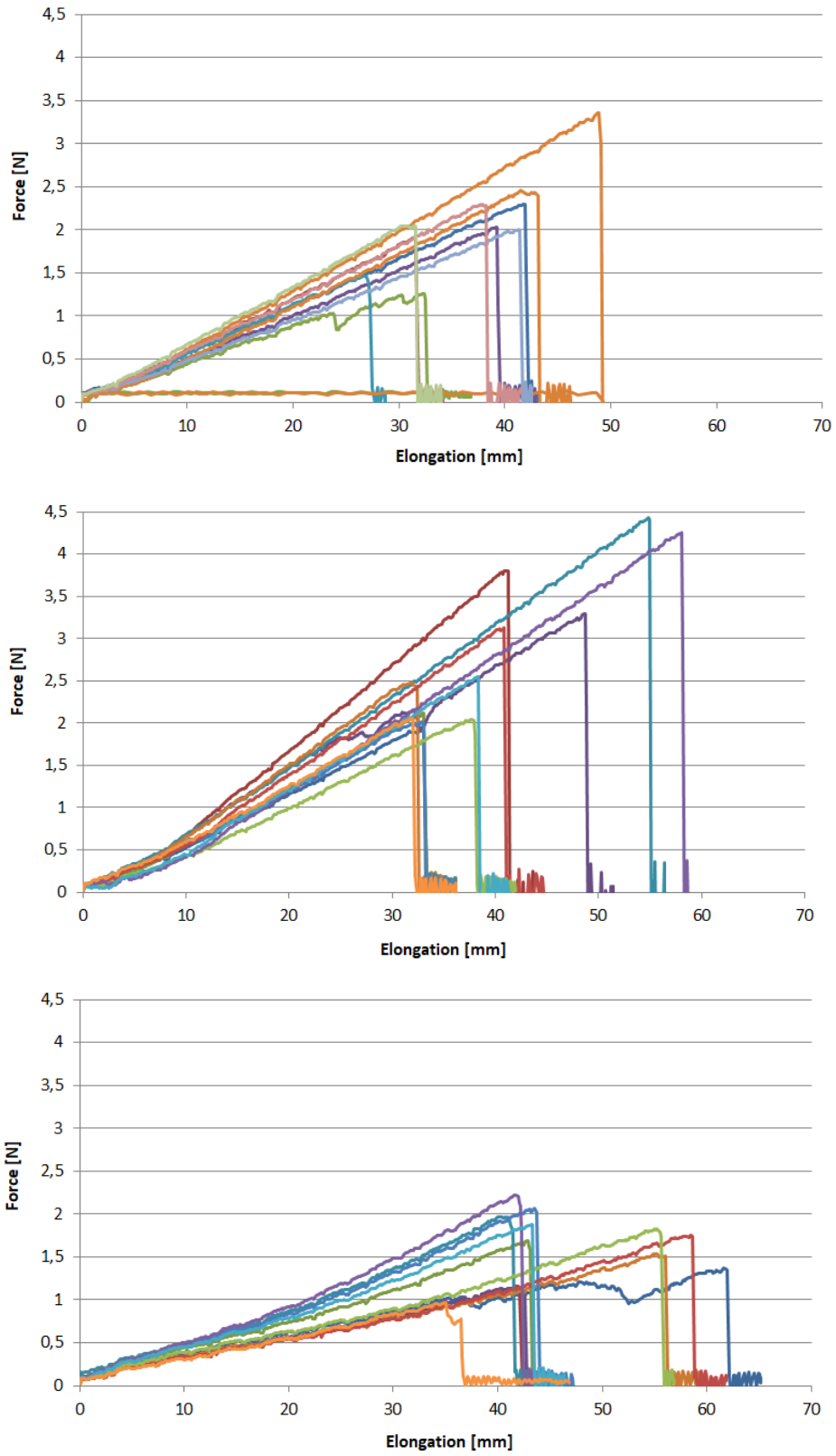

Figure 8.4 Graphs from tensile testings: (up) isotropic PU scaffold; (middle) anisotropic PU scaffold tested parallel to fibre orientation; (down) anisotropic PU scaffold tested perpendicular to fibre orientation. (Figure made by the author of this thesis).

To summarize it, PU scaffolds possess several cell-instructive cues: 1) mechanical properties mimicking natural tissue properties, in particular elasticity of cardiac muscle tissue; 2) structural and mechanical anisotropy mimicking the structure of ECM and significantly affecting *in vitro* cell behaviour; 3) conductivity from PPy coating that helps cells to communicate with each other and is especially intriguing for TE of electrosensitive tissues; 4) bioactivity given from protein coating enhancing cell-surface interactions and thus giving cells cues to adhere.

8.4 Mixed populations behave different from single cell lines

It is usual to culture cells of one selected cell line, but for some purposes, an advanced technique of co-culturing, creating mixed cellular populations, can be utilized.The employment of mixed cell populations not only supports the structural complexity of engineered tissues but also instructs the differentiation and functional promotion of specific cell types through cellular cross-talk and nutrient sharing within a microenvironment. (Bian et al., 2011; Zhang et al., 2015) Furthermore, it has been revealed that incorporating fibroblasts alongside cardiomyocytes is crucial for developing well-functioning cardiac tissues, as fibroblasts play an essential role in supporting cardiomyocyte viability and function. (Hookway et al., 2019; Tulloch et al., 2011)

Therefore, co-culturing of NIH/3T3 fibroblasts and ES R1 embryonic stem cells with LIF in media was applied to the PU scaffold in two different ways:

- Sequential mixed population – Fibroblasts seeded first and after 24 h, embryonic stem cells added, then co-cultured for 2 days.
- Simultaneous mixed population – Fibroblasts and embryonic stem cells seeded at once and co-cultured for 3 days.

Some of the results are shown in Figure 8.6. As expected, fibroblasts pre-cultured on the anisotropic PU scaffold created almost a monolayer of elongated cytoskeletons strictly oriented according to the orientation of fibres. Further, it can be noticed that stem cells seeded on the formed fibroblast monolayer formed clusters with round-like shapes as usual and of very various sizes. Controversially, stem cells seeded together with fibroblasts formed only big uniform clusters with the shape of an oval significantly oriented according to the orientation of fibres. In addition, fibroblasts seeded and grown together with stem cells resulted in even more prolonged and narrowed cytoskeletons.

These results suggest a synergistic effect where both material anisotropy and direct interaction with fibroblasts influence stem cell organization. This could be especially interesting when fibroblasts are co-cultured with stem cells or even embryoid bodies without the presence of LIF, potentially resulting in cell differentiation (without the use of signal molecules). The author will continue to address this topic in the bachelor's and master's theses she is currently supervising.

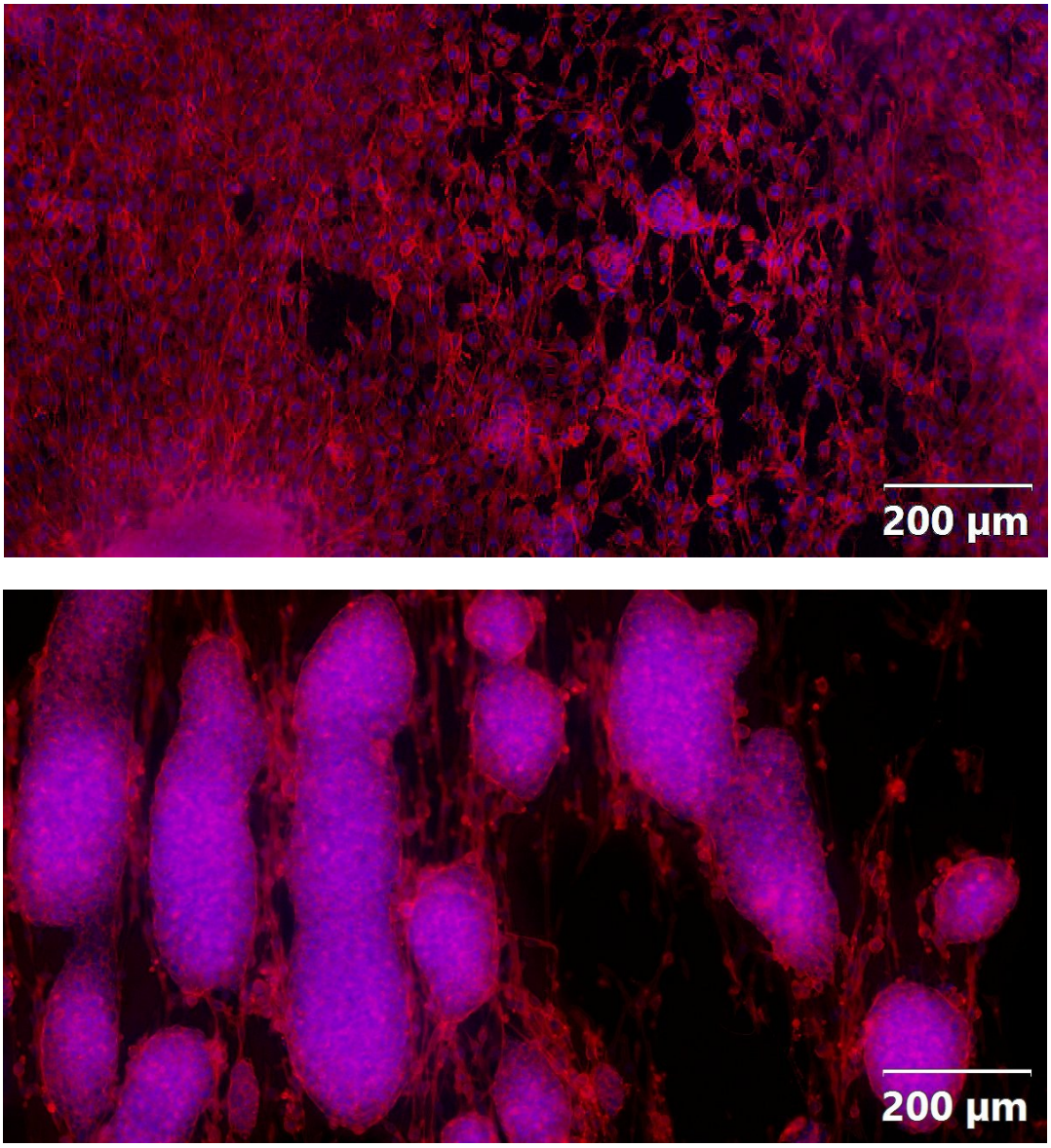

Figure 8.5 Mixed cell population of NIH/3T3 fibroblasts and ES R1 embryonic stem cells grown on anisotropic PU mats: (up) sequential mixed population; (down) simultaneous mixed population. Magnification: 100×, red color – cytoskeletons, blue color – nuclei. (Figure made by the author of this thesis).

8.5 Dynamic cultivation mimics *in vivo* environment

Electrical external stimuli

According to the literature, electrical stimulation effectively modulates cell behavior, promoting proliferation, differentiation, and alignment across various cell types, with proven benefits for osteogenesis, neuroregeneration, and cardiomyocyte function. (Hernández et al., 2016; Tandon et al., 2009; Xia et al., 2024) The research on electrical stimuli is important for TE of electrosensitive tissues, particularly in the context of the use of conductive polymeric substrates.

To create an electrical field in cell culture at the Centre of Polymer Systems, the power source of direct current stimulation was combined with self self-prepared cultivation system of electrodes incorporated in TCDs. Cells were then exposed to either constant or pulsed direct current electrical stimulation, with varying exposure/pause intervals.

The results showed that the combination of conductive substrate and electrical stimuli of the appropriate setup causes cellular changes that are induced by neither of them separately. To summarize it, fibroblasts elongated protrusions and embryonic stem cells (with LIF in medium) formed a less condensed cluster with branched edges.

Mechanical external stimuli

Hydrodynamic shear stress is a key mechanical stimulus in bioreactor systems, significantly influencing cell morphology, signaling, proliferation, and differentiation by mimicking natural *in vivo* physiologically relevant conditions absent in static cultures. (Matějka et al., 2020; Raimondi et al., 2006) Appropriate shear levels enhance tissue engineering outcomes, while excessive stress may trigger apoptosis and metabolic disruption. (Han and Yuan, 2009; Velez-Suberbie et al., 2013)

The Synthecon rotary cell culture system and the Ibidi pump system are the two devices that were used to apply hydrodynamic shear stress to cells. For the former, cells were pre-adhered to a substrate, which was then attached to a self-made holder and placed into the dynamic chamber that rotated at a controlled rate. For the latter, the cells were seeded directly into the narrow testing chamber through which the cell culture media was pumped at a controlled rate. Both systems allowed for the continuous circulation of culture medium over cell monolayers, creating controlled hydrodynamic shear stress conditions that will be further studied by the author and her students.

Multiple external stimuli combined

When different stimuli act on cells simultaneously, a synergistic effect on cellular processes can occur. Moreover, such bioreactors provide the most faithful imitation of the *in vivo* dynamic environment. One such system is the Ebers TC-3 bioreactor, which allows the application of: hydrodynamic shear stress from the flowing medium, electric current supplied by electrodes, and also compressive strain by compression of the scaffold. The compression deformation of the bioreactor was replaced by tensile deformation with a custom 3D printed structure. It was designed and manufactured in collaboration with colleagues from the Faculty of Technology, Tomas Bata University in Zlín, so that it fits into a dynamic chamber (see Figure 8.6) and enables scaffold attachement and stretching.

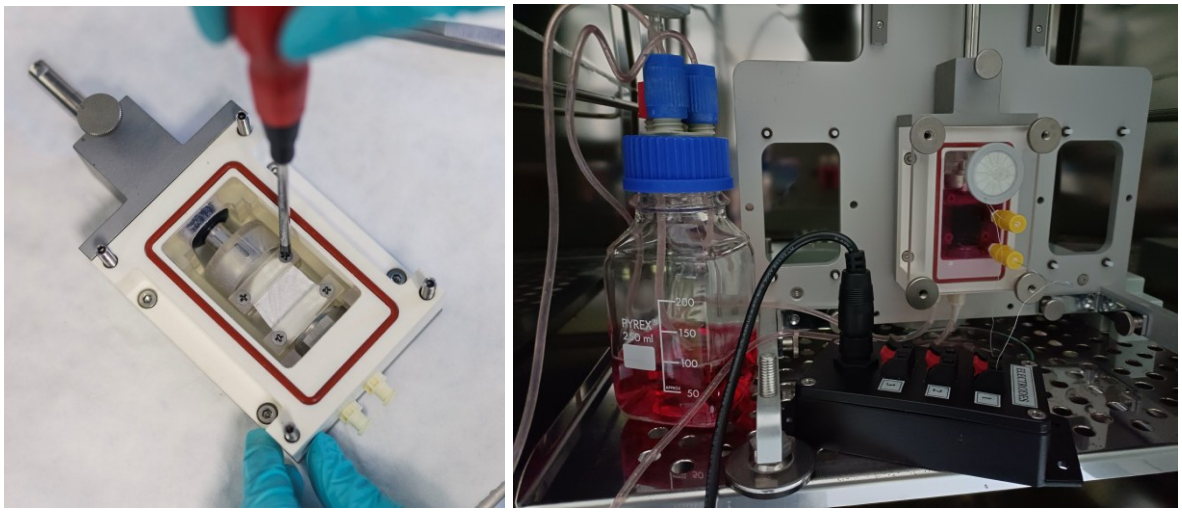

Figure 8.6 TC-3 bioreactor from Ebers: (left) a dynamic cultivation chamber with costum 3D printed holder and PU scaffold attached; (right) the chamber connected to the motion structure, the electrical and medium circuits of the bioreactor system. (Figure made by the author of this thesis).

The initiation of experiments in this bioreactor system was accompanied by a number of significant challenges. After optimizing the process, it was still difficult to achieve consistent results, especially with embryonic stem cells. Stem cells naturally form clusters in which cells attach to other cells more strongly than to the surface of the scaffold. (Abdal Dayem et al., 2018; Mustafa et al., 2022) Thus, the cell clusters are easily released from the scaffold when the flow rate of the medium is slightly increased or when the scaffold is inadvertently bent during handling.

This problem was eventually overcome by using a mixed cell population. Figure 8.7 presents the results of dynamic cultivation of a sequential mixed population of fibroblasts and embryonic stem cells grown on an anisotropic PU scaffold coated with PPy, compared to a reference cells in TCDs under static conditions.

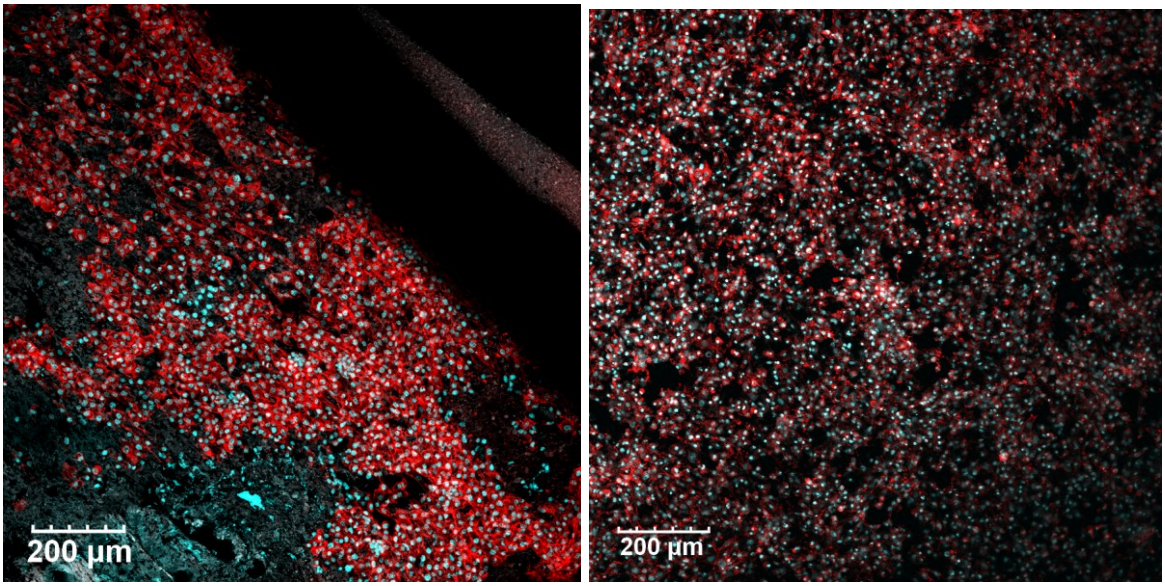

Figure 8.7 Sequential mixed cell population of NIH/3T3 fibroblasts and ES R1 embryonic stem cells grown on: (left) anisotropic PU scaffold coated with PPy under dynamic cultivation conditions of the Ebers TC-3 bioreactor; (right) reference TCD under static cultivation conditions. Magnification: 25×, red color – cytoskeletons, blue color – nuclei. (Figure made by the author of this thesis).

8.6 Image analysis answers 'all' cell-evaluation related questions

Standard evaluation techniques used by the author of this thesis in laboratory practice showed limitation in regard to processing cytocompatibility results of cells seeded on PU/PPy/albumin/gelatin composites. Standard cytocompatibility tests failed due to adverse interactions with the tested materials, for example, MTT solvent damages PU and PPy, ATP assays are disrupted by protein coatings, and neutral red uptake is highly sensitive to serum proteins. Detection methods like UV-VIS spectrophotometry and flow cytometry require cell detachment, which proved unreliable due to strong cell adhesion to PU scaffolds. Additionally, microscopy-based evaluation of cell morphology and migration is time-consuming, subjective, and lacks reproducibility without automation.

All of here addressed limitations can be addressed by image analysis. Cell image analysis offers a comprehensive solution to overcome the limitations encountered during conventional cytocompatibility testing as well as enables quantification of subtle morphological responses beyond the capabilities of conventional assays. Several software programs providing this service exist, but the most versatile and free at the same time is the open-sourced **CellProfiler**. (Carpenter et al., 2006)

Unlike manual cell counting or subjective morphological evaluations, CellProfiler provides automated, high-throughput, and reproducible image analysis across a wide range of experimental conditions.

The author of this thesis used CellProfiler as an alternative to conventional cell viability assays, as shown in ArticleI. To make the quantification of cells on the PU scaffolds precise and reliable, the unified surface area of (22 × 22) mm^2 had to be prepared, seeded, and the total area (forty images of cell nuclei with 40× magnification for each observed sample) was manually acquired and then processed in CellProfiler.

Cell image analysis also enables reliable segmentation of individual cells and objective quantification of multiple parameters simultaneously, such as cell area, circularity, texture, orientation, fluorescence intensity, compactness, and much more. In addition, the software can analyze time-lapse microscopy data, making it particularly powerful for quantifying cell migration, tracking, and wound healing assays.

CellProfiler allows the user to create customizable pipelines without requiring advanced programming skills, while still offering the flexibility needed for complex image-based experiments. One of its main advantages is that it is open-source, freely available, and supported by an active user community. However, challenges may arise during segmentation in low-quality or highly heterogeneous images, and optimization of pipelines can be time-consuming for beginners. Despite these, CellProfiler remains a valuable and robust tool for objective and quantitative cell analysis in biomaterials research.

CONTRIBUTION TO SCIENCE

As demonstrated in this dissertation, a broad range of polymers can be employed in tissue engineering, accompanied by numerous methods to produce, combine, and modify them. Even minor adjustments in the synthesis or processing of these polymers can substantially affect cellular behavior. Moreover, cultivation conditions exert a strong influence on cell physiology. Given the interaction of so many variables, the relationship between polymers and cells is both intricate and sensitive. The work presented here focuses on gaining a deeper understanding of this relationship, particularly in the context of developing electrosensitive tissues. The primary objective has been to identify how material design and cultivation conditions can be tuned to guide and support cells in forming functional, healthy tissue constructs.

At the beginning of the doctoral studies, the author already possessed a set of skills required for fundamental experimental work in laboratories of cell biology, gained through previous experience during bachelor's and master's theses. When the COVID-19 pandemic disrupted regular research workflows, further expertise in academic research management and project preparations was acquired and applied in practice right away (as the lead researcher on a project).

One of the most significant contributions of this dissertation lies in advancing knowledge of the synthesis and characterization of conductive polymers. The author not only prepared polypyrrole thin films with great electrical properties, which is important for the engineering of electrosensitive tissues, but also dug into the practical side of using them in tissue engineering. In particular, the author optimized their synthesis to show how easily they can be prepared and adjusted for successful cell seeding.

Especially new insights were gained with polyazulene, a generally less studied conductive polymer. Due to the lack of information on chemical synthesis protocols, she had to investigate polyazulene empirically. In order to obtain electrochemically prepared polyazulene, she traveled abroad to the University of Silesia in Katowice, Poland, where she became familiar with this method. This work ultimately led to the first study determining the cytocompatibility of polyazulene, which, to the author's knowledge, has not been addressed to date. This opens up exciting new possibilities for future research using polyazulene in tissue engineering.

Another key component of this research involved the development of bioactive fibrous scaffolds – mesh-like structures that provide surfaces for cell attachment and spatial organization during growth. These scaffolds were designed to offer multiple cell-instructive cues: 1) an elastic matrix mimicking the stretchable properties of native tissues, 2) uniaxially aligned fibers reflecting the anisotropic architecture of natural tissues, 3) a polypyrrole coating providing electrical conductivity, and 4) an albumin/gelatin coating emulating the composition of the natural extracellular environment.

Cytocompatibility experiments demonstrated, for instance, that alignment of fibers facilitated easier cell attachment and promoted directional growth along the fiber axis. This effect is particularly beneficial for constructing organized tissues such as myocardium, where cells naturally align in a similar fashion. Additionally, anisotropic scaffolds fabricated from polyvinylidene fluoride exhibited promising potential due to their combination of cytocompatibility and piezoelectric properties. These characteristics make them highly suitable for applications in cardiac and neural tissue engineering.

It is important to note that performing reliable experiments and evaluating cell behavior on these scaffolds posed several challenges. Difficulties such as static electricity, scaffold floating in the culture medium, and low surface wettability initially made it difficult for cells to attach and proliferate. Once these technical hurdles were resolved, the research progressed to investigations on how various types of dynamic stimulation (electrical, magnetic, and mechanical) influence cell behavior on the scaffolds. The rationale for applying such stimuli was to better mimic the dynamic environment experienced by cells in living tissues. The most complex and ambitious experiments involved combining multiple stimuli simultaneously, including hydrodynamic shear stress from media flow, tensile stress from scaffold stretching, and electrical stimulation applied to conductive surfaces. As documented in the dissertation, the implementation of this multifactorial system required extensive optimization due to numerous technical complications. Ultimately, successful cell culture was achieved in a multi-stimuli bioreactor using the multi cell-instructive scaffolds. A key finding of this work was that the creation of mixed cell populations helped embryonic stem cells to survive even under these demanding dynamic conditions.

To effectively analyze the extensive data generated and to overcome limitations in assessing cell viability on conductive thin films and scaffolds, advanced image analysis software was introduced into the cellular laboratory workflow. A comprehensive image analysis pipeline was developed and refined, enabling detailed evaluation of cell viability, morphology, and migration. Once optimized, this methodology proved to be a powerful and reliable tool, with strong potential for future routine use in studies of cell–material interactions.

In summary, this dissertation provides a cohesive account of how conductive polymers, smart scaffold designs, and dynamic cultivation conditions can be integrated to develop more life-like tissue constructs. The discoveries and methodologies presented here establish a solid foundation for continued research aimed at engineering functional tissue replacements. While the potential applications are broad, the findings are particularly promising for cardiac tissue repair and for advancing regenerative medicine more generally.

REFERENCES

Abdal Dayem, A., Lee, S., Y. Choi, H., Cho, S.-G., 2018. The Impact of Adhesion Molecules on the In Vitro Culture and Differentiation of Stem Cells. Biotechnol. J. 13, 1700575. https://doi.org/10.1002/biot.201700575

Adadi, N., Yadid, M., Gal, I., Asulin, M., Feiner, R., Edri, R., Dvir, T., 2020. Electrospun Fibrous PVDF-TrFe Scaffolds for Cardiac Tissue Engineering, Differentiation, and Maturation. Adv. Mater. Technol. 5, 1900820. https://doi.org/10.1002/admt.201900820

Ateh, D. d, Navsaria, H. a, Vadgama, P., 2006. Polypyrrole-based conducting polymers and interactions with biological tissues. J. R. Soc. Interface 3, 741–752. https://doi.org/10.1098/rsif.2006.0141

Baker, T.L., Goodwin, T.J., 1997. Three-dimensional culture of bovine chondrocytes in rotating-wall vessels. Vitro Cell. Dev. Biol. - Anim. 33, 358–365. https://doi.org/10.1007/s11626-997-0006-5

Bardajee, G.R., Hooshyar, Z., 2014. One-pot synthesis of biocompatible superparamagnetic iron oxide nanoparticles/hydrogel based on salep: Characterization and drug delivery. Carbohydr. Polym. 101, 741–751. https://doi.org/10.1016/j.carbpol.2013.10.028

Ben Ayed, E., Ghorbel, N., Kallel, A., Putaux, J.-L., Boufi, S., 2022. Polyaniline-Grafted Chitin Nanocrystals as Conductive Reinforcing Nanofillers for Waterborne Polymer Dispersions. Biomacromolecules 23, 4167–4178. https://doi.org/10.1021/acs.biomac.2c00635

Berg, J., Eriksson, L., Claesson, P., Borve, K., 1994. 3-Component Langmuir-Blodgett-Films with a Controllable Degree of Polarity. Langmuir 10, 1225–1234. https://doi.org/10.1021/la00016a041

Bernhardt, A., Lode, A., Peters, F., Gelinsky, M., 2011. Optimization of culture conditions for osteogenically-induced mesenchymal stem cells in β-tricalcium phosphate ceramics with large interconnected channels. J. Tissue Eng. Regen. Med. 5, 444–453. https://doi.org/10.1002/term.331

Beygisangchin, M., Abdul Rashid, S., Shafie, S., Sadrolhosseini, A.R., Lim, H.N., 2021a. Preparations, Properties, and Applications of Polyaniline and Polyaniline Thin Films—A Review. Polymers 13, 2003. https://doi.org/10.3390/polym13122003

Beygisangchin, M., Abdul Rashid, S., Shafie, S., Sadrolhosseini, A.R., Lim, H.N., 2021b. Preparations, Properties, and Applications of Polyaniline and Polyaniline Thin Films—A Review. Polymers 13, 2003. https://doi.org/10.3390/polym13122003

Bian, L., Zhai, D.Y., Mauck, R.L., Burdick, J.A., 2011. Coculture of Human Mesenchymal Stem Cells and Articular Chondrocytes Reduces Hypertrophy and Enhances Functional Properties of Engineered Cartilage. Tissue Eng. Part A 17, 1137–1145. https://doi.org/10.1089/ten.tea.2010.0531

Borah, R., Das, J.M., Upadhyay, J., 2022. Surface Functionalized Polyaniline Nanofibers:Chitosan Nanocomposite for Promoting Neuronal-like Differentiation of Primary Adipose Derived Mesenchymal Stem Cells and Urease Activity. ACS Appl. Bio Mater. 5, 3193–3211. https://doi.org/10.1021/acsabm.2c00171

Boukamp, P., Petrussevska, R.T., Breitkreutz, D., Hornung, J., Markham, A., Fusenig, N.E., 1988. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. J. Cell Biol. 106, 761–771. https://doi.org/10.1083/jcb.106.3.761

Bursac N., Parker K.K., Iravanian S., Tung L., 2002. Cardiomyocyte Cultures With Controlled Macroscopic Anisotropy. Circ. Res. 91, e45–e54. https://doi.org/10.1161/01.RES.0000047530.88338.EB

Capáková, Z., Radaszkiewicz, K.A., Acharya, U., Truong, T.H., Pacherník, J., Bober, P., Kašpárková, V., Stejskal, J., Pfleger, J., Lehocký, M., Humpolíček, P., 2020. The biocompatibility of polyaniline and polypyrrole 21: Doping with organic phosphonates. Mater. Sci. Eng. C 113, 110986. https://doi.org/10.1016/j.msec.2020.110986

Carpenter, A.E., Jones, T.R., Lamprecht, M.R., Clarke, C., Kang, I.H., Friman, O., Guertin, D.A., Chang, J.H., Lindquist, R.A., Moffat, J., Golland, P., Sabatini, D.M., 2006. CellProfiler: image analysis software for identifying and quantifying cell phenotypes. Genome Biol. 7, R100. https://doi.org/10.1186/gb-2006-7-10-r100

Chen, H.-C., Hu, Y.-C., 2006. Bioreactors for tissue engineering. Biotechnol. Lett. 28, 1415–1423. https://doi.org/10.1007/s10529-006-9111-x

Chen, X., Issi, J.-P., Devaux, J., Billaud, D., 1995. Chemically oxidized polypyrrole: Influence of the experimental conditions on its electrical conductivity and morphology. Polym. Eng. Sci. 35, 642–647. https://doi.org/10.1002/pen.760350803

Cheng, K.-C., Sun, Y.-M., Hsu, S., 2023. Development of double network polyurethane–chitosan composite bioinks for soft neural tissue engineering. J. Mater. Chem. B. https://doi.org/10.1039/D3TB00120B

Citi, S., 2019. The mechanobiology of tight junctions. Biophys. Rev. 11, 783–793. https://doi.org/10.1007/s12551-019-00582-7

Cui, Z., Ni, N.C., Wu, J., Du, G.-Q., He, S., Yau, T.M., Weisel, R.D., Sung, H.-W., Li, R.-K., 2018. Polypyrrole-chitosan conductive biomaterial synchronizes cardiomyocyte contraction and improves myocardial electrical impulse propagation. Theranostics 8, 2752–2764. https://doi.org/10.7150/thno.22599

Cvek, M., Zahoranova, A., Mrlik, M., Sramkova, P., Minarik, A., Sedlacik, M., 2020. Poly(2-oxazoline)-based magnetic hydrogels: Synthesis, performance and cytotoxicity. Colloids Surf. B Biointerfaces 190, 110912. https://doi.org/10.1016/j.colsurfb.2020.110912

Du, V., Luciani, N., Richard, S., Mary, G., Gay, C., Mazuel, F., Reffay, M., Menasché, P., Agbulut, O., Wilhelm, C., 2017. A 3D magnetic tissue stretcher for remote mechanical control of embryonic stem cell differentiation. Nat. Commun. 8, 400. https://doi.org/10.1038/s41467-017-00543-2

Du, X., Chen, Y., Dong, W., Han, B., Liu, M., Chen, Q., Zhou, J., 2016. A nanocomposite-based electrochemical sensor for non-enzymatic detection of hydrogen peroxide. Oncotarget 8, 13039–13047. https://doi.org/10.18632/oncotarget.14308

Fee, T., Downs, C., Eberhardt, A., Zhou, Y., Berry, J., 2016. Image-based quantification of fiber alignment within electrospun tissue engineering scaffolds is related to mechanical anisotropy. J. Biomed. Mater. Res. A 104, 1680–1686. https://doi.org/10.1002/jbm.a.35697

Firda, P.B.D., Jeon, J.-W., 2022. Recovery of Electrochemical Properties of Polyaniline-Based Multilayer Films with Improved Electrochemical Stability. ACS Appl. Polym. Mater. 4, 4850–4859. https://doi.org/10.1021/acsapm.2c00445

Firda, P.B.D., Malik, Y.T., Oh, J.K., Wujcik, E.K., Jeon, J.-W., 2021. Enhanced Chemical and Electrochemical Stability of Polyaniline-Based Layer-by-Layer Films. Polymers 13, 2992. https://doi.org/10.3390/polym13172992

Fu, J., Wang, Y.-K., Yang, M.T., Desai, R.A., Yu, X., Liu, Z., Chen, C.S., 2010. Mechanical regulation of cell function with geometrically modulated elastomeric substrates. Nat. Methods 7, 733–736. https://doi.org/10.1038/nmeth.1487

Gajendiran, M., Choi, J., Kim, S.-J., Kim, Keongsoo, Shin, H., Koo, H.-J., Kim, Kyobum, 2017. Conductive biomaterials for tissue engineering applications. J. Ind. Eng. Chem. 51, 12–26. https://doi.org/10.1016/j.jiec.2017.02.031

Gao, H., Ge, C., Hou, B., Xin, H., Gao, X., 2019. Incorporation of 1,3-Free-2,6-Connected Azulene Units into the Backbone of Conjugated Polymers: Improving Proton Responsiveness and Electrical Conductivity. ACS Macro Lett. 8, 1360–1364. https://doi.org/10.1021/acsmacrolett.9b00657

German, N., Ramanaviciene, A., Ramanavicius, A., 2019. Formation of Polyaniline and Polypyrrole Nanocomposites with Embedded Glucose Oxidase and Gold Nanoparticles. Polymers 11, 377. https://doi.org/10.3390/polym11020377

Gh, D., Kong, D., Gautrot, J., Vootla, S.K., 2017. Fabrication and Characterization of Conductive Conjugated Polymer-Coated Antheraea mylitta Silk Fibroin Fibers for Biomedical Applications. Macromol. Biosci. 17, 1600443. https://doi.org/10.1002/mabi.201600443

Golgovici, F., Cârlan, M.-S., Popescu, A.-G., Anicai, L., 2020. Electrochemical Synthesis of Polypyrrole and Polypyrrole-Indomethacin Coatings on NiCr Alloys Involving Deep Eutectic Solvents. Metals 10, 1130. https://doi.org/10.3390/met10091130

Gorgol, D., Mrlík, M., Mikulka, F., Víchová, Z., Mahelová, L., Ilčíková, M., Minařík, A., 2024. Smart Biopolymer Scaffolds Based on Hyaluronic Acid and Carbonyl Iron Microparticles: 3D Printing, Magneto-Responsive, and Cytotoxicity Study. ACS Appl. Bio Mater. 7, 7483–7493. https://doi.org/10.1021/acsabm.4c00567

Grądzka, E., Makowska, P., Winkler, K., 2018. Chemically formed conducting polyazulene: From micro- to nanostructures. Synth. Met. 246, 115–121. https://doi.org/10.1016/j.synthmet.2018.10.002

Grancarić, A.M., Jerković, I., Koncar, V., Cochrane, C., Kelly, F.M., Soulat, D., Legrand, X., 2018. Conductive polymers for smart textile applications. J. Ind. Text. 48, 612–642. https://doi.org/10.1177/1528083717699368

Guarino, V., Zuppolini, S., Borriello, A., Ambrosio, L., 2016. Electro-Active Polymers (EAPs): A Promising Route to Design Bio-Organic/Bioinspired Platforms with on Demand Functionalities. Polymers 8, 185. https://doi.org/10.3390/polym8050185

Guimard, N.K., Gomez, N., Schmidt, C.E., 2007. Conducting polymers in biomedical engineering. Prog. Polym. Sci., Polymers in Biomedical Applications 32, 876–921. https://doi.org/10.1016/j.progpolymsci.2007.05.012

Hammond, T.G., Hammond, J.M., 2001. Optimized suspension culture: the rotating-wall vessel. Am. J. Physiol.-Ren. Physiol. 281, F12–F25. https://doi.org/10.1152/ajprenal.2001.281.1.F12

Han, P.-P., Yuan, Y.-J., 2009. Metabolic profiling as a tool for understanding defense response of Taxus Cuspidata cells to shear stress. Biotechnol. Prog. 25, 1244–1253. https://doi.org/10.1002/btpr.209

He, X., Li, L., Tang, M., Zeng, Y., Li, H., Yu, X., 2019. Biomimetic electrical stimulation induces rat bone marrow mesenchymal stem cells to differentiate into cardiomyocyte-like cells via TGF-beta 1 in vitro. Prog. Biophys. Mol. Biol., Biomembranes and Related Systems: From Molecular Structure to Cellular Function 148, 47–53. https://doi.org/10.1016/j.pbiomolbio.2017.09.023

Hernández, D., Millard, R., Sivakumaran, P., Wong, R.C.B., Crombie, D.E., Hewitt, A.W., Liang, H., Hung, S.S.C., Pébay, A., Shepherd, R.K., Dusting, G.J., Lim, S.Y., 2016. Electrical Stimulation Promotes Cardiac Differentiation of Human Induced Pluripotent Stem Cells. Stem Cells Int. 2016, 1718041. https://doi.org/10.1155/2016/1718041

Hippler, M., Lemma, E.D., Bertels, S., Blasco, E., Barner-Kowollik, C., Wegener, M., Bastmeyer, M., 2019. 3D Scaffolds to Study Basic Cell Biology. Adv. Mater. 31, 1808110. https://doi.org/10.1002/adma.201808110

Hirata, A., Takano, Y., Kamimura, Y., Fujiwara, O., 2010. Effect of the averaging volume and algorithm on the in situ electric field for uniform electric- and magnetic-field exposures. Phys. Med. Biol. 55, N243. https://doi.org/10.1088/0031-9155/55/9/N03

Hookway, T.A., Matthys, O.B., Mendoza-Camacho, F.N., Rains, S., Sepulveda, J.E., Joy, D.A., McDevitt, T.C., 2019. Phenotypic Variation Between Stromal Cells Differentially Impacts Engineered Cardiac Tissue Function. Tissue Eng. Part A 25, 773–785. https://doi.org/10.1089/ten.tea.2018.0362

Hoque, M.E., 2017. Robust formulation for the design of tissue engineering scaffolds: A comprehensive study on structural anisotropy, viscoelasticity and degradation of 3D scaffolds fabricated with customized desktop robot based rapid prototyping (DRBRP) system. Mater. Sci. Eng. C 72, 433–443. https://doi.org/10.1016/j.msec.2016.11.019

Hou, B., Li, J., Zhou, Z., Tan, W.L., Yang, X., Zhang, J., McNeill, C.R., Ge, C., Wang, J., Gao, X., 2022. Incorporation of Electron-Rich Indacenodithiophene Units into the Backbone of 2,6-Azulene-Based Conjugated Polymers for Proton-Responsive Materials and p-Type Polymeric Semiconductors. ACS Mater. Lett. 4, 392–400. https://doi.org/10.1021/acsmaterialslett.1c00767

Huang, J., Lu, L., Zhang, J., Hu, X., Zhang, Y., Liang, W., Wu, S., Luo, Z., 2012. Electrical Stimulation to Conductive Scaffold Promotes Axonal Regeneration and Remyelination in a Rat Model of Large Nerve Defect. PLoS ONE 7, e39526. https://doi.org/10.1371/journal.pone.0039526

Humpolicek, P., Kasparkova, V., Pachernik, J., Stejskal, J., Bober, P., Capakova, Z., Radaszkiewicz, K.A., Junkar, I., Lehocky, M., 2018a. The biocompatibility of polyaniline and polypyrrole: A comparative study of their cytotoxicity, embryotoxicity and impurity profile. Mater. Sci. Eng. C-Mater. Biol. Appl. 91, 303–310. https://doi.org/10.1016/j.msec.2018.05.037

Humpolicek, P., Radaszkiewicz, K.A., Capakova, Z., Pachernik, J., Bober, P., Kasparkova, V., Rejmontova, P., Lehocky, M., Ponizil, P., Stejskal, J., 2018b. Polyaniline cryogels: Biocompatibility of novel conducting macroporous material. Sci. Rep. 8, 135. https://doi.org/10.1038/s41598-017-18290-1

Hursán, D., Ábel, M., Baán, K., Fako, E., Samu, G.F., Nguyën, H.C., López, N., Atanassov, P., Kónya, Z., Sápi, A., Janáky, C., 2022. CO2 Conversion on N-Doped Carbon Catalysts via Thermo- and Electrocatalysis: Role of C–NOx Moieties. ACS Catal. 12, 10127–10140. https://doi.org/10.1021/acscatal.2c01589

Iwasaki, K., Matsumoto, K., Hino, S., Yasunami, M., 1993. Electrochemical polymerization of alkyl-substituted azulenes. Synth. Met., Proceedings of the International Conference on Science and Technology of Synthetic Metals 55, 1062–1066. https://doi.org/10.1016/0379-6779(93)90200-G

Jasenská, D., Kašpárková, V., Radaszkiewicz, K.A., Capáková, Z., Pacherník, J., Trchová, M., Minařík, A., Vajďák, J., Bárta, T., Stejskal, J., Lehocký, M., Truong, T.H., Moučka, R., Humpolíček, P., 2021. Conducting composite films based on chitosan or sodium hyaluronate. Properties and cytocompatibility with human induced pluripotent stem cells. Carbohydr. Polym. 253, 117244. https://doi.org/10.1016/j.carbpol.2020.117244

Jeong, J.-W., Shin, G., Park, S.I., Yu, K.J., Xu, L., Rogers, J.A., 2015. Soft Materials in Neuroengineering for Hard Problems in Neuroscience. Neuron 86, 175–186. https://doi.org/10.1016/j.neuron.2014.12.035

Jin, C., Wu, Y., Zhang, H., Xu, B., Liu, W., Ji, C., Li, P., Chen, Z., Chen, B., Li, J., Wu, X., Jiang, P., Hu, Y., Xiao, Z., Zhao, Y., Dai, J., 2022. Spinal cord tissue engineering using human primary neural progenitor cells and astrocytes. Bioeng. Transl. Med. 8, e10448. https://doi.org/10.1002/btm2.10448

Joung, I.S., Iwamoto, M.N., Shiu, Y.-T., Quam, C.T., 2006. Cyclic strain modulates tubulogenesis of endothelial cells in a 3D tissue culture model. Microvasc. Res. 71, 1–11. https://doi.org/10.1016/j.mvr.2005.10.005

Kaewchingduang, R., Paradee, N., Sirivat, A., Niamlang, S., 2019. Effects of conductive polyazulene and plasticizer embedded in deproteinized natural rubber transdermal patch on electrically controlled naproxen release-permeation. Int. J. Pharm. 561, 296–304. https://doi.org/10.1016/j.ijpharm.2019.02.046

Kamble, H., Vadivelu, R., Barton, M., Shiddiky, M.J.A., Nguyen, N.-T., 2018. Pneumatically actuated cell-stretching array platform for engineering cell patterns in vitro. Lab. Chip 18, 765–774. https://doi.org/10.1039/C7LC01316G

Kašpárková, V., Humpolíček, P., Capáková, Z., Bober, P., Stejskal, J., Trchová, M., Rejmontová, P., Junkar, I., Lehocký, M., Mozetič, M., 2017. Cell-compatible conducting polyaniline films prepared in colloidal dispersion mode. Colloids Surf. B Biointerfaces 157, 309–316. https://doi.org/10.1016/j.colsurfb.2017.05.066

Kašpárková, V., Humpolíček, P., Stejskal, J., Kopecká, J., Kuceková, Z., Moučka, R., 2016. Conductivity, impurity profile, and cytotoxicity of solvent-extracted polyaniline. Polym. Adv. Technol. 27, 156–161. https://doi.org/10.1002/pat.3611

Kihara, N., Nakayama, H., Fukutomi, T., 1997. True Polyazulene: Soluble Precursor of So-Called "Polyazulene." Macromolecules 30, 6385–6387. https://doi.org/10.1021/ma970510m

Kim, S., Jang, L.K., Jang, M., Lee, S., Hardy, J.G., Lee, J.Y., 2018. Electrically Conductive Polydopamine–Polypyrrole as High Performance Biomaterials for Cell Stimulation in Vitro and Electrical Signal Recording in Vivo. ACS Appl. Mater. Interfaces 10, 33032–33042. https://doi.org/10.1021/acsami.8b11546

Kitsara, M., Revet, G., Vartanian-Grimaldi, J.-S., Simon, A., Minguy, M., Miche, A., Humblot, V., Dufour, T., Agbulut, O., 2022. Cyto- and bio-compatibility assessment of plasma-treated polyvinylidene fluoride scaffolds for cardiac tissue engineering. Front. Bioeng. Biotechnol. 10.

Krukiewicz, K., Czerwińska-Główka, D., Turczyn, R.M., Blacha-Grzechnik, A., Vallejo-Giraldo, C., Erfurt, K., Chrobok, A., Faure-Vincent, J., Pouget, S., Djurado, D., Biggs, M.J.P., 2023. Flexible, Transparent, and Cytocompatible Nanostructured Indium Tin Oxide Thin Films for Bio-optoelectronic Applications. ACS Appl. Mater. Interfaces 15, 45701–45712. https://doi.org/10.1021/acsami.3c10861

Kucekova, Z., Humpolicek, P., Kasparkova, V., Perecko, T., Lehocký, M., Hauerlandová, I., Sáha, P., Stejskal, J., 2014. Colloidal polyaniline dispersions: Antibacterial activity, cytotoxicity and neutrophil oxidative burst. Colloids Surf. B Biointerfaces 116, 411–417. https://doi.org/10.1016/j.colsurfb.2014.01.027

Kulkarni, M., Junkar, I., Humpolíček, P., Capáková, Z., Radaszkiewicz, K.A., Mikušová, N., Pacherník, J., Lehocký, M., Iglič, A., Hanáčková, M., Mozetič, M., 2017. Interaction of nanostructured TiO2 biointerfaces with stem cells and biofilm-forming bacteria. Mater. Sci. Eng. C 77, 500–507. https://doi.org/10.1016/j.msec.2017.03.174

Latonen, R.-M., Meana Esteban, B., Kvarnström, C., Ivaska, A., 2009. Electrochemical polymerization and characterization of a poly(azulene)-TiO2 nanoparticle composite film. J. Appl. Electrochem. 39, 653–661. https://doi.org/10.1007/s10800-008-9705-1

Le, H.Q., Ghatak, S., Yeung, C.C., Tellkamp, F., Günschmann, C., Dieterich, C., Yeroslaviz, A., Habermann, B., Pombo, A., Niessen, C.M., Wickström, S.A., 2016. Mechanical regulation of transcription controls Polycomb-mediated gene silencing during lineage commitment. Nat. Cell Biol. 18, 864–875. https://doi.org/10.1038/ncb3387

Lee, C.H., Shin, H.J., Cho, I.H., Kang, Y.-M., Kim, I.A., Park, K.-D., Shin, J.-W., 2005. Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. Biomaterials 26, 1261–1270. https://doi.org/10.1016/j.biomaterials.2004.04.037

Lee, J.H., Yoon, Y.C., Kim, H.S., Lee, J., Kim, E., Findeklee, C., Katscher, U., 2022. In vivo electrical conductivity measurement of muscle, cartilage, and peripheral nerve

around knee joint using MR-electrical properties tomography. Sci. Rep. 12, 73. https://doi.org/10.1038/s41598-021-03928-y

Li, Y., Liao, C., Tjong, S.C., 2019. Electrospun Polyvinylidene Fluoride-Based Fibrous Scaffolds with Piezoelectric Characteristics for Bone and Neural Tissue Engineering. Nanomaterials 9, 952. https://doi.org/10.3390/nano9070952

Lins, L.C., Wianny, F., Livi, S., Dehay, C., Duchet-Rumeau, J., Gérard, J.-F., 2017. Effect of polyvinylidene fluoride electrospun fiber orientation on neural stem cell differentiation. J. Biomed. Mater. Res. B Appl. Biomater. 105, 2376–2393. https://doi.org/10.1002/jbm.b.33778

MacNee, W., 2006. Pathology, pathogenesis, and pathophysiology. BMJ 332, 1202–1204. https://doi.org/10.1136/bmj.332.7551.1202

Mahmoodian, M., Pourabbas, B., Mohajerzadeh, S., 2015. Effect of anionic dopants on thickness, morphology and electrical properties of polypyrrole ultra-thin films prepared by in situ chemical polymerization. Thin Solid Films 583, 255–263. https://doi.org/10.1016/j.tsf.2015.03.043

Maity, S., Sarkar, A., 2017. 4 - Monitoring fibrous capsule formation, in: Narayan, R.J. (Ed.), Monitoring and Evaluation of Biomaterials and Their Performance In Vivo. Woodhead Publishing, pp. 69–80. https://doi.org/10.1016/B978-0-08-100603-0.00004-3

Man, K., Liu, J., Phan, K.M., Wang, K., Lee, J.Y., Sun, X., Story, M., Saha, D., Liao, J., Sadat, H., Yang, Y., 2022. Dimensionality-Dependent Mechanical Stretch Regulation of Cell Behavior. ACS Appl. Mater. Interfaces 14, 17081–17092. https://doi.org/10.1021/acsami.2c01266

Maráková, N., Humpolíček, P., Kašpárková, V., Capáková, Z., Martinková, L., Bober, P., Trchová, M., Stejskal, J., 2017. Antimicrobial activity and cytotoxicity of cotton fabric coated with conducting polymers, polyaniline or polypyrrole, and with deposited silver nanoparticles. Appl. Surf. Sci. 396, 169–176. https://doi.org/10.1016/j.apsusc.2016.11.024

Matějka, R., Koňařík, M., Štěpanovská, J., Lipenský, J., Chlupáč, J., Turek, D., Pražák, Š., Brož, A., Šimůnková, Z., Mrázová, I., Forostyak, S., Kneppo, P., Rosina, J., Bačáková, L., Pirk, J., 2020. Bioreactor Processed Stromal Cell Seeding and Cultivation on Decellularized Pericardium Patches for Cardiovascular Use. Appl. Sci. 10, 5473. https://doi.org/10.3390/app10165473

Mohammadi Amirabad, L., Massumi, M., Shamsara, M., Shabani, I., Amari, A., Mossahebi Mohammadi, M., Hosseinzadeh, S., Vakilian, S., Steinbach, S.K., Khorramizadeh, M.R., Soleimani, M., Barzin, J., 2017. Enhanced Cardiac Differentiation of Human Cardiovascular Disease Patient-Specific Induced Pluripotent Stem Cells by Applying Unidirectional Electrical Pulses Using Aligned Electroactive Nanofibrous Scaffolds. ACS Appl. Mater. Interfaces 9, 6849–6864. https://doi.org/10.1021/acsami.6b15271

Mojena-Medina, D., Martínez-Hernández, M., de la Fuente, M., García-Isla, G., Posada, J., Jorcano, J.L., Acedo, P., 2020. Design, Implementation, and Validation of a Piezoelectric Device to Study the Effects of Dynamic Mechanical Stimulation on

Cell Proliferation, Migration and Morphology. Sensors 20, 2155. https://doi.org/10.3390/s20072155

Mukasheva, F., Adilova, L., Dyussenbinov, A., Yernaimanova, B., Abilev, M., Akilbekova, D., 2024. Optimizing scaffold pore size for tissue engineering: insights across various tissue types. Front. Bioeng. Biotechnol. 12. https://doi.org/10.3389/fbioe.2024.1444986

Murai, M., Amir, E., Amir, R.J., Hawker, C.J., 2012. Azulene-based conjugated polymers: unique seven-membered ring connectivity leading to stimuli-responsiveness. Chem. Sci. 3, 2721–2725. https://doi.org/10.1039/C2SC20615C

Mustafa, A.I., Ebrahim, A.A., Abel Halim, W.A.L., Fawzy, E., Abdou, A.F., 2022. Serum Soluble Intercellular Adhesion Molecule-1 (sICAM-1): A Novel Potential Biomarker in Severe Acne Vulgaris. Indian J. Dermatol. 67, 512. https://doi.org/10.4103/ijd.ijd_387_21

Navale, S.T., Mane, A.T., Ghanwat, A.A., Mulik, A.R., Patil, V.B., 2014. Camphor sulfonic acid (CSA) doped polypyrrole (PPy) films: Measurement of microstructural and optoelectronic properties. Measurement 50, 363–369. https://doi.org/10.1016/j.measurement.2014.01.012

Nazari, H., Kehtari, M., Rad, I., Ashtari, B., Joghataei, M.T., 2020. Electrical stimulation induces differentiation of human cardiosphere-derived cells (hCDCs) to committed cardiomyocyte. Mol. Cell. Biochem. 470, 29–39. https://doi.org/10.1007/s11010-020-03742-6

Neoh, K.G., Kang, E.T., Tan, T.C., 1988. Chemical synthesis and characterization of electroactive and partially soluble polyazulene. Polym. Bull. 19. https://doi.org/10.1007/BF00263932

Nikkhah, M., Edalat, F., Manoucheri, S., Khademhosseini, A., 2012. Engineering microscale topographies to control the cell–substrate interface. Biomaterials 33, 5230–5246. https://doi.org/10.1016/j.biomaterials.2012.03.079

Nimmo, C.M., Owen, S.C., Shoichet, M.S., 2011. Diels−Alder Click Cross-Linked Hyaluronic Acid Hydrogels for Tissue Engineering. Biomacromolecules 12, 824–830. https://doi.org/10.1021/bm101446k

Ning, C., Zhou, Z., Tan, G., Zhu, Y., Mao, C., 2018. Electroactive polymers for tissue regeneration: Developments and perspectives. Prog. Polym. Sci. 81, 144–162. https://doi.org/10.1016/j.progpolymsci.2018.01.001

Nishimura, I., Hisanaga, R., Sato, T., Arano, T., Nomoto, S., Ikada, Y., Yoshinari, M., 2015. Effect of osteogenic differentiation medium on proliferation and differentiation of human mesenchymal stem cells in three-dimensional culture with radial flow bioreactor. Regen. Ther. 2, 24–31. https://doi.org/10.1016/j.reth.2015.09.001

Nokhbatolfoghahaei, H., Bohlouli, M., Paknejad, Z., Rad, M.R., Amirabad, L.M., Salehi-Nik, N., Khani, M.M., Shahriari, S., Nadjmi, N., Ebrahimpour, A., Khojasteh, A., 2020. Bioreactor cultivation condition for engineered bone tissue: Effect of various bioreactor designs on extra cellular matrix synthesis. J. Biomed. Mater. Res. A 108, 1662–1672. https://doi.org/10.1002/jbm.a.36932

Nur Hidayah, S., Dania Adila, A.R., Sharaniza, A.R., Muhammad Abid, A., Mohd Muzamir, M., 2024. Sequentially crosslinked collagen-based hydrogel to form a semi-interpenetrating network for enhanced stability to hydrolytic degradation and electrochemical properties. Polym. Adv. Technol. 35, e6546. https://doi.org/10.1002/pat.6546

Pang, A.L., Arsad, A., Ahmadipour, M., 2021. Synthesis and factor affecting on the conductivity of polypyrrole: a short review. Polym. Adv. Technol. 32, 1428–1454. https://doi.org/10.1002/pat.5201

Patois, T., Lakard, B., Martin, N., Fievet, P., 2010. Effect of various parameters on the conductivity of free standing electrosynthesized polypyrrole films. Synth. Met. 160, 2180–2185. https://doi.org/10.1016/j.synthmet.2010.08.005

Peng, Z., Shu, B., Zhang, Y., Wang, M., 2019. Endothelial Response to Pathophysiological Stress. Arterioscler. Thromb. Vasc. Biol. 39, e233–e243. https://doi.org/10.1161/ATVBAHA.119.312580

Pourkhodadad, S., Hosseinkazemi, H., Bonakdar, S., Nekounam, H., 2023. Biomimetic engineered approaches for neural tissue engineering: Spinal cord injury. J. Biomed. Mater. Res. B Appl. Biomater. 111, 701–716. https://doi.org/10.1002/jbm.b.35171

Puhl, D.L., Funnell, J.L., Fink, T.D., Swaminathan, A., Oudega, M., Zha, R.H., Gilbert, R.J., 2023. Electrospun fiber-mediated delivery of neurotrophin-3 mRNA for neural tissue engineering applications. Acta Biomater. 155, 370–385. https://doi.org/10.1016/j.actbio.2022.11.025

Qi, F., Wang, Y., Ma, T., Zhu, S., Zeng, W., Hu, X., Liu, Z., Huang, J., Luo, Z., 2013. Electrical regulation of olfactory ensheathing cells using conductive polypyrrole/chitosan polymers. Biomaterials 34, 1799–1809. https://doi.org/10.1016/j.biomaterials.2012.11.042

Raimondi, M.T., Moretti, M., Cioffi, M., Giordano, C., Boschetti, F., Laganà, K., Pietrabissa, R., 2006. The effect of hydrodynamic shear on 3D engineered chondrocyte systems subject to direct perfusion. Biorheology 43, 215–222. https://doi.org/10.1177/0006355X2006043003004006

Ravichandran, R., Sundarrajan, S., Venugopal, J.R., Mukherjee, S., Ramakrishna, S., 2010. Applications of conducting polymers and their issues in biomedical engineering. J. R. Soc. Interface 7. https://doi.org/10.1098/rsif.2010.0120.focus

Rezaeeyazdi, M., Colombani, T., Memic, A., Bencherif, S.A., 2018. Injectable Hyaluronic Acid-co-Gelatin Cryogels for Tissue-Engineering Applications. Materials 11, 1374. https://doi.org/10.3390/ma11081374

Rodriguez-Boulan, E., Kreitzer, G., Müsch, A., 2005. Organization of vesicular trafficking in epithelia. Nat. Rev. Mol. Cell Biol. 6, 233–247. https://doi.org/10.1038/nrm1593

Rosenfeld, D., Landau, S., Shandalov, Y., Raindel, N., Freiman, A., Shor, E., Blinder, Y., Vandenburgh, H.H., Mooney, D.J., Levenberg, S., 2016. Morphogenesis of 3D vascular networks is regulated by tensile forces. Proc. Natl. Acad. Sci. 113, 3215–3220. https://doi.org/10.1073/pnas.1522273113

Sasso, C., Beneventi, D., Zeno, E., Chaussy, D., Petit-Conil, M., Belgacem, N., 2011. Polypyrrole and Polypyrrole/Wood-Derived Materials Conducting Composites: A Review. BIORESOURCES 6, 3585–3620.

Shrestha, B.K., Shrestha, S., Baral, E.R., Lee, J.Y., Kim, B.-S., Park, C.H., Kim, C.S., 2019. π-Conjugated polyaniline-assisted flexible titania nanotubes with controlled surface morphology as regenerative medicine in nerve cell growth. Chem. Eng. J. 360, 701–713. https://doi.org/10.1016/j.cej.2018.12.027

Skopalova, K., Radaszkiewicz, K.A., Kasparkova, V., Stejskal, J., Bober, P., Junkar, I., Mozetic, M., Capakova, Z., Lehocky, M., Kasparova, M., Pachernik, J., Humpolicek, P., 2021. Modulation of Differentiation of Embryonic Stem Cells by Polypyrrole: The Impact on Neurogenesis. Int. J. Mol. Sci. 22, 501. https://doi.org/10.3390/ijms22020501

Smith, J.A., Mele, E., 2021. Electrospinning and Additive Manufacturing: Adding Three-Dimensionality to Electrospun Scaffolds for Tissue Engineering. Front. Bioeng. Biotechnol. 9.

Tallawi, M., Rosellini, E., Barbani, N., Cascone, M.G., Rai, R., Saint-Pierre, G., Boccaccini, A.R., 2015. Strategies for the chemical and biological functionalization of scaffolds for cardiac tissue engineering: a review. J. R. Soc. Interface 12, 20150254. https://doi.org/10.1098/rsif.2015.0254

Tanasa, E., Zaharia, C., Hudita, A., Radu, I.-C., Costache, M., Galateanu, B., 2020. Impact of the magnetic field on 3T3-E1 preosteoblasts inside SMART silk fibroin-based scaffolds decorated with magnetic nanoparticles. Mater. Sci. Eng. C 110, 110714. https://doi.org/10.1016/j.msec.2020.110714

Tandon, N., Goh, B., Marsano, A., Chao, P.-H.G., Montouri-Sorrentino, C., Gimble, J., Vunjak-Novakovic, G., 2009. Alignment and elongation of human adipose-derived stem cells in response to direct-current electrical stimulation, in: 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society. Presented at the 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, pp. 6517–6521. https://doi.org/10.1109/IEMBS.2009.5333142

Thunberg, J., Kalogeropoulos, T., Kuzmenko, V., Hägg, D., Johannesson, S., Westman, G., Gatenholm, P., 2015. In situ synthesis of conductive polypyrrole on electrospun cellulose nanofibers: scaffold for neural tissue engineering. Cellulose 22, 1459–1467. https://doi.org/10.1007/s10570-015-0591-5

Tulloch, N.L., Muskheli, V., Razumova, M.V., Korte, F.S., Regnier, M., Hauch, K.D., Pabon, L., Reinecke, H., Murry, C.E., 2011. Growth of Engineered Human Myocardium With Mechanical Loading and Vascular Coculture. Circ. Res. 109, 47–59. https://doi.org/10.1161/CIRCRESAHA.110.237206

Uda, M., Kawashima, H., Mayama, H., Hirai, T., Nakamura, Y., Fujii, S., 2021. Locomotion of a Nonaqueous Liquid Marble Induced by Near-Infrared-Light Irradiation. Langmuir 37, 4172–4182. https://doi.org/10.1021/acs.langmuir.1c00041

Unadkat, H.V., Hulsman, M., Cornelissen, K., Papenburg, B.J., Truckenmüller, R.K., Carpenter, A.E., Wessling, M., Post, G.F., Uetz, M., Reinders, M.J.T., Stamatialis,

D., van Blitterswijk, C.A., de Boer, J., 2011. An algorithm-based topographical biomaterials library to instruct cell fate. Proc. Natl. Acad. Sci. 108, 16565–16570. https://doi.org/10.1073/pnas.1109861108

Van Vlierberghe, S., Vanderleyden, E., Boterberg, V., Dubruel, P., 2011. Gelatin Functionalization of Biomaterial Surfaces: Strategies for Immobilization and Visualization. Polymers 3, 114–130. https://doi.org/10.3390/polym3010114

Velez-Suberbie, M.L., Tarrant, R.D.R., Tait, A.S., Spencer, D.I.R., Bracewell, D.G., 2013. Impact of aeration strategy on CHO cell performance during antibody production. Biotechnol. Prog. 29, 116–126. https://doi.org/10.1002/btpr.1647

Venkatesh, S., Vishista, K., 2018. Identification of the best chemical equivalent ratio to produce emeraldine salt exhibiting better pseudo capacitance. Electrochimica Acta 263, 76–84. https://doi.org/10.1016/j.electacta.2018.01.032

Vítková, L., Musilová, L., Achbergerová, E., Kolařík, R., Mrlík, M., Korpasová, K., Mahelová, L., Capáková, Z., Mráček, A., 2022. Formulation of Magneto-Responsive Hydrogels from Dually Cross-Linked Polysaccharides: Synthesis, Tuning and Evaluation of Rheological Properties. Int. J. Mol. Sci. 23, 9633. https://doi.org/10.3390/ijms23179633

Vogler, E.A., 1998. Structure and reactivity of water at biomaterial surfaces. Adv. Colloid Interface Sci. 74, 69–117. https://doi.org/10.1016/S0001-8686(97)00040-7

Wang, F., Lai, Y.-H., Kocherginsky, N.M., Kosteski, Yu.Yu., 2003. The First Fully Characterized 1,3-Polyazulene: High Electrical Conductivity Resulting from Cation Radicals and Polycations Generated upon Protonation. Org. Lett. 5, 995–998. https://doi.org/10.1021/ol0274615

Wang, X.D., Gu, X.S., Yuan, C.W., Chen, S.J., Zhang, P.Y., Zhang, T.Y., Yao, J., Chen, F., Chen, G., 2004. Evaluation of biocompatibility of polypyrrole in vitro and in vivo. J. Biomed. Mater. Res. A 68A, 411–422. https://doi.org/10.1002/jbm.a.20065

Wong, D.W., Gan, W.L., Teo, Y.K., Lew, W.S., 2018. Interplay of cell death signaling pathways mediated by alternating magnetic field gradient. Cell Death Discov. 4, 1–9. https://doi.org/10.1038/s41420-018-0052-7

Wrzecionko, E., Minařík, A., Smolka, P., Minařík, M., Humpolíček, P., Rejmontová, P., Mráček, A., Minaříková, M., Gřundělová, L., 2017. Variations of Polymer Porous Surface Structures via the Time-Sequenced Dosing of Mixed Solvents. ACS Appl. Mater. Interfaces 9, 6472–6481. https://doi.org/10.1021/acsami.6b15774

Wu, M., Zhong, C., Deng, Y., Zhang, Q., Zhang, X., Zhao, X., 2020. Resveratrol loaded glycyrrhizic acid-conjugated human serum albumin nanoparticles for tail vein injection II: pharmacokinetics, tissue distribution and bioavailability. Drug Deliv. 27, 81–90. https://doi.org/10.1080/10717544.2019.1704944

Xia, Z., Zhang, H., Li, Q., Yi, C., Xing, Z., Qin, Z., Zhao, H., Jing, J., Zhao, C., Cai, K., 2024. The Biomimetic Electrical Stimulation System Inducing Osteogenic Differentiations of BMSCs. ACS Appl. Mater. Interfaces 16, 56730–56743. https://doi.org/10.1021/acsami.4c11890

Yan, H., Wang, Y., Li, L., Zhou, X., Shi, X., Wei, Y., Zhang, P., 2020. A micropatterned conductive electrospun nanofiber mesh combined with electrical stimulation for

synergistically enhancing differentiation of rat neural stem cells. J. Mater. Chem. B 8, 2673–2688. https://doi.org/10.1039/C9TB02864A

Yang, J., Zhang, J., Ding, C., Dong, D., Shang, P., 2018. Regulation of Osteoblast Differentiation and Iron Content in MC3T3-E1 Cells by Static Magnetic Field with Different Intensities. Biol. TRACE Elem. Res. 184, 214–225. https://doi.org/10.1007/s12011-017-1161-5

Yin, Z., Chen, X., Chen, J.L., Shen, W.L., Hieu Nguyen, T.M., Gao, L., Ouyang, H.W., 2010. The regulation of tendon stem cell differentiation by the alignment of nanofibers. Biomaterials 31, 2163–2175. https://doi.org/10.1016/j.biomaterials.2009.11.083

Zhang, F., Su, K., Fang, Y., Sandhya, S., Wang, D.-A., 2015. A mixed co-culture of mesenchymal stem cells and transgenic chondrocytes in alginate hydrogel for cartilage tissue engineering. J. Tissue Eng. Regen. Med. 9, 77–84. https://doi.org/10.1002/term.1641

Zhuang, X., 2020. Azulene-Based Molecules, Polymers, and Frameworks for Optoelectronic and Energy Applications. Small Methods 4. https://doi.org/10.1002/smtd.202000628

Zuščíková, L., Greifová, H., Bažány, D., Lukáč, N., Jambor, T., 2024. Current approaches and techniques of 3D cell culture systems: a review. Arch. Ecotoxicol. 6, 22–27. https://doi.org/10.36547/ae.2024.6.1.22-27

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LIST OF ABBREVIATIONS AND SYMBOLS

AFM	Atomic force microscopy
APS	Ammonium persulfate
CIPs	Carbonyl iron particles
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
FeCl3	Iron(III) chloride
FTIR	Fourier transform infrared
HA	Hyaluronic acid
hMSC	Human mesenchymal stem cell
ITO	Indium tin oxide
LIF	Leukemia inhibitory factor
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PANI	Polyaniline
PAz	Polyazulene
PPy	Polypyrrole
PU	Polyurethane
PVDF	Polyvinylidene fluoride
SEM	Scanning electron microscopy
TCDs	Tissue culture dishes
TE	Tissue engineering
TruePAz	True Polyazulene
UV	Ultraviolet
VIS	Visible

LIST OF PUBLICATIONS

Articles published in journals indexed on Web of Science:

Vítková, L., Musilová, L., Achbergerová, E., Kolařík, R., Mrlík, M., Korpasová, K., **Mahelová, L.**, Capáková, Z., Mráček, A., 2022. *Formulation of Magneto-Responsive Hydrogels from Dually Cross-Linked Polysaccharides: Synthesis, Tuning and Evaluation of Rheological Properties.* Int. J. Mol. Sci. 23, 9633. https://doi.org/10.3390/ijms23179633 IF: 5.6

Mahelová, L., Slobodian, P., Kocourková, K., Minařík, A., Moučka, R., Trchová, M., Martínková, M., Skopalová, K., Víchová, Z., Kašpárková, V., Humpolíček, P., 2024. *Method for in situ polypyrrole coating, and the example of its use for functionalization of polyurethane anisotropic electrospun mats.* Heliyon 10. https://doi.org/10.1016/j.heliyon.2024.e27883 IF: 3.6

Gorgol, D., Mrlík, M., Mikulka, F., Víchová, Z., **Mahelová, L.**, Ilčíková, M., Minařík, A., 2024. *Smart Biopolymer Scaffolds Based on Hyaluronic Acid and Carbonyl Iron Microparticles: 3D Printing, Magneto-Responsive, and Cytotoxicity Study.* ACS Appl. Bio Mater. 7, 7483–7493. https://doi.org/10.1021/acsabm.4c00567 IF: 4.7

Articles submitted to the editors of international journals with an impact factor:

Mahelová, L., Trchová, M., Kotowicz, S., Škoda, D., Kocourková, K., Víchová, Z., Vícha, J., Kašpárková, V., Humpolíček, P., 2025. *Is polyazulene cytocompatible? It depends.* Currently under review.

Three other co-authored articles are currently being prepared for submission.

Meeting abstracts from conferences indexed on Web of Science:

Mahelová, L., Slobodian, P., Humpolíček, P., 2023. *Aligned Polyurethane Nanofibers Coated with Polypyrrole: Anisotropy and Conductivity as Cell-Instructive Cues.* TISSUE Eng. PART A 29, 1166–1166.

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Šandová, K. *Effect of electric field on cell behaviour.* Zlín, 2024. Bachelor thesis. Tomas Bata University in Zlín, Faculty of Technology, Department of Fat, Surfactant and Cosmetics Technology.

Zubková, S. *Využití elektrického pole k léčbě kožních poranění.* Zlín, 2024. Bachelor thesis. Tomas Bata University in Zlín, Faculty of Technology, Department of Fat, Surfactant and Cosmetics Technology.

Stuchlíková, S. *Polyazulen a jeho biologické vlastnosti.* Zlín, 2023. Master thesis. Tomas Bata University in Zlín, Faculty of Technology, Department of Fat, Surfactant and Cosmetics Technology.

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Use of polymers in tissue engineering

Využití polymerů pro tkáňové inženýrství

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