Modification of polymers to the targeted cell response

Mgr. Petra Rejmontová, Ph.D.

Doctoral Thesis Summary

Tomas Bata University in Zlín

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Faculty of Technology

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Modification of polymers to the targeted cell response

Modifikace polymerních materiálů za účelem cílené buněčné reakce

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ABSTRACT

Polymers play a crucial role in the preparation of biomaterials for tissue engineering and regenerative medicine. The biocompatibility and ability to be combined with other materials to produce desirable 3D structure are critical properties for their application. The thesis is focused on the modification of polymers in its native form, composites or copolymers with mentioned properties to achieve desirable interaction with the eukaryotic cells in terms of the cell adhesion, growth, proliferation, differentiation and death. The interaction of materials with the cells was studied in the cell laboratory using advanced *in vitro* techniques.

ABSTRAKT

Polymery hrají klíčovou roli v oblasti vývoje biomateriálů využitelných v oblasti tkáňového inženýrství a regenerativní medicíny. Předpokladem pro jejich aplikaci je jejich biokompatibilita a kombinovatelnost s dalšími materiály vytvářejícími vhodnou 3D strukturu s požadovanými objemovými vlastnostmi. Předmětem práce je modifikace polymerů ať již samostatných či v kopolymerech a kompozitech za účelem cíleného ovlivnění buněčné vazby, růstu, proliferace, diferenciace a smrti. Interakce materiálu s buňkami byla studována pomocí pokročilých *in vitro* metod v rámci laboratoře buněčných kultur.

1. INTRODUCTION

Today, polymers represent irreplaceable material in many fields of human activities. Various types of polymers already found their firm place in many areas of industry including building, electronics and textile industry or products for daily use. Moreover, polymers have become very attractive for biomedical applications - either as materials utilized as sensors, medical devices or scaffolds in tissue engineering (TE).

In the first instance, the interest was focused on natural polymers, because of their good material-tissue compatibility. However, the use of natural polymers can bring also several disadvantages – e.g. limitation of availability, purity, the risk of transfection of disease or inducing immune response, batch-to-batch consistency and mainly insufficient of mechanical properties for some applications (Yao, Tao et al. 2011). The synthetic polymers lack some of these disadvantages and, moreover, exhibit additional advantages. Various low-cost products with controlled properties including mechanical properties (strength), biodegradation rate, chemical composition or (micro) structure can easily be prepared (Liu and Ma 2004). Synthetic polymers are also much more reproducible and show in general longer shelf-life in comparison with the natural ones (Ozdil and Aydin 2014). Furthermore, some synthetic polymers can offer some advanced properties such as intrinsic conductivity.

2. POLYMERIC BIOMATERIALS

Synthetic polymers can be divided into two main categories - non-biodegradable and biodegradable. Biodegradation is occurred hydrolytically or enzymatically under *in vivo* conditions (Migonney 2014).

Focusing on synthetic biodegradable polymers, aliphatic polyesters create one of the most discussed groups. Their biodegradation is based on the hydrolysis of the ester groups presented in the polymer backbone (Albertsson, Hakkarainen et al. 2008). Based on their nature, biodegradable polymers can be used as temporary devices - e.g. transient implants, TE scaffolds and drug delivery system (Nair and Laurencin 2007). Polylactide, polyglycolide or poly(ε-caprolactone) and their copolymers are commonly utilized in medical devices (Lopes, Jardini et al. 2012).

Non-biodegradable polymers have their irreplaceable position in biomedical devices, where long term stability is required (Migonney 2014). As an example, the poly(methyl methacrylate) based bone cement (Teo, Mishra et al. 2016), polytetrafluoroethylene based heart valves (Jaganathan, Supriyanto et al. 2014) or polyamide sutures (Maitz 2015) can be mentioned.

3. TISSUE ENGINEERING

The aim of TE is to regenerate living tissues damaged by disease, injury or trauma (Dhandayuthapani, Yoshida et al. 2011). Conventional treatment of damaged tissues comprises the transplantation of the tissue from one side to another. However, even the lifesaving, the described approach could bring not neglected side effects, e.g. shortage of donors or the rejection of the transplant by the patient´s immune systems and also the possibility of the disease transmission from the donor (in case of allografts) (Amini, Laurencin et al. 2012).

Tissue engineering combines the biomaterial scaffolds, cells from the patients´ body and growth factors to restore, maintain or improve tissue function (Howard, Buttery et al. 2008).

One of the possible processes of TE starts with the isolation of tissue-specific cells from the patient´s biopsy. The isolated cells are cultivated and expanded to obtain their sufficient amount. The cells are seeded along with the growth factors into biomaterial scaffold, which acts as a template for regenerated tissue formation. Subsequently, the two ways are possible. The seeded scaffold is directly implanted into the injured site, or the scaffold with seeded cells is firstly cultured *in vitro* to enable the tissue formation and then it is implanted back into the patient´s body (Uchegbu and Schatzlein 2006).

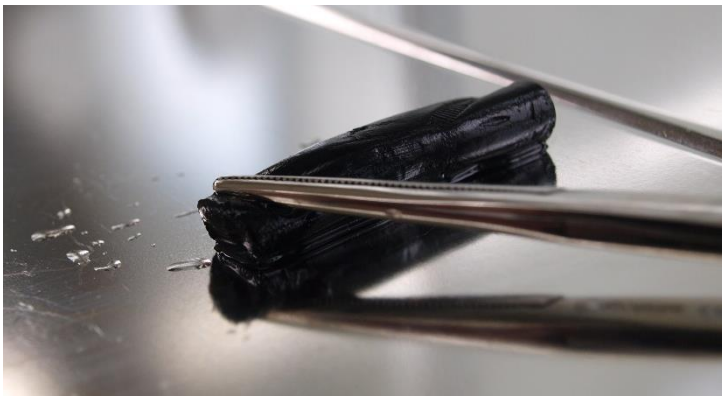

Figure 1. Polyaniline cryogel mimicking the properties of native tissue (Humpolicek, Radaszkiewicz et al. 2018).

As each biomaterial, scaffolds for TE must be biocompatible. Moreover, the scaffold should allow cell adhesion, proliferation, migration and ingrowth of cells through the bulk of the scaffold. In the case of stem cells, the scaffold should promote their differentiation into the desired cell line.

4. TECHNIQUES OF CELL CULTIVATION

The standard (two dimensional, 2D) *in vitro* cell cultivation represents technique, in which cells grow in a monolayer on a flat bottom of tissue culture plastic (mostly polystyrene) flask or plate. Traditional and still routinely utilized "2D" monolayer cell culturing methods are insufficient for TE demands. For the purpose of TE, a bioreactor represents one of the key components of 3D cell

culturing. The cell culturing in the bioreactor mimics the *in vivo* cellular behaviour and therefore purvey more physiologically relevant outcomes (Edmondson, Broglie et al. 2014). The differences between both cultivation methods are aptly described in a review by Knight and Przyborski (Knight and Przyborski 2015). In conventional 2D cultivation, cells create monolayer and are flatter. In contrast, cells adopt a natural structure in all dimensions under 3D cultivation in bioreactors. All mentioned facts can be observed in Figure 2, in which confocal images of a single fibroblast cell are depicted.

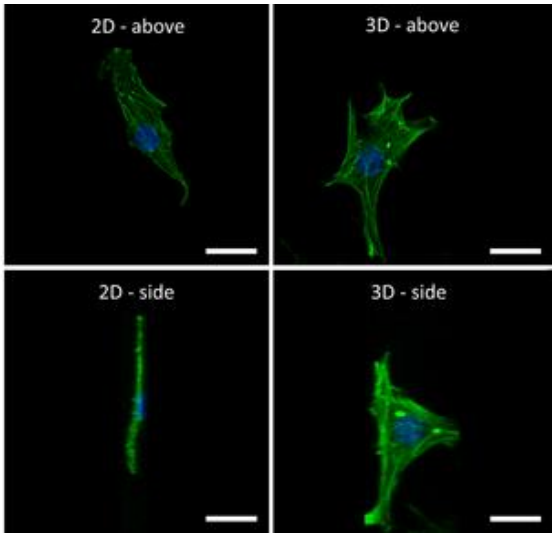

Figure 2. The differences between the cell morphology in "2D" and 3D cultivation conditions (Knight and Przyborski 2015).

It should seem like a non-essential factor, but this shift in natural morphology influences the spatial organization of the cell surface receptors participated in cell-cell interactions. Therefore, it can also affect the outside-inside signal transduction pathways and this impact can finally lead to modified gene expression and altered cell physiology (Edmondson, Broglie et al. 2014).

Bioreactors enable the dynamic cell cultivation, which assumes the homogenous concentration of nutrients, oxygen and other required elements as well as the toxic by-products removal and pH level preservation. This is allowed due to continuous culture media mixing or perfusion.

There are three basic types of bioreactors including spinner flasks, perfusion bioreactor and low gravity bioreactor (Chaudhuri 2005). A lot of research papers focused on the effect of microgravity[1] on the behaviour of various cell types have been published. The modulation of gene expression and consequent protein synthesis, signalling pathways or morphology has been previously described (Chaudhuri 2005). Several devices were constructed to simulate microgravity.

[1] *Contrary to physics, in regenerative medicine the term "microgravity" (or "micro-g") is frequently used as a synonym of "weightlessness" and "zero-g", which indicates that the g-forces are not actually zero but just "very small" (Herranz, R., R. Anken, et al. (2013). "Ground-Based Facilities for Simulation of Microgravity: Organism-Specific Recommendations for Their Use, and Recommended Terminology." Astrobiology **13**(1): 1-17.).*

The four basic types of simulators have been already developed – 2D or 3D clinostats, random position machine, rotating wall vessel and diamagnetic levitation. The two basic types of rotating wall vessel bioreactor exist. The slow turning lateral vessel and high aspect rotating vessel (HARV).

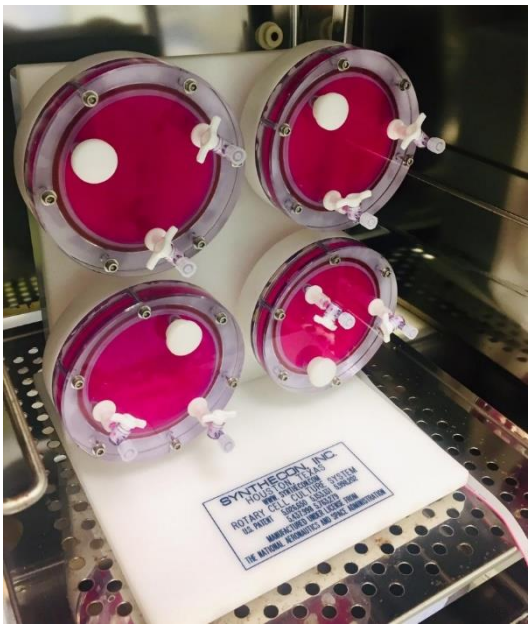

Figure 3. The rotary cell culture system (HARV type). Source: Cell biology laboratories, Centre of polymer systems, Tomas Bata University in Zlin.

Certain cells require different physical stimuli based on the function of tissue, e.g. shear stress, mechanical or electrical stimulation. The modern bioreactors should mediate all of the mentioned stimulation. Therefore, the specific types of bioreactors have been constructed to fulfil all the requirements of individual tissue.

5. MATERIAL PROPERTIES AFFECTING CELLULAR BEHAVIOUR

Considering the surface properties of material, surface energy, surface topography and chemistry should be primarily mentioned. Moreover, considering 3D scaffolds, the mechanical characteristics and scaffold architecture are particularly discussed in scientific articles.

5.1. Surface energy and topography

The surface energy determines the wettability and significantly influence cellular behaviour, and also the protein absorption (Saini, Singh et al. 2015). Although synergic effect of both surface energy and topography is expected to be a crucial factor for cell adhesion and proliferation (Borges, Benetoli et al. 2013), it has been described the greater importance of surface topography in several research papers.

The importance of surface topography is most likely related to the structure of the extracellular matrix (ECM), which surrounds cells in natural environment. The ECM is a component of all tissues and organs and its composition and topography are specific for each tissue/organ (Frantz, Stewart et al. 2010). As structures of ECM have a size in micrometre/nanometre range, the effort of researchers was focused on the preparation of micro- and nanostructured material (Grespan, Martewicz et al. 2016, Skoog, Kumar et al. 2018).

Surface texturing can be achieved using lithography techniques (Acikgoz, Hempenius et al. 2011) self-assembly approaches (Gunkel-Grabole, Palivan et al. 2017), thermal processing (Lizundia, Saenz-Perez et al. 2017), chemical (Chun, Khang et al. 2009) or laser treatment (Nedela, Slepicka et al. 2017). Electrospinning or inkjet printing could be also used (Skoog, Kumar et al. 2018).

The texture of the surface comprises roughness (a complex of irregular elements with variable sizes) or creating of regularly repeated features (pits, pillars/protrusions, grooves etc). The curvature of a single feature (convex, concave), as well as its "sharpness" (sharped or curved edges) also influence the cell response (Harvey, Hill et al. 2013). It has been already described that surface topography influence proliferation (Taskin, Xia et al. 2017), migration (Kim and Kim 2018), differentiation (Chen, Han et al. 2018), influence the strength of adhesion (Hampe, Li et al. 2018) or altered gene expression (Gasiorowski, Liliensiek et al. 2010).

However, the results describing relationships between material topography are ambiguous and vary with the cell types, substrate material, and surface feature character (Skoog, Kumar et al. 2018). Moreover, cell source also affects the cellular behaviour on structured surfaces (Rebollar, Frischauf et al. 2008).

5.2. Surface chemistry

The term surface chemistry is mainly connected with the presence of functional groups on the surface of the material. Considering the cell interaction with the material on molecular level, cells interact with the artificial surface *via* the cell adhesion molecules (integrin, cadherin or selectin receptors) (Petruzzelli, Takami et al. 1999). In fact, the extracellular domain of adhesion molecules bonds to specific functional groups, more precisely to specific amino acid sequence (Srichai and Zent 2010).

The most famous sequence is artificial RGD (a tripeptide composed of Arginine, Glycine and Aspartic acid). Based on the significance of RGD sequence, it is commercially available and commonly utilized for material modification with the aim to improve the biocompatibility of material (Hersel, Dahmen et al. 2003).

Multifarious methods of chemical modification of polymer surface exist. Plasma treatment, UV/ozone treatment, chemical grafting or their combinations can be used for introducing of new functional groups (Rashidi, Yang et al. 2014). The chemical composition of the surface could be subsequently modified with

bioactive substances (frequently with growth factors, ECM components or above mentioned adhesive motif) by physical adsorption or covalent bonding (Tallawi, Rosellini et al. 2015).

5.3. Scaffold architecture

The most important feature of each scaffold to be fulfilled is an appropriate porosity. Moreover, the pores must be interconnected (Loh and Choong 2013). The interconnected pores enable the cell to migrate within the scaffold. Therefore, the pore interconnection is a crucial characteristic for appropriate cell ingrowth and penetration within the whole bulk of the material.

Another important factor is the mean pore size. The recommended pore size of scaffold depends on tissue, which is proposed to be regenerated. Moreover, the significance of pore size could vary more or less with the used scaffold, the condition of cell cultivation and also with the type of used cells. Additionally, it should be mentioned that several tissues exhibit a gradient porous structure (e.g. skin, in which structure the pore sizes increase with the distance away from the surface of the skin) (Loh and Choong 2013). The biomimetic scaffolds with gradient structure have been already prepared (Wang, Xu et al. 2016). Finally, the shape of pores is also important (e.g. the long pores are beneficial in the engineering of tissue of peripheral axons) (Bruzauskaite, Bironaite et al. 2016).

6. POLYMERIC BIOMATERIALS WITH ADVANCED PROPERTIES

In following chapter, only two chosen properties of many possibilities will be mentioned - the electrical conductivity and structured surfaces. These two properties are in the centre of attention of the presented doctoral thesis.

6.1. Conductive polymers

As electrical signalling plays a non-substitutable role in the living system and several cell types require electrical stimulation for its proper function, the conductive polymers (CP) have the indispensable site in TE.

The intrinsic conductivity of conductive polymers is grounded in their conjugated backbone consisted of alternating single and double bonds (Cui, Yang et al. 2016). This unique structure enables the electrons to move along the chain (Cui, Yang et al. 2016). The conductivity could be modulated by doping (generating charge carriers). Dopants utilized for this purpose could vary from small ions (chlorine ions) to large (polymers) (Kaur, Adhikari et al. 2015). Used dopant agent (especially in case of large ones) could influence also the surface properties (e.g. roughness, surface energy) and subsequently cellular behaviour (Collier, Camp et al. 2000).

As the most famous representatives of CP, polypyrrole, polyaniline (PANI) and poly(3,4-ethylenedioxythiophene) could be mentioned. The utilization of conductive polymers as biosensors or drug delivery devices have been already described (Inzelt 2017, Sankoh, Vagin et al. 2017). Nowadays, the interest of scientists have been directed to TE (Kaur, Adhikari et al. 2015). In this context, it is important to produce 3D electrically conductive scaffolds. There are generally three possible ways how the electrically conductive scaffolds can be prepared: A) electrospun fibres containing conductive colloids; B) independently prepared scaffold covered with a thin conductive films; C) conductive polymer scaffold.

6.2. Structured polymer surfaces

One of the initial studies that opens this field of science, was published in Nature in 2011 (Baker 2011). In this and the following works, the cell line dependent effect of topography was described.

The cellular response to topography is really complex and various factors of surface geometry could altered cellular behaviour. Therefore, the advanced approach in an effort to reveal the relationship between cell behaviour and surface topography is necessary. Unadkat *et al.* (Unadkat, Hulsman et al. 2011) prepared chips of poly(lactic acid) with different topographies. They described an effective tool for deciphering the "Braille code" of cell-topography interactions. Beside influenced stem cell proliferation and differentiation, it was also evaluated the different effect on cell morphology (see fluorescent micrographs in Figure 4 A-D or scanning electron microscopy images 4 E-H).

Lately, the effect of topography on pluripotency of human-induced pluripotent stem cells was determined using the same design of micro-TopoChips (Reimer, Vasilevich et al. 2016). Recently, the nano-TopoChip has been developed to study the effect of nano-sized topography on cell behaviour (Hulshof, Zhao et al. 2017).

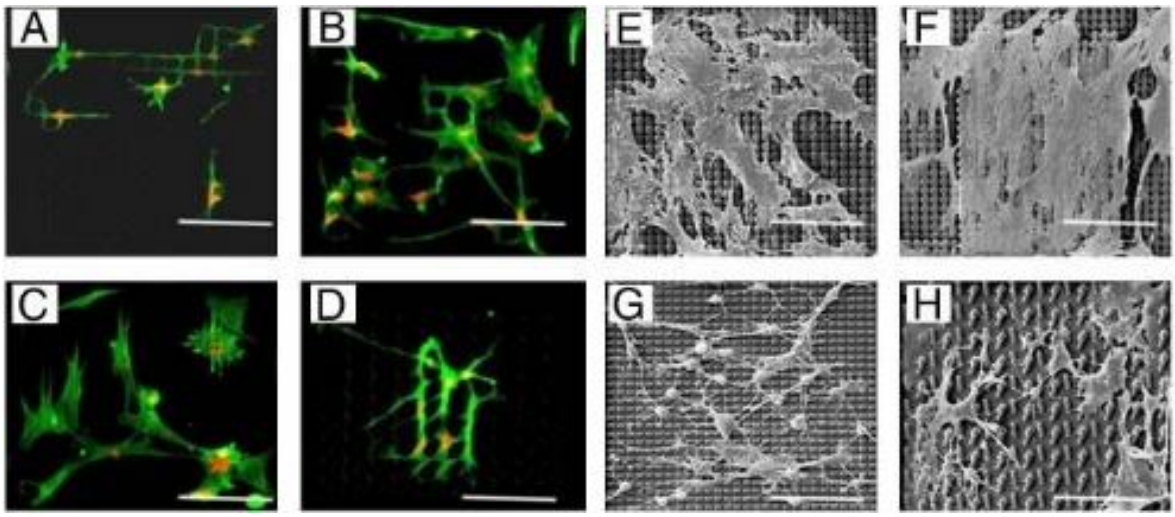

Figure 4. Diverse cell morphologies on structured surfaces (Unadkat, Hulsman et al. 2011).

AIMS OF DOCTORAL THESIS

The aim of the presented thesis is the study of the interaction of eukaryotic cells and polymer materials. This aim has been defined in accordance with formerly achieved results of research conducted in laboratories at our institution and with the aid of information gathered from literature review summarized in previous sections. According to the current state of knowledge, the aims of work were defined:

1) Determination of interaction of eukaryotic cells with conductive polymers.

2) Study of cell response to structured polymer surfaces with focusing on cell adhesion, proliferation and cytoskeletal alternations.

3) Implementation of methodology for testing the ability of eukaryotic cells to incorporate within 3D polymeric scaffolds with the utilization of bioreactors. Testing of cell ability to expand within 3D porous polymeric scaffolds.

7. EXPERIMENTAL PART

7.1. Materials and sample preparation

Various polymeric materials were used during the study. It has been intensively worked with conductive polymers, mainly with PANI. For this purpose, the IUPAC protocol were mostly used (and modified) for preparation of PANI films (Stejskal and Sapurina 2005). PANI films modified with acids and PANI films prepared in colloidal dispersion mode were prepared. The green approach was also applied and set of PANI powders were synthesized based on previously described methodology (Falletta, Costa et al. 2014). Moreover, PANI cryogel were prepared according to methodology summarized in (Humpolicek, Radaszkiewicz et al. 2018). Besides PANI, also poly(p-phenylenediamine) (PpPDA) powders were prepared by chemical oxidation of p-phenylenediamine in acidic media (Kuceková, Rejmontová et al. 2017). Both PANI cryogel and PpPDA powders were synthesized at the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic.

Furthermore, for the purpose of study of cell-material surface interaction, the micro-structure polystyrene structures were prepared at the Department of physics and materials engineering, Tomas Bata University in Zlín.

Finally, scaffolds based on PVA and fractionated Kraft lignin (KL^f) were prepared in cooperation with Assoc. Prof. Adriana Kovalčik. The detailed preparation procedure is described in (Rejmontová, Kovalcik et al. 2019, in press).

7.2. Characterization of material properties

Within the study, measurement of electrical conductivity (the four-point van der Pauw method) and the surface energy evaluation were utilised. To determine the surface energy of tested materials, the Surface Energy Evaluation System (Advex Instruments, Czech Republic) was used. Moreover, to reveal the internal structure of scaffolds based on PVA and KL^f, scanning electron microscopy (SEM) was utilized in cooperation with the Department of Physics and Materials Engineering, Tomas Bata University in Zlin. Finally, the amount of toxic residual impurities in PANI cryogel was determined by the high-performance liquid chromatography (HPLC). For HPLC analysis, samples were extracted in accordance with ISO 10993-12.

7.3. Biological properties

Prior to biological *in vitro* testing, materials were disinfected. PANI films (both modified with acids and prepared in colloidal dispersion mode) were disinfected by exposure to a UV-radiation source operating at a wavelength of 258 nm emitted from a low-pressure mercury lamp for 30 min. PpPDA powders were disinfected by dry heat at 120°C for 40 min. Finally, scaffolds based on PVA and KL^f were disinfected by 70% ethanol.

Cell lines

For biological testing, the mouse embryonic fibroblast cell line (ATCC CRL-1658 NIH/3T3, USA) and primary mouse embryonic fibroblasts (a kind gift of Jiří Pacherník from the Institute of Experimental Biology, Faculty of Science, Masaryk University) were utilized.

Cytotoxicity in direct contact: Cell proliferation around the tested samples was evaluated after three days using an inverted Olympus phase contrast microscope (Olympus IX81, Japan). Cells seeded on tissue plastic (TPP; Switzerland) were used as a reference.

Cytotoxicity testing of extracts of tested samples was performed according to ISO 10 993-5 procedure. Samples were extracted according to ISO 10993-12.

Cell adhesion, proliferation, migration

Cell adhesion was evaluated after 1 hour, the cells were gently rinsed and micrographs were taken (Olympus IX81, Japan). Nuclei counterstaining using Hoechst was utilized for evaluation of cell adhesion to sliced scaffolds.

To evaluate the cell proliferation, micrographs were taken every 24 hours (Olympus IX81, Japan). To simulate wound healing, the modified Scratch assay was performed according to Liang *et al*. (Liang, Park et al. 2007).

The impact on cell cytoskeleton was evaluated using an ActinRed 555 staining (Thermo Fisher Scientific, United States).

Cell seeded on culture dishes (TPP; Switzerland) were used as reference.

Testing the ability of the cells to ingrowth within the biomaterial

For this purpose, a Rotary Cell Culture systems™ RCCS-4 (Synthecon Incorporated, Texas) was used. The cell suspension at a concentration of $1x10^6$ cells per mL was gently injected by syringe into each scaffold and cultivation medium was added. The cells were let to adhere in an incubator for 24 hours. After this pre-cultivation period, the seeded scaffolds were inserted separately into the bioreactor vessel, cultivation medium was added and the forward rotational speed was adjusted to 15.5 RPM. Cultivation in the bioreactor lasted 14 days with the medium changing after seven days.

Evaluation of cell incorporation within the scaffold: The fixed and permeabilized cells were stained using ActinRed™ 555 and the cell morphology was observed using fluorescent microscopy (Olympus IX81, Japan).

Skin irritation test

The skin irritation potential was tested *in vitro* after application of tested sample onto 3D reconstructed human tissue model EpiDerm (MatTek, Slovakia). The test was performed according to OECD Guideline for the Testing of Chemicals, No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method (OECD 2015).

7.4. Results and discussion

The presented doctoral thesis deals with the relationship between polymer-based materials and eukaryotic cells or artificial tissue. The understanding of the mentioned relationship is crucial for development of new materials with anticipated biological properties and with application potential in rapidly developing field of biomedicine.

7.4.1. Cell interaction with conductive polymers

Polyaniline (PANI)

Within the work on the thesis, the modified PANI films were prepared based on the IUPAC methodology (Stejskal and Sapurina 2005). Concretely, either films modified with acids or films prepared in colloidal suspension were synthesized. Additionally, PANI powder was also prepared using green synthesis. Finally, PANI-based scaffold was tested as a novel 3D material.

a) PANI films modified with acids

Concerning the biomedical application, the interaction of PANI with blood must be also taken into account. The anticoagulation effect of PANI reprotonated with poly(2-acrylamido-2-methyl-1-propanesulfonic) acid (PAMPSA) has been already described. (Humpolíček, Kuceková et al. 2015). Apart from PAMPSA, other acids may also be utilized, e.g. sulfamic and phosphotungstic acid.

Within doctoral study, an investigation was made into the adhesion, proliferation and migration of mouse embryonic fibroblasts (NIH/3T3) on films doped with sulfamic (PANI-SULF) and phosphotungstic acids (PANI-PT). In addition, PANI films supplemented with PAMPSA at various mole ratios were tested (PANI-PAMPSA-1:1, PANI-PAMPSA-2:1).

Table 1. Surface energy evaluation of different polyaniline surfaces.

Sample	Surface Energy Components ($mN.m^{-1}$)			
	γ^{tot}	γ^{LW}	γ^{AB}	γ^{dif}
PANI-S	52.54 *	46.05 *	6.49 *	3.33
PANI-B	50.88 *	46.54 *	4.35 *	1.67
PANI-SULF	52.13	44.97	7.17	2.92
PANI-PT	51.89	47.39	4.50	2.68
PANI-PAMPSA-1:1	41.85	40.98	0.87	7.36
PANI-PAMPSA-2:1	56.35	43.91	12.45	7.14
Cells	49.21	23.21	26.00	-

* The values presented in (Humpolíček, Radaszkiewicz et al. 2015).

Firstly, the surface energy of prepared films was evaluated. Moreover, the surface energy of cell monolayer was measured, because cell surface properties could affect the ability of cells to adhere to a solid surface (discussed particularly in the context of bacteria) (Popovici, White et al. 2014). Therefore, the total surface energy (γ^{tot}) was obtained and the absolute value of the difference between

the surface energy of the cells and the sample was calculated (γ^{dif} = |γ^{tot} of the sample - γ^{tot} of cells|). Obtained results are summarized in Table 1.

An interesting phenomena was observed for PANI samples containing PAMPSA in the reaction mixture. The value of the γ^{tot} of PANI-PAMPSA-1:1 obtained in this study significantly corresponded to the value of total surface energy of PANI doped with PAMPSA published previously in study of Humpolíček and co-workers (Humpolíček, Radaszkiewicz et al. 2015). This may indicate that the characteristics of PAMPSA predominated and significantly impacted the surface properties of PANI-PAMPSA-1:1 sample. Surface energy changed dramatically when a reduced amount of PAMPSA was used during PANI synthesis (the ratio of aniline hydrochloride to PAMPSA for synthesis was 2:1). In this case, the measured value of γ^{tot} of PANI-PAMPSA-2:1 approximated to γ^{tot} of pristine PANI-S and PANI-B. Thus, it can be concluded that the surface properties of PANI-PAMPSA-2:1 are primarily governed by PANI, and only to a lesser extent by PAMPSA. Moreover, as can be seen, doping PANI with both sulfamic acid and phosphotungstic acid did not influence the surface properties in terms of γ^{tot} in comparison with PANI-S and PANI-B. Furthermore, the obtained γ^{tot} resembled that for the cell monolayer should indicate that suitable biological properties exist.

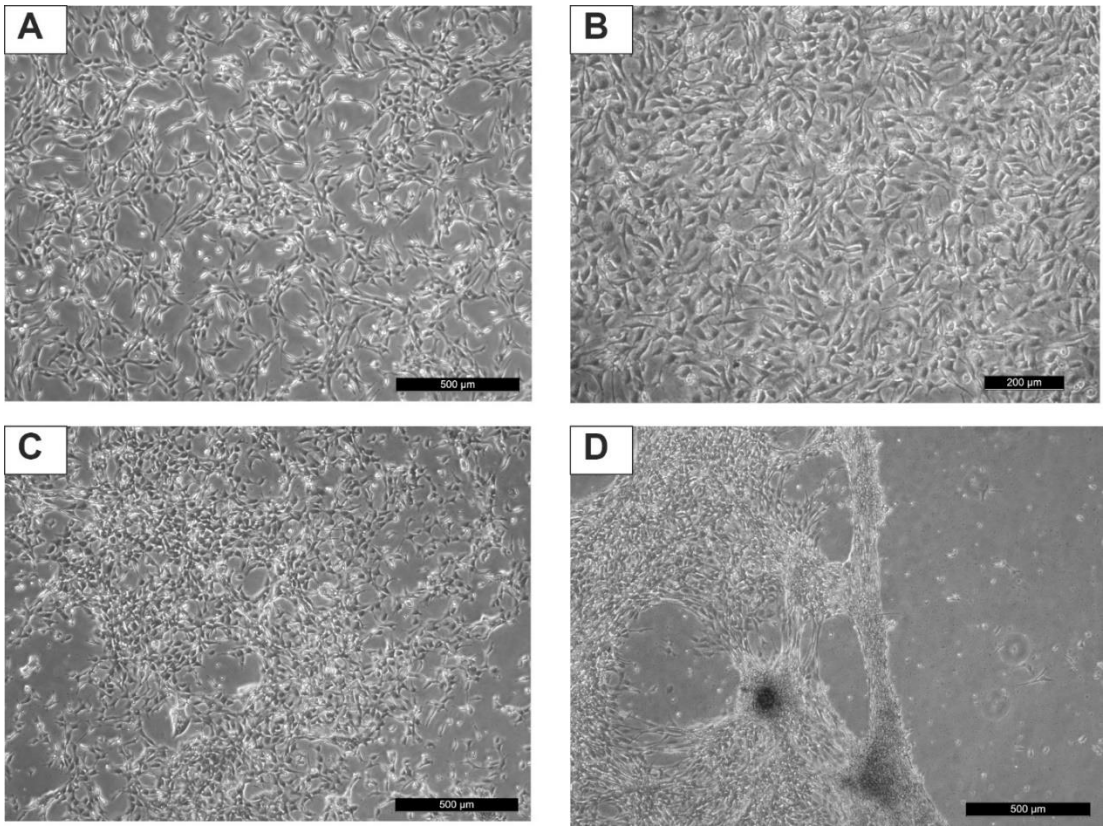

Figure 5. Proliferation: A) Reference 24h; B) PANI-SULF 24h; C) PANI-PAMPSA-1:1 144h; D) PANI-PAMPSA-2:1 144h.

Cell behaviour corresponds to the surface energy of individual samples. The cells were able to adhere to all the tested surfaces in a similar way as the reference, however, remarkable differences in the subsequent cell proliferation existed (see Figure 5). It was clearly demonstrated that the cells reached semi-confluence on the reference sample (Figure 5A), as well as on PANI-SULF and PANI-PT surfaces after 24h (see Figure 5B, in which only PANI-SULF is depicted). In contrast, proliferation significantly decreased on the PANI-PAMPSA-1:1 sample, and the cells reached a semi-confluent state after 144 h (see Figure 5C). Cell proliferation was improved on PANI-PAMPSA-2:1. Nevertheless, the attachment of cells was weak and cells adhered on PANI-PAMPSA-2:1 easily detached from its surface (Figure 5D). Consequently, it can be concluded that introducing PAMPSA into the polymer bulk during synthesis notably affected NIH/3T3 cell proliferation in comparison with reference and pristine PANI. These results correspond to observed preferable behaviour of mouse embryonic stem cells on pristine PANI forms, compared to PANI sample deposited with PAMPSA (Humpolíček, Radaszkiewicz et al. 2015).

The results of cell migration (micrographs can be found in doctoral thesis) fully correspond to the limited cell proliferation observed on these surfaces and correlates with the results obtained for the surface energy.

To conclude this first study, the NIH/3T3 fibroblasts were able to adhere, proliferate and migrate on PANI doped with sulfamic or phosphotungstic acids. These PANI films could therefore be suitable for utilization, e.g. in TE of electrically responsive tissues. Nevertheless, the incorporation of PAMPSA affected the surface properties of the PANI films, significantly influencing the cell proliferation and migration. Therefore, their application in the biomedical sector is limited, but opens the door for utilization as a biosensor or bio-interface, where limited cell adhesion can be desirable. Moreover, cell behaviour corresponds to the surface energy of individual samples and the surface energy constitutes the crucial factor that influences cell/surface interaction. Obtained results were presented in "Article Adhesion, Proliferation and Migration of NIH/3T3 Cells on Modified Polyaniline Surfaces" by Rejmontová *et al*., published in International Journal of Molecular Science (Rejmontova, Capakova et al. 2016).

b) PANI films prepared in colloidal dispersion mode

In the presence of suitable stabilizer, PANI could create a colloidal dispersion (Stejskal, Trchová et al. 2015). Films proceeded during dispersion polymerization are in general thinner and smoother compared to those proceeded from the reaction mixture without stabilizer (Stejskal and Sapurina 2004). The PANI films synthesized in colloidal dispersion mode could, therefore, represent interesting conducting material with application potential in TE or as biosensors.

PANI films were synthesized in presence of four stabilizers, namely poly-N-vinylpyrrolidone (PANI-PVP-H_2O), sodium dodecyl sulfate (PANI-SDS-H_2O), Tween 20 (PANI-T20-H_2O) and Pluronic F108 (PANI-F108-H_2O). The

polymerization was also carried out in 1M hydrochloric acid instead of water (PANI-PVP-HCl, PANI-SDS-HCl, PANI-T20-HCl, PANI-F108-HCl).

Since prepared PANI films represent novel materials without any described biological properties, the standard tests of cell adhesion, proliferation and migration on PANI films were performed. Moreover, the biological evaluation was extended by testing of skin irritation potential of selected samples.

For the purpose of doctoral thesis summary, the obtained results will be briefly summarized. Concerning the cell adhesion, cell behaviour was similar to reference material. However, significant differences were observed in the subsequent behaviour of cells. Cells were able to grow and proliferate only on PANI-SDS-H_2O and PANI-SDS-HCl samples. Explanation of the exceptional cell behaviour on these films can be attributed to the fact that SDS contains sulfate groups, which were reported to activate the spreading of cells, and influence cytoskeleton reorganization (Kowalczyńska and Nowak-Wyrzykowska 2003).

Furthermore, the best performing film with respect to cell compatibility (PANI-SDS-HCl) was tested for the production of reversible damage to the skin using epidermal model. Utilization of this model shifted the testing from study single cell response to the response of a more complex system and brought improved information about biological properties of tested sample. Based on the results obtained using skin irritation test, this film was classified as a non-irritating material, since the viability of cells in the epidermal model treated with the tested sample reached (112 ± 6) %. The obtained result supported previous findings of Humpolíček *et al.* (Humpolíček, Kašpárková et al. 2012). They determined no skin irritation potential of standard PANI powders without stabilizers.

To conclude this section, the novel PANI films were synthesized and their biological properties were described. The presence of stabilizers during the synthetizes of PANI films significantly affected cell response within the meaning of cell proliferation and migration. Among used stabilizers, incorporation of SDS had the best positive impact on the cell biocompatibility of prepared PANI films. Moreover, PANI-SDS-HCl films were non-irritating and showed no harmful effects on human skin. This significantly enhances the applicability of PANI in biomedicine. In particular, its use in biosensors capable of conductometric monitoring of ongoing changes on the skin surface should be of particular interest. Described results were presented in Article "Cell-compatible conducting polyaniline films prepared in colloidal dispersion mode" by Kašpárková *et al*, published in Colloids and Surfaces B: Biointerfaces (Kašpárková, Humpolíček et al. 2017).

c) Green synthesis of PANI

Considering PANI synthesis, new "green" approach could involve the utilization of environmentally friendly oxidants, e.g. molecular oxygen or hydrogen peroxide, instead of ordinarily used toxic persulfates or silver nitrate. Moreover, aniline, which is commonly used as starting reactant, is also regarded to be toxic (Pauluhn 2004).

For the purpose of presented study, two basic approaches were applied (both instructed during the Erasmus traineeship at the chemical department of University degli Studi di Milano). First, PANI synthesis was based on the oxidation of N-(4-aminodiphenyl)aniline (aniline dimer) with hydrogen peroxide in the presence of Fe^{3+} as a catalyst. This novel "green" approach allow to obtain water as co-product and avoid the formation of toxic pollutants, e.g. benzidine (Della Pina, Capáková et al. 2017). The second synthesis way of PANI considers the presence of ethanol in the reaction mixture. It has been already described that presence of alcohol during PANI synthesis altered e.g. the morphology of product (Zhou, Wu et al. 2007). All prepared samples were finally doped by salicylic acid, intended to improve biological properties.

The aim of this study was focused on the description of the cytotoxic effect of prepared PANI powders. For this purpose, cytotoxicity testing was performed using NIH/3T3 cell line. The test was conducted according to the EN ISO 10993-5 standard procedure to be comparable with previously published results about PANI cytotoxicity. The completed results are discussed in full text of doctoral thesis. Briefly, all tested samples showed slightly higher cytotoxic effect toward to NIH/3T3 cell line compared to the cytotoxicity of reprotonated PANI powder prepared by Humpolíček and co-workers towards to HaCaT and HepG2 cell lines (Humpolíček, Kašpárková et al. 2012). The toxicity could be addressed to decreased pH of extract (indicated by the observed colourless of prepared extract). The observed phenomenon could be caused by the insufficient purification of samples after final doping step by salicylic acid. Therefore, the additional washing could decrease the toxic effect of all tested PANI powder samples.

d) PANI cryogel

For the purpose of this thesis, the PANI cryogel was prepared at the Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic. The prepared scaffold combines intrinsic electrical conductivity and the material properties of hydrogels (Humpolicek, Radaszkiewicz et al. 2018). This properties make it more than desirable for application in TE and regenerative medicine.

Cytotoxicity of extract was primarily studied as the basic parameter of biocompatibility. Two cell lines were utilized for this purpose - mouse fibroblasts NIH/3T3 cell line and mouse embryonic fibroblasts (MEF). The cytotoxic effect was tested both on native and purified PANI cryogels (repeated extraction of 5 g of cryogel with 50 mL of ultrapure water). The selected results of cytotoxicity testing are clearly illustrated in Figure 6. Concerning the cytotoxic effect on both

cell lines, the results obtained using NIH/3T3 and MEF were comparable. For that reason, only the results obtained using MEF cell line are presented.

Cell viability was also comparable for native and purified samples, up to their extract concentration of 75%, which exhibited no cytotoxic effect. The parent 100% extracts of both native and purified cryogel showed mild cytotoxicity.

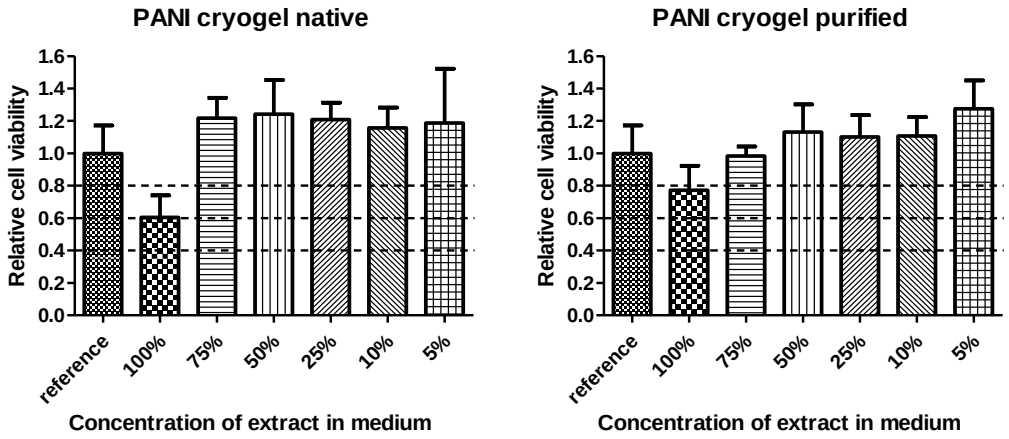

Figure 6. Cytotoxicity of extracts of native (A) or purified (B) PANI cryogel determined as a relative number of viable MEF cells cultivated in the presence of extracts for 24 h. The dashed lines highlight the limits of viability according to EN ISO 10993-5: viability >0.8 corresponds to no cytotoxicity, >0.6–0.8 mild cytotoxicity, >0.4–0.6 moderate toxicity and <0.4 severe cytotoxicity.

Based on the promising results obtained from cytotoxicity test, an ability of cells to ingrowth within the scaffold was also performed. However, the cells were not able to ingrowth inside the scaffold, likely due to insufficient pore interconnection.

To conclude, the novel PANI-based scaffold exhibits suitable biocompatibility. However, for the utilization in TE, the improvement of synthesis procedure is necessary to produce scaffold with pores, which enable cells to ingrowth within the bulk of the material. The results were summarized in Article "Polyaniline cryogels: Biocompatibility of novel conducting macroporous material" published in Scientific Reports by Humpolíček *et al.* (Humpolicek, Radaszkiewicz et al. 2018).

e) Poly(p-phenylenediamine)

Although the polyphenylenediamines are considered as an electric non-conductor with very low conductivity varied between 10^{-10} and 10^{-9} $S.cm^{-1}$ (Stejskal 2015), they exhibit properties related to conductive polymers, especially PANI. Therefore, besides their already established potential as corrosion protection or sensors, they could be also attractive for application in biomedicine.

The fundamental cytotoxic effect of poly(*p*-phenylenediamine) (PpPDA) was described. Two samples were synthesized at Institute of Macromolecular Chemistry, Academy of Science in Prague differing in the concentration of

oxidant in reaction mixture – PpPDA-1 powder (0.25M ammonium persulfate) and PpPDA-2 powder (0.5M ammonium persulfate). The flow cytometer was used for the cytotoxic effect evaluation. The harmful effect of the extracts of both PpPDA-1 and PpPDA-2 on NIH/3T3 cell line was described (results are summarised in doctoral thesis). Both PpPDA samples exhibited severe toxicity and their application in biomedicine is conditioned by modification of preparation procedure and/or purification of resulting products, with the aim of significantly decreasing their cytotoxicity.

Described results were presented in Article "Cytotoxicity of poly(p-phenylenediamine)" published in Chemical papers by (Kuceková, Rejmontová et al. 2017).

7.4.2. Cell interaction with micro-structured surfaces

Micro-structured polystyrene surfaces prepared at the Department of Physics and Materials Engineering, Tomas Bata University in Zlín were utilized for the study of cell interaction with topographic features. Polystyrene (PS) Petri dishes (TPP Tissue culture dishes, BioTech, Czech Republic), were modified with micropores (M-PS) *via* the time-sequenced dispensing of good and poor solvent mixtures on the rotating surface of treated substrate (Wrzecionko, Minařík et al. 2017). The micro-structured surfaces were subsequently modified with plasma treatment (M-PS-20W, M-PS-50W, M-PS-100W). The parent PS Petri dishes were also treated with plasma (PS-50W, PS-100W).

Pore size/depth/distribution as well as surface energy, significantly influence the cells in a various manner including their adhesion (Wan, Wang et al. 2005), proliferation (McGlohorn, Holder et al. 2004), morphology (Arai, Tanaka et al. 2008) gene expression (DeRosa, Hong et al. 2014) etc. The scheme of biological testing was following: 1) Cell adhesion test; 2) Cell proliferation test; and 3) Evaluation of impact on cell cytoskeletons.

No impact on cell adhesion was observed. Concerning the cell proliferation, the number of viable cells was lower on M-PS, M-PS-20W, and M-PS-50W compared to that on the reference (PS) (data presented in doctoral thesis).

The impact of topography on the cytoskeleton formation was detected by actin staining. From the micrographs presented in Figure 7, it can be concluded that the cytoskeletons of cells cultivated on samples PS-50W and M-PS-20W were similar to those in the reference.

Despite the dramatic change of topography (samples marked as M-PS), the cytoskeletal structure did not change significantly with moderate hydrophilicity increase (plasma treatment 20 W). At more hydrophilic surfaces (plasma treatment 100 W for PS and 50/100 W for M-PS), the cell's cytoskeleton was altered noticeably at both substrates: the referential PS and modified M-PS. This suggests that the surface energy plays a primary role, and the surface relief plays a secondary role, probably because the structured and (at the same time) hydrophilic surfaces better mimic the native 3D structure of biological tissue.

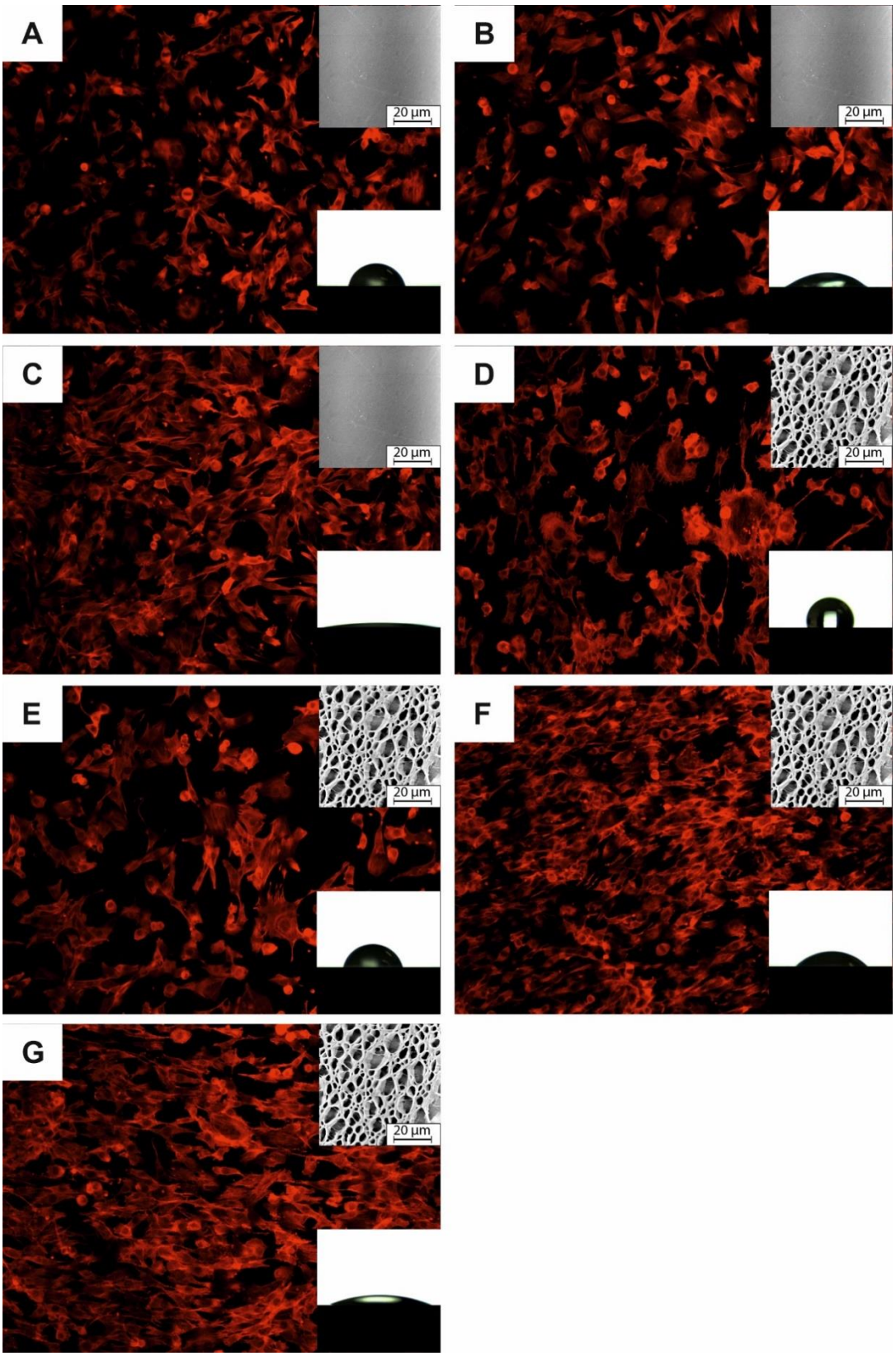

Figure 7. The cytoskeleton of NIH/3T3 visualized by ActinRed on (A) reference PS, (B) PS-50W, (C) PS-100W, (D) M-PS, (E) M-PS-20W, (F) M-PS-50W, and (G) M-PS-100W. Magnification x100. Top-right corner: SEM image of the used surface (scale bars correspond to 20 μm); bottom-right corner: water drop on the studied surface as a measure of surface hydrophilicity (Wrzecionko, Minařík et al. 2017).

In the case of surfaces M-PS and M-PS-20W, the cells are more compact, spherical, and create filopodia. On the M-PS-50W and M-PS-100W surfaces, the cells seem to be preferentially oriented, and their morphology is closer to the normal fibroblast morphology on planar surfaces. Therefore, it can be concluded that, based on both the cell viability and cell morphology experiments, the M-PS-100W represents the most promising surface for biological applications.

To conclude this section, presented study enable the deeper insight into the issue of cell interaction with micro-structured polymer surfaces, which is still not sufficiently described due to its complexity. Obtained results were presented in Article "Variations of Polymer Porous Surface Structures *via* the Time-Sequenced Dosing of Mixed Solvents" of Wrzecionko *et al.*, published in ACS Applied Materials and Interfaces (Wrzecionko, Minařík et al. 2017).

7.4.3. Cell interaction with the 3D polymeric material (scaffold)

Contrary to above mentioned tests, no standardised protocol for cell cultivation in bioreactor existed in Cell biology laboratories at Centre of Polymer Systems. Moreover, no uniform method has been discovered in published articles. Therefore, prior to testing itself, the reliable pre-cultivation procedure was necessary to be established. Primarily, the optimal cell seeding was determined ($1x10^6$ cells per mL). The sufficient pre-cultivation period was adjusted to 24 hours under static cultivation conditions in incubator allowing cell adhesion prior to cultivation in bioreactor. Finally, the minimum cultivation period of 14 days and the efficient rotational speed of 15.5 RPM was confirmed.

Scaffolds based on poly(vinyl alcohol) and Kraft lignin

Poly(vinyl alcohol) (PVA) has already been utilized in tissue replacement (Hayes and Kennedy 2016). However, limited mechanical properties of PVA was described (Karimi and Navidbakhsh 2014). This limitation was presumed to be improved by Kraft lignin.

The biological properties of Kraft lignin have not yet been satisfactorily described. However, it could exhibit several properties desirable for TE, e.g. stiffening effect (Naseem, Tabasum et al. 2016), antioxidant efficiency (Kaur and Uppal 2015) or antibacterial activity (Dong, Dong et al. 2011). However, its utilization in TE is challenging due to its polydispersity. Therefore, a fractionation of KL is highly recommended before its utilization in any application.

A set of scaffolds based on PVA and fractionated Kraft lignin (KL^f) were prepared in cooperation with the Graz University of Technology. Hydrogels were designated as PVA_X-KL^f, where X indicates the concentration of lignin (1, 5, 10, 15 and 20 wt%). The arrangement of biological testing included cytotoxicity testing (both in direct contact and cytotoxicity of extracts) and the evaluation of cell ability to ingrowth within the bulk of tested samples.

Firstly, the testing of cytotoxicity in direct contact was employed. It was found that cells were able to proliferate in direct contact with all samples.

For quantitative cytotoxicity assessment, extracts of the studied scaffolds were tested using flow cytometry and SYTO staining. The results for 50, 75 and 100% extracts of PVA_5wt-KL^f and PVA_20wt-KL^f are presented in Figure 8. A significant dependence between the cytotoxic effect and the amount of KL^f in the tested samples was observed.

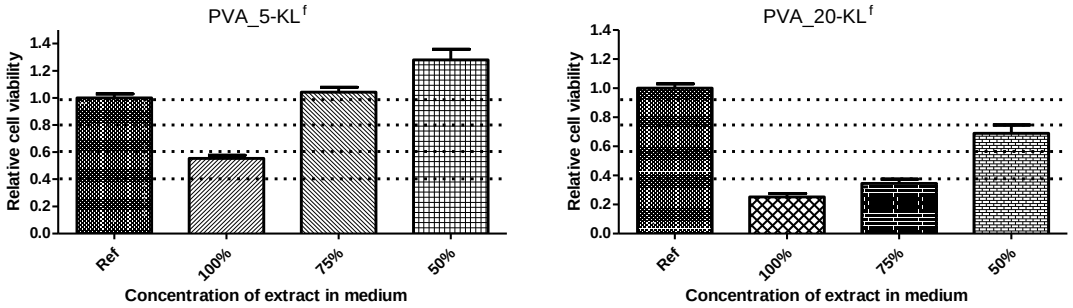

Figure 8. Cytotoxicity of scaffold extracts of various concentrations presented as a relative number of viable cells ± the standard deviation compared to reference according to ISO 10993-5 standard. The dashed lines highlight the critical viabilities to be assessed according to requirements of EN ISO 10993-5, where viability > 0.8 means no cytotoxicity; 0.6–0.8, mild cytotoxicity; 0.4–0.6, moderate toxicity; and <0.4, severe cytotoxicity.

The obtained results are discussed in the context of more general studies of lignin, because of a lack of articles dealing with the biocompatibility of KL and especially its fractionated form. In spite of the fact that lignin-based copolymers or material with incorporated lignin are perceived as nontoxic (Kai, Low et al. 2015, Musilová, Mráček et al. 2018), it must be noted that lignin could have an adverse impact on cell viability. The data presented in this thesis is in a good agreement with the study performed by Kai *et al.* (Kai, Ren et al. 2016). They described the limitations of the application of alkali lignin resulting from its cytotoxicity at high concentrations. A concentration of 6 % by mass was evaluated as the optimal amount of alkali lignin in PLLA/PLA-lignin nanofibers. Based on the results obtained in our study, the highest possible concentration of KL^f exhibiting no or very low cytotoxicity was assessed to be around 5 wt%.

The ability of cells to ingrowth and survive within the internal structure of scaffolds is another crucial parameter. Thus, NIH/3T3 cells were seeded inside the tested samples and cultivated in a Rotary Cell Culture systems™ RCCS-4. It was found that the cells were able to grow into all the tested scaffolds (as can be seen in Figure 9, in which only PVA, PVA_1-KL^f, PVA_5-KL^f, and PVA_20-KL^f are depicted as examples). A significant correlation was found between the ability of cells to grow into scaffolds and the amount of KL^f contained in samples. Greater cell growth into samples with lower amounts of KL^f was observed similarly to what was observed during the testing of cytotoxicity of extracts. The samples PVA_1- KL^f and PVA_5- KL^f are advantageous for mouse fibroblasts. However, according to the comparison of stained cell cytoskeletons inside

scaffolds, cell growth was homogeneous only in the case of PVA_1- KL^f (Figure 9B). In contrast, the cells inside the PVA_5- KL^f (Figure 9C) scaffold created clusters. The cell ingrowth is an important parameter because TE scaffold should enable cells to grow homogeneously into its structure and gradually form a continuous tissue. The creation of separate clusters is undesirable. On the basis of advanced biological testing, it must be noted that only scaffold containing one wt% of KL^f can be proposed as advanced materials suitable for use in TE.

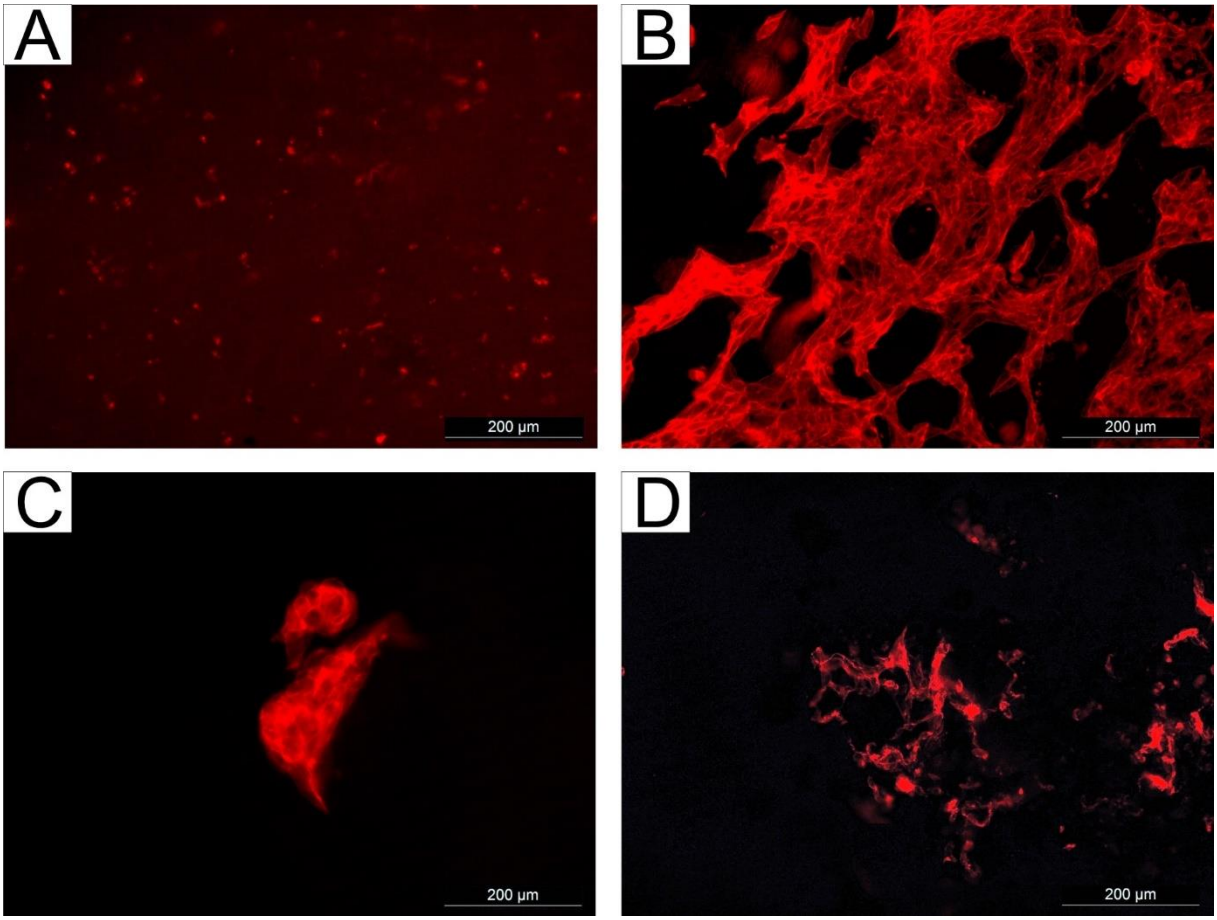

Figure 9. NIH/3T3 fibroblasts stained with AnctinRed™ 555 grown within A) PVA, B) PVA_1- KL^f, C) PVA_5- KL^f , D) PVA_20- KL^f. Scale bars correspond to 200 µm.

To conclude this part, the biological properties of novel PVA and Kraft lignin-based scaffolds were described using both standardized *in vitro* methods and bioreactors simulating *in vivo* culturing conditions. The cell-3D material interaction under simulated *in vivo* conditions was evaluated at Cell biology laboratories of the Centre of polymer systems for the first time. The results were summarized in Manuscript entitled "The use of fractionated Kraft lignin to improve the mechanical and biological properties of PVA-based scaffolds" accepted in RSC Advances in May, 2019 (Rejmontová, Kovalcik et al. 2019, in press).

CONCLUDING SUMMARY

The understanding of the principles of cell-material interaction is essential for developing of functional biomaterials with desired biological properties. Toxicity (in polymers mainly connected to the presence of residual impurities), surface and bulk properties (consider 3D scaffolds) could be mentioned as crucial aspects influencing the biological properties of any material. Based on its complexity and despite the perpetual effort of scientists, this topic has been still poorly understood.

The thesis is focused on cell interaction with a polymeric material, particularly with conductive ones. The topic of the interaction of eukaryotic cells with artificial materials is discussed in relatively broad scope extended from already well-established 2D *in vitro* to 3D simulated *in vivo* cell cultivation conditions. *In vitro* cell cultivation conditions enable controlled, quantifiable experimental characterization, however, it lacks the complexity occurs *in vivo*. The bioreactors simulated *in vivo* conditions could be considered as an intermediate step between *in vitro* and *in vivo* conditions combining the advantages of both approaches.

Experiments performed during the doctoral study cover all above mentioned basic aspects related to the biological properties of polymeric material. Obtained results could be divided according to defined aims of doctoral thesis into three groups: 1) cell interaction with conductive polymers; 2) cell interaction with micro-structured surfaces; and 3) the implementation of methodology and subsequent testing of cell interaction with the polymeric scaffolds.

The first part, cell interaction with conductive polymers (especially with PANI), comprised the majority of doctoral study. PANI in several forms (including film, powder or novel 3D scaffold) was studied. In summary, PANI films with modified surface properties were prepared and the principal cell response to surfaces this of individual films was investigated. Firstly, the surface energy of the sample, especially its correlation with a surface energy of cells, was indicated to be the key factor influencing the cytocompatibility of PANI films modified with acids. Secondly, the using of colloidal dispersion during PANI synthesis can lead to improving the interaction of cells with the surface of the material. Focusing on the powder form of material, the residual impurities (such as non-bounded monomers, oligomers, used chemicals or additives) was decided to be the major source of the toxic effect of polymeric material. Therefore, the selection of appropriate purification step (to avoid the unwanted releasing toxic impurities under physiological conditions) is necessary for successful utilization of polymeric material in biomedicine. Moreover, a preliminary study focused on the biocompatibility of novel soft 3D porous PANI-based cryogel was presented. The PANI cryogel represents promising material for TE showing appropriate biological properties.

Concerning the second aim, the cell response to micro-structured PS surfaces exhibited various surface hydrophilicity was evaluated. The results suggested the

principal effect of surface energy on cell behaviour, primarily on cell morphology, more than surface topography alone.

Finally, the methodology for testing the ability of eukaryotic cells to incorporate within 3D polymeric scaffolds was introduced in Cell biology laboratories at the Centre of polymer systems. The appropriate seeding concentration of the cells, parameters of cell cultivation as well as the procedure suitable for the evaluation of cell ingrowth were defined. During the doctoral study, this novel method was utilized for evaluation of cell ingrowth within several polymeric scaffolds. The cell interaction with 3D polymeric scaffolds based on PVA and fractionated KL under simulated *in vivo* conditions was also described in the presented thesis. The appropriate amount of KL^f within scaffolds in the sense of lack of toxicity and homogeneous cell ingrowth was determined.

CONTRIBUTION TO SCIENCE

The presented topic of cell interaction with artificial material remains still not fully explored. The presented thesis deals not only with the cell interaction under standard 2D cultivation conditions, but also extend the knowledge about cell-material interaction under conditions closed to the cells natural environment (under simulated *in vivo* conditions). The methodology for testing of cell ingrowth within 3D porous scaffold was established. Therefore the range of routine biological tests employed in Cell biology laboratory at Centre of polymer systems has been extended. The cell interaction within porous 3D polymeric scaffolds can be studied using both rotary cell culture vessel and continuous flow perfusion systems.

During the study, the biological properties of several novel materials were described and their possible application in biomedicine were discussed. To be concrete, PANI films modified with acids or prepared in colloidal mode, green synthesized PANI powders, PANI cryogel and scaffold based on PVA and KL^f were studied. Of them, the PANI cryogel could be mentioned as one of the most interesting materials mimicking the mechanical properties of native tissue, with promising application potential in TE.

The acquired knowledge can be used in the process of development of novel material with properties desired for utilization in biomedicine and TE. Nowadays, the nontoxicity itself is not sufficient property of any biomaterial. High demand is paid to stimuli-responsive material, which is able to specifically modulate cellular behaviour and trigger a targeted cell response (e.g. differentiation). Therefore, the understanding of the interaction between cells and materials at the interface remains a crucial precondition for the successful application of any material in modern medicine.

Moreover, the results described in this thesis were presented at several national and international conferences and were published in impacted international journals.

FUTURE PROSPECTIVE

Based on the cell sensitivity to diverse stimuli previously described in the theoretical part and continuing researcher effort to become cell culturing closer to physiological conditions, the presented study could be further extended in several areas. Firstly, the cell interaction with polymeric materials under dynamic cell cultivation condition could be studied (determine the impact of perfusion rate on cell behaviour could be mentioned as an example). Additionally, as the predominant part of the presented thesis was focused on the conductive polymer, the cell response to electrical and/or mechanical stimuli could be considered as an interesting topic for further investigations. Finally, the attention could also be paid to development of porous scaffolds mimicking tissue properties prepared by the combination of conductive polymers with natural biopolymers.

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LIST OF ABBREVIATIONS AND SYMBOLS

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2D	Two-dimensional material/ cell culturing technique
3D	Three-dimensional material/ cell culturing technique
CP	Conductive polymers
ECM	Extracellular matrix
HARV	High aspect rotating vessel
HCl	Hydrochloric acid
HPLC	High-performance liquid chromatography
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
KL^f	Fractionated Kraft lignin
MEF	Primary mouse embryonic fibroblast
M-PS	Micro-structured Polystyrene
OECD	The Organisation for Economic Co-operation and Development
PAMPSA	Poly(2-acrylamido-2-methyl-1-propanesulfonic) acid
PANI	Polyaniline
PANI-B	Polyaniline base
PANI-PT	Polyaniline doped with phosphotungstic acid
PANI-S	Polyaniline salt
PANI-SULF	Polyaniline doped with sulfamic acid
PpPDA	Poly(p-phenylenediamine)
PS	Polystyrene
PVA	Poly(vinyl alcohol)
PVP	Poly(N-vinylpyrrolidone)
RCCS	The rotary cell culturing system
RGD	Tripeptide composed of Arginine, Glycine and Aspartic acid
RPM	Revolutions per minute
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscopy
SYTO	Red fluorescent nucleic acid stain
TE	Tissue engineering
TPP	Tissue culture plastic
UV	Ultraviolet
wt%	Percentage by mass
γ^{AB}	Polar part of surface energy of material
γ^{DIF}	Difference between the surface energy of the cells and the sample
γ^{LW}	Apolar part of surface energy of material
γ^{TOT}	Total surface energy of material

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