Tomas Bata University in Zlín
Faculty of Technology

Summary of habilitation thesis

Conducting Polymer Scaffolds – the Technology of Preparation and Cytocompatibility

Vodivé polymerní scaffoldy – technologie přípravy a cytokompatibilita

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ABSTRACT

This habilitation thesis summarises studies performed on conducting polymers, especially polyaniline and polypyrrole, during more than ten years of research. In the course of time, various procedures for polymer synthesis and a number of modification techniques were employed, giving rise to a wide variety of conducting materials or their composites. Prepared conducting polymers and their composites were deeply characterized in terms of their material properties, with special interest paid to their surface properties. The thesis also sums up unique and original results documenting improvements in the cytocompatibility of these conducting polymers and composites. Especially the composites with biopolymers are unique in terms of their cytocompatibility. The new knowledge gained in this field resulted in the preparation of cytocompatible conducting scaffolds with target applications in tissue engineering.

ABSTRAKT

Teze habilitační práce shrnují více než desetiletou práci uchazečky v oblasti studia elektricky vodivých polymerů, zejména pak polyanilinu a polypyrrolu. Za oněch deset let využila uchazečka ve své práci řadu postupů syntézy vodivých polymerů a jejich modifikací. Výsledkem je široká škála připravených vodivých polymerů a jejich kompozitů. Připravené materiály byly detailně studovány z hlediska materiálových, především povrchových, vlastností. Teze sumarizují především oblast originální uchazeččiny práce zaměřené na zlepšení buněčné kompatibility vodivých polymerů a jejich kompozitů. Obzvláštní pozornost zasluhují kompozity s biopolymery, které vykazují unikátní biologické vlastnosti. Nové znalosti, které vzniky díky práci uchazečky, vyústily v přípravu tkáňových lešení vhodných pro oblast tkáňového inženýrství.

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Article X. Rejmontova, P.; Kovalcik, A.; Humpolicek, P.; **Capakova, Z**.; Wrzecionko, E.; Saha, P. The Use of Fractionated Kraft Lignin to Improve the Mechanical and Biological Properties of PVA-Based Scaffolds. RSC Adv. 2019, 9 (22), 12346–12353. https://doi.org/10.1039/c8ra09757g.

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INTRODUCTION

Thousands of surgical procedures are performed globally on a daily basis due to the need to replace or repair of tissues which have been damaged by disease or injury. This is made possible by the enormous progress in material science, which has led to the preparation of biocompatible materials suitable for the fabrication of medical devices – otherwise known as biomaterials. Biomaterial science has matured in the last few decades and become a highly interdisciplinary field. An important part of biomaterial science is the field of polymer science. The development of biocompatible polymers and their utilization as biomaterials have significantly contributed to progress in modern medicine. The chief advantage of using polymers is the ability to control their shape and size, as well as their functionalities and mechanical properties, in order to fabricate products with the desired properties for a specific use. However, considering the complexity of the human body, the field of biomaterials is still open to new discoveries and inventions. Hence, new biomaterials with new applications are continually being developed in the biomedical area. This includes the synthesis, fabrication, design, and selection of such materials. The increasing demand for the development of superior biomaterials with new applications has resulted in efforts to create smart biomaterials.

Smart biomaterials are sophisticated materials which are able to respond to various external chemical and physical stimuli, and to changes in physiological parameters. They can be sensitive to pH, temperature, redox potential, stress, and electrical fields. The development of such stimuli-responsive materials with properties tailored to specific applications is a very challenging task for researchers worldwide. Electro-conducting materials are classified as one such smart biomaterial. They allow for current conduction and thus the modification of cell behaviour. Conducting polymers are an excellent choice for the preparation of such smart materials. However, a lack of fundamental understanding of the impact of various forms of conducting polymers on cell behaviour has been the major limitation on their utilization in biomaterials sciences. Hence, the biological characterization of various conducting polymers and their composites, prepared by a variety of means, is the main topic of this work, this thesis, and my research career. My interest focuses especially on two of them: polyaniline and polypyrrole.

1 BIOMATERIALS

Nowadays, modern medicine is unthinkable without the use of biomaterials. Thanks to the utilization of various biomaterials, the quality of healthcare has rapidly increased. Biomaterials are used in a broad range of applications, from disposable medical devices (e.g. blood bags), through contact lenses, to medical implants. Although humans have used biomaterials since the dawn of history, their development has accelerated over the last 50 years. However, there is still demand for new biomaterials with better properties. Indeed, it is not sufficient for a biomaterial merely to be biocompatible and to serve as a simple replacement; it should also have additional advanced properties, such as health-promoting function(s).

Biomaterials can be defined in several ways. Indeed, definitions have been evolving in the same manner as biomaterials themselves. Originally (in 1987), the European Society for Biomaterials Consensus Conference II proposed the following simple definition "*A biomaterial is a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body*"[1]. Currently, one of the most broadly accepted definitions states that "*A biomaterial is any substance or combination of substances, other than drugs, synthetic or natural in origin, which can be used for any period of time, which augments or replaces partially or totally any tissue, organ or function of the body, in order to maintain or improve the quality of life of the individual*"[2,3]. However, both these definitions agree that a biomaterial is essentially a material which interacts with the human body. The material can be both natural and synthetic and it can come into contact with organisms in a direct or indirect way. Consequently, the material which is in contact with the body can influence its physiology or more generally its biological function. Therefore, biocompatibility is its first and most crucial characteristic, which is to say that biomaterials cannot elicit any harmful effects in or on organisms.

Each of a substance's material properties, however, is able to affect the substance's overall biocompatibility. All of them together, including chemical composition and surface, mechanical and physical properties, must therefore be considered in this context. Unfortunately, there is no exact "recipe" for preparing materials with a combination of properties which would ensure biocompatibility. Which properties are desirable depends on where the biomaterial is to be applied and on its expected function. The basic classification of material properties affecting host response covers surface and bulk properties and will be described in more detail below.

Metals, ceramics, glass, and polymers or their composites can be utilised as biomaterials[4,5]. Metals and their alloys are widely used as load bearing implants or internal fixation materials, such as screws, wires etc. The biggest advantages

are their strength and easy sterilization[5]. However, among their disadvantages includes corrosion due to chemical reaction with body enzymes or acids[6]. Ceramics are also known for their strength, stiffness, and hardness[7]. They generally find applications in the repair of the skeletal system, i.e. bones, joints, and teeth[8]. Biomaterials based on glass are also intended for use in the skeletal system, e.g. for the correction of bone defects or as composites in dentistry[9]. All the above mentioned materials find application mainly in hard tissues, and their mechanical properties are not suitable for soft tissue engineering. The sophisticated nature of soft tissue can, however, be mimicked by polymers,[10] whose utilization in biomedical sciences is more diverse than that of metallic, ceramic, or glass materials.

1.1 Properties of biomaterials

The properties of biomaterials can be classified into the two main groups: 1) material properties and 2) biological properties[11]. As already mentioned, these two groups are closely connected and affect each other. No matter whether we are considering chemical composition, or mechanical or physical properties, all such properties can directly influence the biocompatibility of the material.

1.1.1 Material properties

Material properties play an important role in the development of new biomaterials. No matter whether we are talking about physical, chemical or mechanical properties, all are able to directly influence the host response. Material properties cover both surface and bulk properties[12]. This topic is described in more detail in teaching materials prepared by myself within the framework of project No. CZ.02.2.69/0.0/0.0/16_018/0002720 .

1.1.1.1 Surface properties

The surface is the first "part" of any material which comes into contact with the organism (i.e., when considering materials used in direct contact with the body). The very first phenomenon which occurs after contact between the biomaterial and the host organism is protein adsorption onto the material surface. The type as well as amount of adsorbed proteins affects the adhered cells in respect of their physiology and cellular signalling pathways. Therefore, protein adsorption plays an important role in the future fate of cell adhesion, proliferation, and differentiation, etc. The surface immobilization of proteins is explained by a process called the Vroman effect[13].

Protein adsorption is influenced by a broad range of factors including the physical as well as chemical properties of the material surface[11]. For example, surface properties such as free energy, charge, wettability, functional groups, or topography affect protein adsorption and, therefore, the final biocompatibility of

the material. In the case of conducting polymers, conductivity should also be included.

1.1.1.2 Bulk properties

After the cells have adhered to the surface of material, the physical, chemical and material properties of the bulk material directly influence the dynamic interactions at the interface between the cells and the biomaterial, which subsequently affect cell fate.

Generally, the first prerequisite for successful cell adhesion is conditioned by the requirement that biomaterials should mimic the properties of native tissue, according to the exact location of the intended use[12]. Therefore, it is very important to consider whether the biomaterial will be utilized in soft or hard tissue, etc. As their names suggest, soft or elastic materials are desirable for soft tissues. By contrast, rigid and tight materials are more suitable for hard tissues.

Bulk properties of materials directly influence dynamic interactions at tissue/biomaterial interfaces. Among the most important are two interrelated characteristics - mechanical properties and the architecture (porosity) of the material. The size, shape, orientation, and distribution of pores influence the mechanical properties of the bulk, as they define the structure and dimensions[14,15]. It is desirable to mimic the physicochemical properties of native tissues that the biomaterials are intended to replace or augment.

Nevertheless, even if the "perfect material" – one exhibiting "perfect" chemical physical and structural properties – were to be created, biological compatibility would still have to be ensured. Thus, biocompatibility testing is, and always will be, necessary.

1.1.2 Biological properties – biocompatibility

The most common term characterising the suitability of the biological properties of biomaterials is biocompatibility[16]. The first definition of biocompatibility came from Williams, who defined biocompatibility in 1987 as "*the ability of a material to perform with an appropriate host response in a specific situation*"[17]. However, over time, this simple definition proved insufficient, which led to a re-definition of the term in 2008 by Williams himself. This definition is commonly used until today. Its exact wording is: "*The ability of a material to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most appropriate beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy*"[18].

The biocompatibility of a material is a very complex characteristic, which involves various biological properties. A biocompatible material has to fulfil

several conditions, e.g. it has to be non-cytotoxic, non-immunogenic, non-irritant, non-inflammatory, and non-carcinogenic[19]. To test the biocompatibility of a material, a series of different tests has to be performed. The exact types of tests depend on the anticipated future utilization of the material. There are numerous aspects determining the extent of biological testing. Here, we can mention, for example, the nature of biomaterial contact with the organism, which is classified as external contact, surface contact, or implantation. The duration of the contact (limited, prolonged, or permanent) is another important aspect of biocompatibility. Indeed, there is a legislative framework defining specifications which the material or device has to meet[20].

The evaluation of cytotoxicity, in contrast, has to be accomplished no matter what the intended utilization of the biomaterial is. It is one of the first tests performed for every biomaterial[20].

After the evaluation of cytotoxicity, the material undergoes other types of biological testing, which may include carcinogenicity, chronic toxicity, genotoxicity, immune response, skin sensitization and irritation, intracutaneous reactivity, and reproductive toxicity, among others.

2 BIOELECTRICITY

The effect of electric fields on the behaviour of cells and tissues, especially muscle and nerve, has been of interest for a long time[21,22]. The impact of electricity on muscle contractions was demonstrated as early as 1791 by Luigi Galvani[23]. In his work, he uncovered the fact that electricity occurs in living tissues. Later, in the 19th century, Emil du Bois-Reymond, following up the work of Galvani, reported the presence of electricity in wounds[24]. The electrical phenomena occurring in or generated by living organisms are termed bioelectricity. Interest in bioelectricity grew in the 20th century and led to the design of many commonly used medical devices. Today, it is well-known that electricity plays a role in many cellular processes, such as cell differentiation, cell division, and cell migration, and in signalling systems[25].

It is worth mentioning that the conduction of electric current, which is a stream of charged particles, can be accomplished either *via* the flow of electrons or the flow of ions, the latter being typical for bioelectric current[26]. This means that ions are the charge carriers in living tissues and organisms. This relates to one of the advantages of conducting polymers compared to other conducting materials such as metals. Conducting polymers (which will be discussed further) exhibit mixed conductivity – ionic and electronic – and can thus better "communicate" with living objects[27].

2.1 Electrically conducting polymers

There is currently an enormous effort to develop biomaterials with "added value" – in literature, called "smart" or "intelligent" materials. Smart materials should have tailored properties and controlled functions which directly influence cell behaviour. These biomaterials should be able to respond to various environmental stimuli by changing their properties, such as biomechanical or drug-releasing features[28]. This means, for example, that they can be temperature or pH sensitive, or enzymatically active. As reported in the previous chapter, electric fields are able to influence cell behaviour. Therefore, conducting polymers could be useful with respect to the preparation of such smart biomaterials.

The materials with the best electrical conductivity are metals, of which the highest conductivity was measured for silver (6.3 S m^{-1} at 20 °C)[29]. However, the limited compatibility of some metals with living systems is often encountered in tissue engineering. The limitations are mainly connected to 1) their mechanical properties making them incompatible with soft tissues, and 2) the fact that the electric current is carried by electrons and not by ions. In light of these facts, conducting polymers are suitable candidates for the replacement of metals in the area of tissue engineering[21].

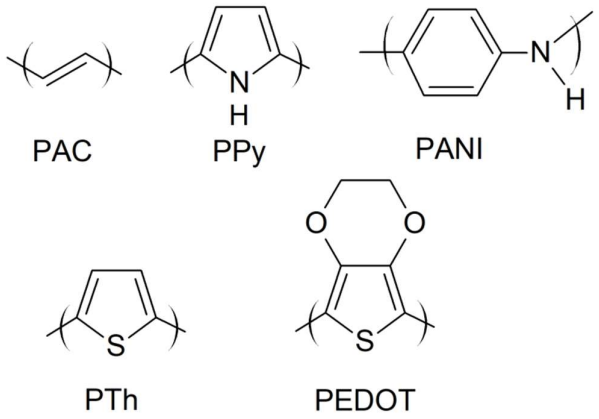

Fig. 1. *Examples of conductive polymers. PAC – polyacetylene, PPy – polypyrrole, PANI – polyaniline, PTh – polythiophene, PEDOT –poly(3,4-ethylenedioxythiophene).*

Polyacetylene (PAC), polypyrrole (PPy), polyaniline (PANI), and polythiophene (PTh) and its derivatives, such as poly(3,4-ethylenedioxythiophene) (PEDOT) belong to the family of conducting polymers (Fig. 1). Among these polymers, PAC exhibits the highest conductivity. On the other hand, PAC exhibits very low stability in air [30]. Therefore, this polymer is not suitable for further processing. With respect to processability, PANI and PPy are the best studied conducting polymers. They exhibit several advantages for applications in tissue engineering[31]. Among the most important is their mixed ionic and electronic conductivity, already mentioned in the previous chapter. Also their chemical, electrical and physical properties can be tailored for specific applications by using various dopants or by the incorporation of biological active substances such as enzymes and proteins, etc. The dopant agents are able to affect the conductivity of these polymers as well as their stability[32].

Conducting polymers can be prepared in two ways, chemically or electrochemically[33]. Thin films with a well-controlled thickness and morphology are commonly obtained by electrochemical synthesis, while various forms of these polymers can be produced by chemical synthesis. Depending on the polymer type, powders, films, colloidal suspensions, or hydrogels/cryogels can be prepared[34]. Therefore, chemical synthesis provides a wide range of suitable conducting materials for various applications. On the other hand, the electrochemical route leads to lower concentrations of unwanted by-products formed in the polymer during synthesis.

As mentioned above, most attention is devoted to two conducting polymers, PANI and PPy. This has also been true of my research. Therefore, a short description of their chemical preparation and properties is presented in the next chapter.

2.1.1 Preaparation of conducting polymers

The conducting polymers were for the first time produced several decades ago[34]. Today, they can be prepared by many methods. The most common is the oxidative polymerization[35]. Here, we will focus on the preparation of conducting polymers which are in the interest of this thesis – PANI and PPy.

2.1.1.1 Polyaniline

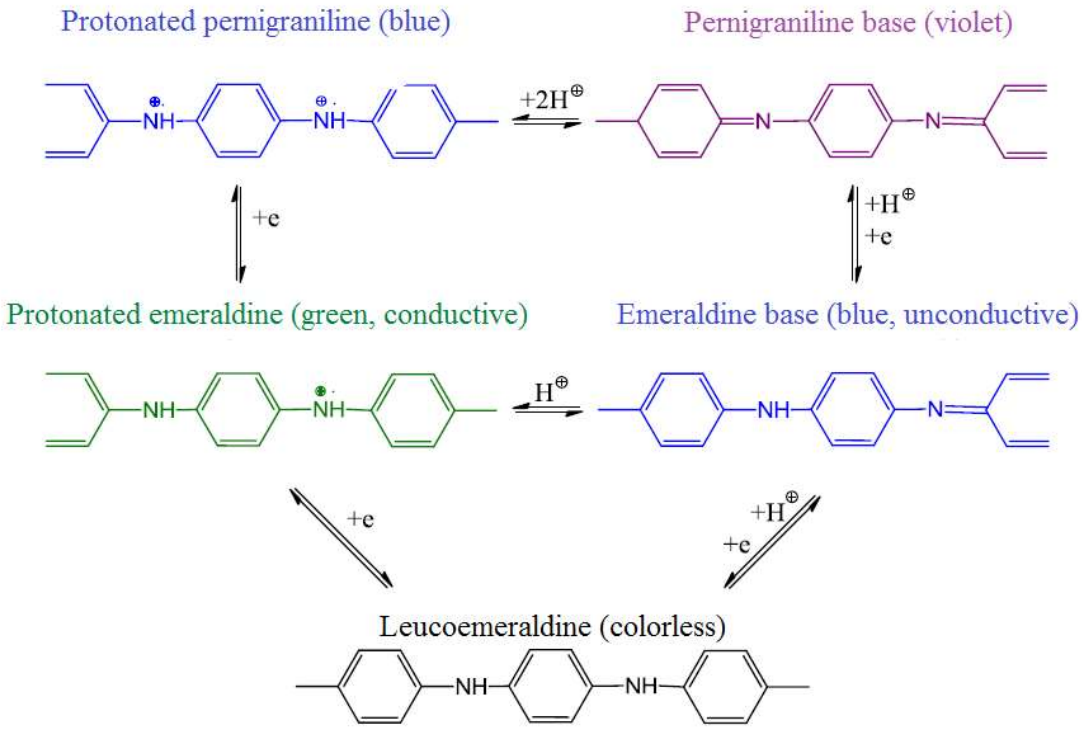

Fig. 2. *Oxidation forms of PANI.*

Polyaniline is an intensively studied conducting polymer. It has excellent electrical and optical properties, is easy to synthesize in high yields, and achieves good conductivity. However, its conductivity highly depends on its oxidation state (Fig. 2). Three types of polyaniline, differing in the degree of oxidation/reduction, are known: 1) the fully reduced form – leucoemeraldine, 2) the semi-oxidized form – emeraldine, and 3) the fully oxidized form - pernigraniline[36]. Emeraldine is the most stable and the most highly conducting PANI form.

Pristine PANI can be manufactured in the form of a powder, film or colloidal dispersion (Fig. 3) depending on the preparation procedure. The standard preparation of all three forms is well described in IUPAC technical reports[37,38]. Now, we will briefly focus on these procedures.

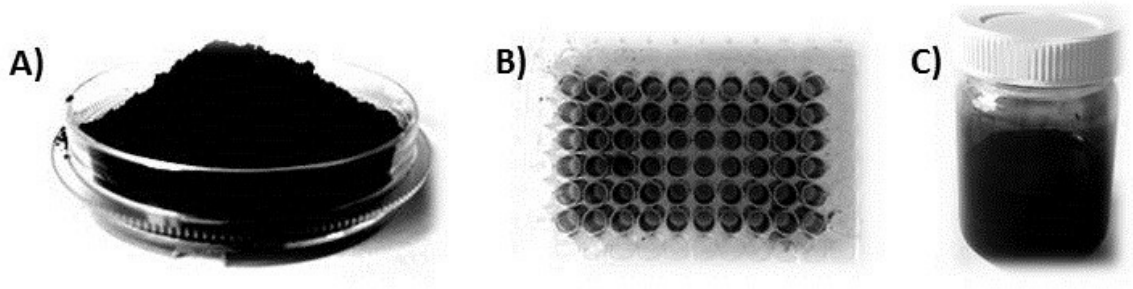

Fig. 3. *PANI in the formo f A) powder, B) film, C) colloidal dispersion.*

The preparation of PANI powders builds on the work in an *IUPAC technical report* written by Stejskal and Gilbert in 2002[37], in which PANI powder was prepared at various laboratories and the results were compared. The standard procedure is based on the oxidation of monomer aniline hydrochloride (AH) by an oxidation agent, namely ammonium peroxydisulfate (APS), in aqueous medium at laboratory temperature using concentrations of 0.2 M for AH and 0.25 M for APS. Both precursors are prepared in aqueous solutions, which are mixed together, stirred, and left to polymerize overnight. Thereafter, the polymerization mixture is filtered and the PANI precipitate is collected on the filter and washed with 0.2 M HCl and subsequently with acetone, before being dried. The greenish powder of PANI hydrochloride (emeraldine) is obtained by this procedure. According to the IUPAC technical report, the electrical conductivity of PANI prepared by this method was 4.4 ± 1.7 S cm^{-1} (the average of 59 samples).

The preparation of colloidal dispersions and thin films was also described in an IUPAC technical report three years later, in 2005. This report was written by Stejskal and Sapurina[38]. Similarly to the previous report, the procedure was conducted by various laboratories in six countries.

2.1.1.2 Polypyrrole

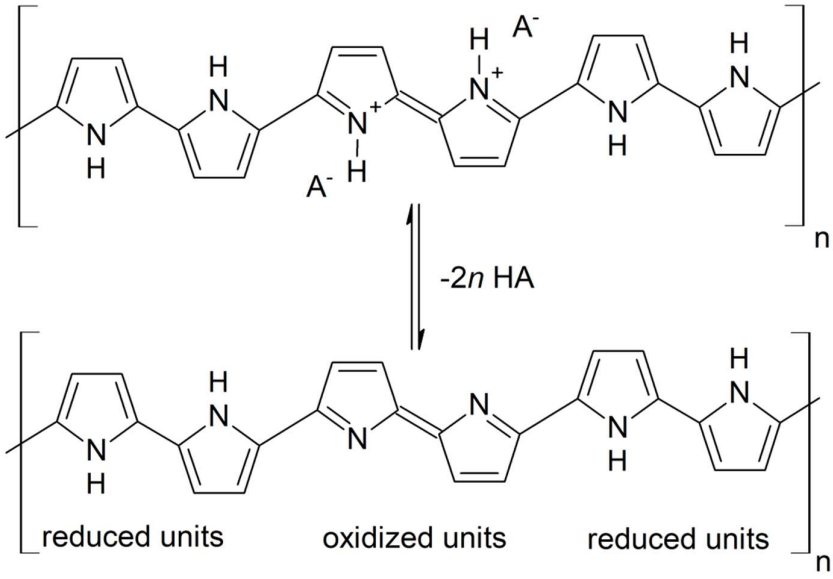

Fig. 4. *The molecular structure of protonated and deprotonated PPy.*

Similarly to PANI, the advantage of PPy is its easy preparation, environmental stability, and electrochemical activity[39]. PPy can be prepared in the form of powder[40], film[41] or colloid[42].

The oxidation of pyrrole can be performed by electrochemical[43] or chemical[44] means. The properties of PPy change depending on the degree of oxidation. The molecular structures of protonated and deprotonated PPy are depicted in fig. 4. For example, PPy films change colour from blue to dark black as the degrees ofoxidation and film thickness increase[45]. PPy conductivity ranges from units to tens of S cm^{-1} depending on the mode of preparation[46]. The most common procedure to synthesise polypyrrole is *via* the oxidation of pyrrole by iron (III) chloride[47,48]. The typical conductivity of PPy prepared in this way is 10^{-2} S cm^{-1} [49]. Additionally, ferric sulfate[50], ammonium persulfate[51], hexycyanoferrate[52], or ferric percholate[53] can be used as oxidizing agents.

2.1.1.3 Modification of conducting polymers

The modification and functionalization of conducting polymers can lead to improvements in their properties with respect to their various applications. For example, the incorporation of functional groups can improve the biocompatibility of such polymers and open them up for utilization in tissue engineering and biomedical areas. As a further example, the wettability of PANI can be changed simply through reprotonation by an acid. In the study of Stejskal et al.[54], forty two various acids were used for the reprotonation of PANI. While the contact angle of pristine PANI hydrochloride is above 49° [55], the measured contact angles after reprotonation varied from 29° to 102°. Therefore, it can be concluded that reprotonation can both increase and decrease the wettability of conducting polymers.

Another aspect which can be changed by a modification procedure is the morphology of PANI, which can vary according to the acidity of the reaction mixture during polymerization, this allowing PANI to be prepared with globular, nanofibrilar, or nanotubular morphology[56]. For example, globular PANI is obtained by the oxidation of monomer in strongly acidic conditions, while nanotubular PANI is obtained if the polymerization is conducted in less acidic conditions (e.g. in the presence of sulfuric acid)[57].

In the case of PPy, the morphology can be controlled by polymerization conducted in the presence of different azo dyes. For example, in the studies by Hu et al.[58] and Yang et al.[59] methyl orange was used to obtain nanotubes with circular profiles. In another work, the replacement of methyl orange with ethyl orange led finally to PPy with a globular morphology[60]. In contrast, Yan and Han[61] used Acid red 1 for the synthesis of PPy with rectangular nanotube morphology.

When dealing with conducting polymers, the aspect of conductivity should not be forgotten. The most common method of influencing the conductivity of these

polymers is to dope them with various agents, which may increase the conductivity by several orders of magnitude[34,62,63]; however, changes in the preparation technique can also influence the conductivity. For example, the polymerization temperature can strongly affect the conductivity of PANI[64,65]. Polymerization in the presence of various reaction media or the addition of other polymers are other approaches that can lead to changes in electrical properties[66].

In my work, several types of conducting polymer modifications have been employed, with the main focus on improving the biocompatibility of the materials. Now, I will briefly discuss examples of this research, which clearly document changes in material characteristics after modification.

In **article I.** the effect of PANI surface modifications on surface energy and their impact on biocompatibility were studied. Pristine PANI hydrochloride (PANI-S) and its deprotonated form (PANI-B) were prepared according to the IUPAC protocol[38]. Sulfamic, phosphotungstic, and poly(2-acrylamido-2-methyl-1-propanesulfonic) (PAMPSA) acids were used for modifications. Two types of preparation routes were studied: 1) using the acids as doping agents or 2) direct incorporation of the acids into the reaction mixture. Sulfamic and phosphotungstic acid were used as doping agents for PANI-B films, resulting in reprotonated films named PANI-SULF and PANI-PT. Polymeric acid PAMPSA was added to the reaction mixture in different concentrations. The mole ratio of aniline hydrochloride to PAMPSA was adjusted to 1:1 (PANI-PAMPSA-1:1) or 2:1 (PANI-PAMPSA-2:1).

Table 1. Surface energy evaluation of different surfaces.

	Surface energy components (mN m^{-1})		
Sample	γ^{tot}	γ^{LW}	γ^{AB}
PANI-S	52.54	46.05	6.49
PANI-B	50.88	46.54	4.35
PANI-SULF	52.13	44.97	7.17
PANI-PT	51.89	47.39	4.50
PANI-PAMPSA-1:1	41.85	40.98	0.87
PANI-PAMPSA-2:1	56.35	43.91	12.45

Note: γ^{tot} - total surface energy, γ^{LW} - disperse part of surface energy, γ^{AB} - polar part of surface energy. Data obtained from article I.[67].

The effect of the modifications on surface energy is shown in Tab. 1. Samples PANI-S, PANI-B, PANI-SULF and PANI-PT produced similar results. However, the modification with PAMPSA resulted in changes in surface energies. The most significant differences, compared to pristine PANI-S, were observed for PANI-PAMPSA-1:1, where the total surface energy decreased by about 20 %. By contrast, PANI-PAMPSA-2:1 slightly increased the surface energy compared to pristine PANI-S.

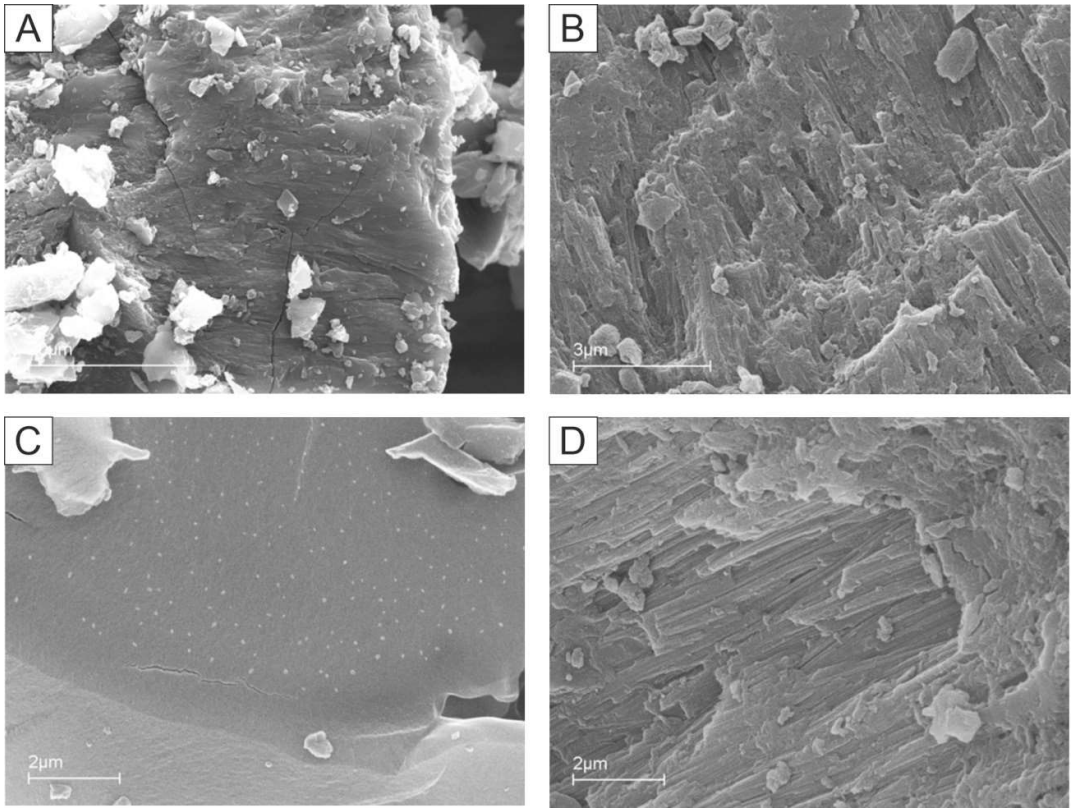

Figure 5. *SEM images of A) P4APA/HCl, B) P4APA/HCl after the post-treatment, C) P4APA/SA, B) P4APA/SA after the post-treatment. Adapted from article II.*[68].

Modification of the conducting polymer poly(4-aminodiphenylaniline) (P4APA) by acids was also studied in **article II**. Here, the effect of P4APA doping on the cytotoxicity, morphology, and molecular weight distribution of P4APA was examined. P4APA powder was prepared in the presence of HCl according to standard procedure; this sample was named P4APA/HCl. Similarly to the previous article, pristine P4APA/HCl was deprotonated with ammonium hydroxide. This deprotonated form was further subjected to a reprotonation process. The following acids were used as doping agents: phosphoric acid (H_3PO_4), salycilic acid (SA), dodecylbenzenesulfonic acid (DBSA), and camphorsulfonic acid (CSA). Furthermore, the samples were exposed to a post-treatment procedure in which they were purified by soaking in phosphate buffer saline (PBS) with pH 7.3. The results showed that the post-treatment procedure influenced both the morphology of the modified samples and the content of oligomers. Data from size exclusion chromatography showed the presence of aniline oligomers before post-treatment. Aniline dimer, hexamer, and octamer derivatives were present in all the powders. The post-treatment process allowed the removal of these oligomers, which are potentially cytotoxic species. In addition, from the SEM images (Fig. 5) it is obvious that the sample morphology changed. Before the post-treatment procedure, the samples exhibited

an irregular morphology whilst after post-treatment, layer-by-layer oriented rod-like structures were observed.

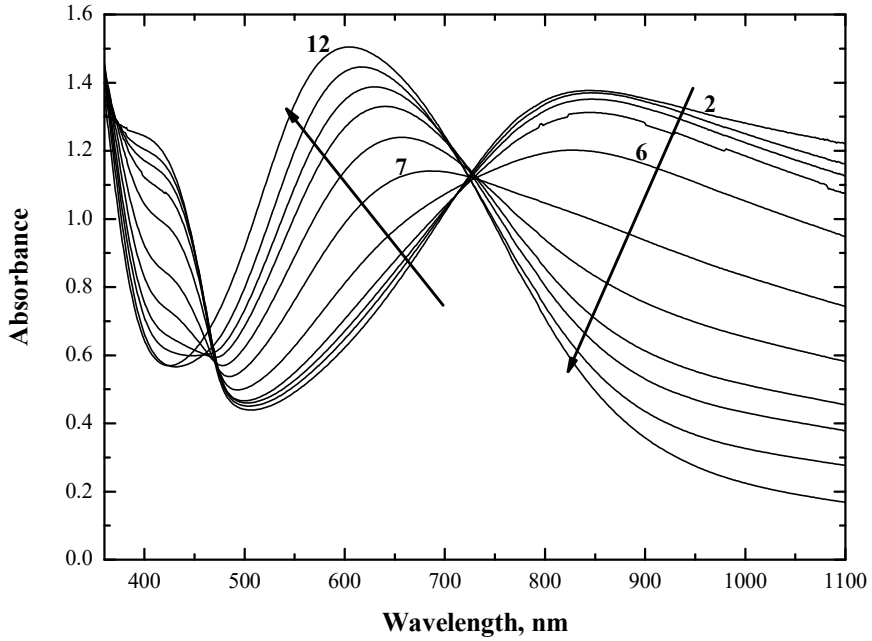

Fig. 6. *UV–vis spectra of PANI–PAMPSA films between pH 2–12. Adapted from article III.*[69].

Another important aspect related to bio-applications is the stability of polyaniline conductivity under physiological pH. Standard PANI is non-conducting in physiological pH. The transition from conducting PANI salt to non-conducting PANI base occurs at a pH of around 4[70]. This transition can be shifted to higher pH by various modifications. In **article III.** the pH stability of conductivity was improved by reprotonation of the PANI surface by PAMPSA. The acceptable level of conductivity for this modified polymer was retained at pH 6 (Fig. 6), which is a very notable improvement over pristine PANI.

3 CYTOCOMPATIBILITY OF CONDUCTING POLYMERS

The main effort of my research has been focused on the preparation of a stimuli-responsive biomaterial which can be used to prepare scaffolds with excellent mechanical properties together with good biocompatibility. At the beginning of my work, I chose conducting polymers as potentially stimuli-responsive materials. Considering all the issues mentioned so far, the utilization of conducting polymers in biomaterials requires deep knowledge about various aspects of their biocompatibility. However, at the start of my research, the impact of conducting polymers on cell behaviour had not been adequately studied and knowledge was insufficient. Therefore, it was first necessary to widen our grasp of this field to gather new and advanced knowledge on these materials.

First, the biological properties of pristine conducting polymers were at the centre of interest. Then, various modifications were employed to improve the biological response of these polymers. In addition, various forms of these polymers were prepared and tested. The research began with the testing of PANI powders and continued with colloidal dispersions and thin films, and the knowledge acquired was applied to the preparation of conducting polymer-based scaffolds. Over time, the level of biological testing was also notably extended and improved, from basic cytotoxicity studies conducted on standard cell lines to cell cultivations in bioreactors with stem cells.

3.1 PANI - powders

To the best of my knowledge, the biocompatibility of pure PANI powders was tested for the first time in 2012 by Humpolíček at al.[71]. Previous investigations were targeted on various PANI composites or complexes[72,73], but not on pristine PANI. Tests on PANI salt and base to determine levels of skin irritation, sensitization, and cytotoxicity were performed in the mentioned study, which showed that pristine PANI did not induce any sensitization or skin irritation either. However, both forms of PANI exhibited significant cytotoxic effects. This study showed that the modification of pure PANI is needed for it to be used in biological applications.

For the reasons given above, **article IV.** focuses on one of the possible ways of purifying PANI, and on the determination of leached impurities. The aim of this work was to overcome the limitations of PANI described by Humpolicek et al., 2012. PANI powder was therefore prepared by the same procedure and subsequently purified in a Soxhlet extractor using the following solvents: methanol, 1,2-dichloroethane, acetone, ethyl acetate, hexane, and 0.2 M aqueous hydrochloric acid. The impurities removed from PANI were determined by size exclusion chromatography and their contents are shown in Tab. 2. The cytotoxicity of purified samples was evaluated using mouse embryonic fibroblasts

(NIH/3T3 cell line). After purification with HCl and methanol, the cytotoxic effect of extracted samples was decreased compared to pristine PANI. In addition, it was found that the cytotoxicity of pristine PANI is mostly connected with the presence of low-molecular-weight fractions in the polymer. The knowledge summarised in the article led to an understanding of the causes of PANI cytotoxicity and contributed to further improvement in the purity of this polymer.

Table 2. The impurities removed from PANI after treatment with different solvents in a Soxhlet extractor (given in normalized peak area) determined by SEC and the extracted matter.

Solvent	Monomers and oligomers	Dissolved polymer	Total impurities	Extracted Matter (mg/ 100 mL)
0.2 M HCL	1445	15	1460	113
Methanol	840	80	920	87
Acetone	50	410	460	14
Dichloroethane	125	195	320	17
Ethyl acetate	80	480	560	9
Hexane	12	43	55	8

Note: Data obtained from article IV.[74].

3.2 PANI – colloidal dispersions

The powders studied in **manuscript IV.** are regrettably insoluble in aqueous media and common organic solvents. Therefore, it is difficult to process them to any suitable product useful in biomedical applications. Here, thin films or colloidal dispersions are much more desirable. Colloidal forms of conducting polymers can be easily dispersed in aqueous media, and the advantages of films lie in their ability to easily cover various substrates.

Knowledge about the biological activity of PANI was extended in the work published in **article V**. Here, the biological characteristics of colloidal PANI were reported for the first time. Colloidal dispersions were prepared according the standard protocol of the IUPAC by oxidation of aniline hydrochloride with ammonium persulfate in the presence of poly (N-vinylpyrrolidine) (PVP) as a stabilizer[38].

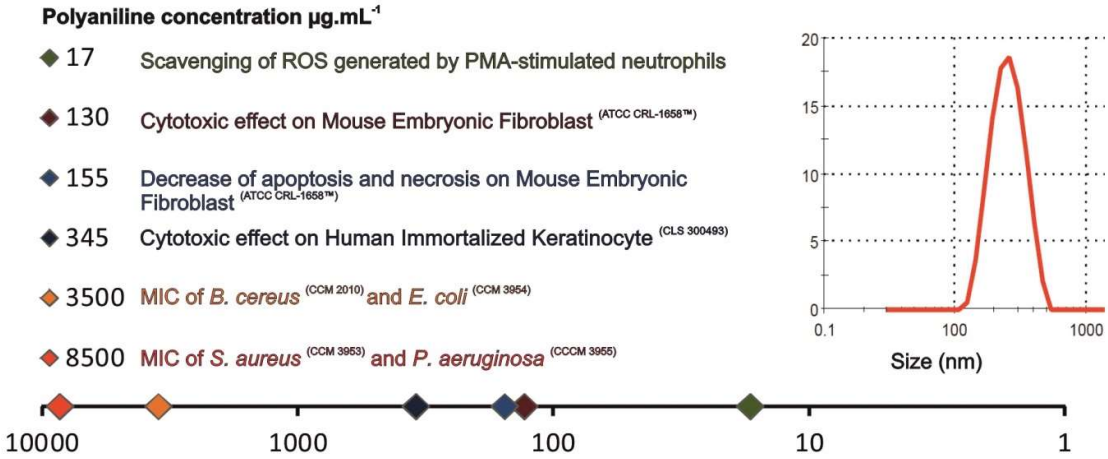

Fig. 7. *Dependence of biological properties on concentration of PANI in colloidal dispersion. Adapted from article V.*[75].

Particle size, PANI concentration, and biological properties were determined (Fig. 7). The biological testing of the colloid focused on its antibacterial activity, cytotoxic effect, the type of cell death, and oxidative burst in neutrophils and whole blood. The tests revealed that PANI dispersion was homogenous, with a nearly uniform single population of particles of size 226.5 ± 0.5 nm and a polydispersity index of 0.145 ± 0.004. These data showed particles with the expected size range, meaning that the dispersions were prepared correctly. The type of cell death, apoptosis or necrosis, was recognized by means of an annexin/propidium iodide assay on flow cytometry. The results indicated that the safe concentration of PANI in colloid for biological applications is of the order of 150 µg mL^{-1}. At the same time, this concentration did not provoke neutrophil activity, as measured through the detection of reactive oxygen species. These observations suggest that colloidal PANI is a potentially good candidate for biological applications.

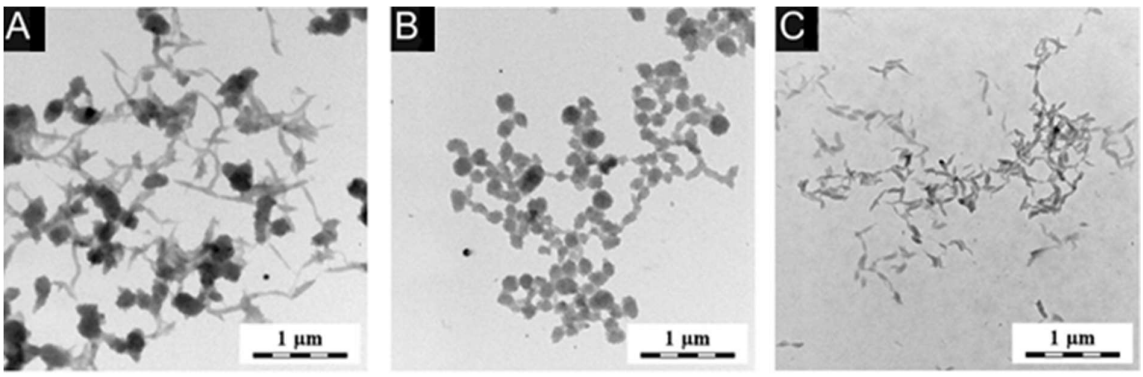

Fig. 8. *Transmission electron micrographs of PANI colloids stabilized with A) sodium hyaluronate of lower molecular weight, B) sodium hyaluronate of higher molecular weight, and C) chitosan. Adapted from article VI.*[76].

The advantageous properties of colloidal dispersions described in **Article V.** led to their further research and an effort to utilise such colloids in advanced applications. To improve their biological properties, the PANI colloids were stabilized with biocompatible polysaccharides. The preparation and characterization of the composite polysaccharide-PANI particles is described in the **article VI**. Sodium hyaluronate (HA) and chitosan (CH) were used for this experiment. Both biopolymers were used in two different molecular weights for the stabilization (HA: M=1 800 – 2 100 kDa and 50 kDa; CH: M = 50 –190 000 Da and 400 kDa). The material characterization involved the determination of UV-Vis spectra, the particle size distribution, and morphology which is shown in Fig. 8. The behaviour of the colloids in contact with prokaryotic and eukaryotic cells was studied and the cytotoxicity and antibacterial activity were determined. The cytotoxic effect depended mainly on the concentration of PANI in the respective colloidal samples. Colloids stabilized with higher molecular weight HA exhibited the best properties, with the absence of cytotoxicity observed for a PANI concentration of 465 μg mL^{-1} (Fig. 9), which was a significant improvement over pristine PANI. In addition, this colloid exhibited an antibacterial effect against *Staphylococcus aureus*. Thus, these formulations of PANI colloids can be considered as promising candidates for use as stimuli-responsive biomaterials.

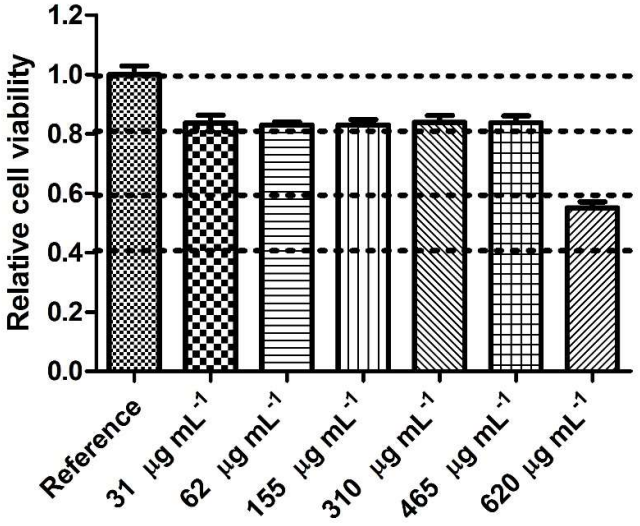

Fig. 9. *Cytotoxicity of colloidal PANI stabilized with sodium hyaluronate (molecular weights 1 800 – 2 100 kDa) for individual concentrations of PANI in colloid. Adapted from article VI.*[76].

3.3 PANI – films

Many potential applications for conducting polymers in medicine are related to the formation of surfaces. Here, biosensing or the control of the fate of adhered cells can be given as examples. PANI films were studied in **Article III**. Here, pristine PANI films were prepared according to the IUPAC procedure in the form of PANI salt and base[38]. Subsequently, the films were modified with PAMPSA. The polymeric acid PAMPSA was chosen as a representative of heparin-like substances. Heparin is the most common compound used as an anticoagulant and its efficacy is attributed to the structure of its polysaccharide backbone with a combination of sulfo and carboxyl groups [77]. The molecular structures of heparin and PAMPSA are shown in Fig. 10.

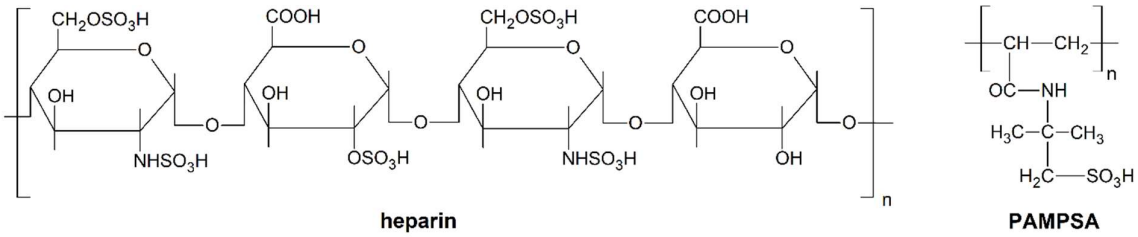

Fig. 10. *The molecular structure of heparin and PAMPSA.*

The prepared samples were tested for selected parameters of their hemocompatibility, namely blood coagulation and platelet adhesion. Two different procedures for the preparation of PANI/PAMPSA films were employed. The first consisted in the reprotonation of PANI base with PAMPSA (PANI-PAMPSA). In the second procedure, PAMPSA was used directly in the reaction mixture (PANI-1:1) under PANI synthesis. The films were also tested for their surface properties and contact angle measurements were performed. The contact angle increased in the samples with PAMPSA incorporated into the reaction mixture compared to standard PANI salt. In contrast, after the reprotonation of PANI base with PAMPSA, the contact angle decreased. As it is a commonly accepted fact that plasma proteins prefer hydrophobic surfaces to hydrophilic ones[78], these results correspond to the results on platelet adhesion on the film surfaces, where the lowest adhesion was observed for the most hydrophobic surface (PANI-PAMPSA). This surface not only reduced platelet adhesion, but also had a notable impact on blood coagulation. Moreover, the modification of PANI with PAMPSA improved the pH stability of PANI under physiological conditions by increasing the salt-base transition from pH 4 to pH 6. These findings suggest that this procedure can be a possible means of preparing PANI-based biomaterials.

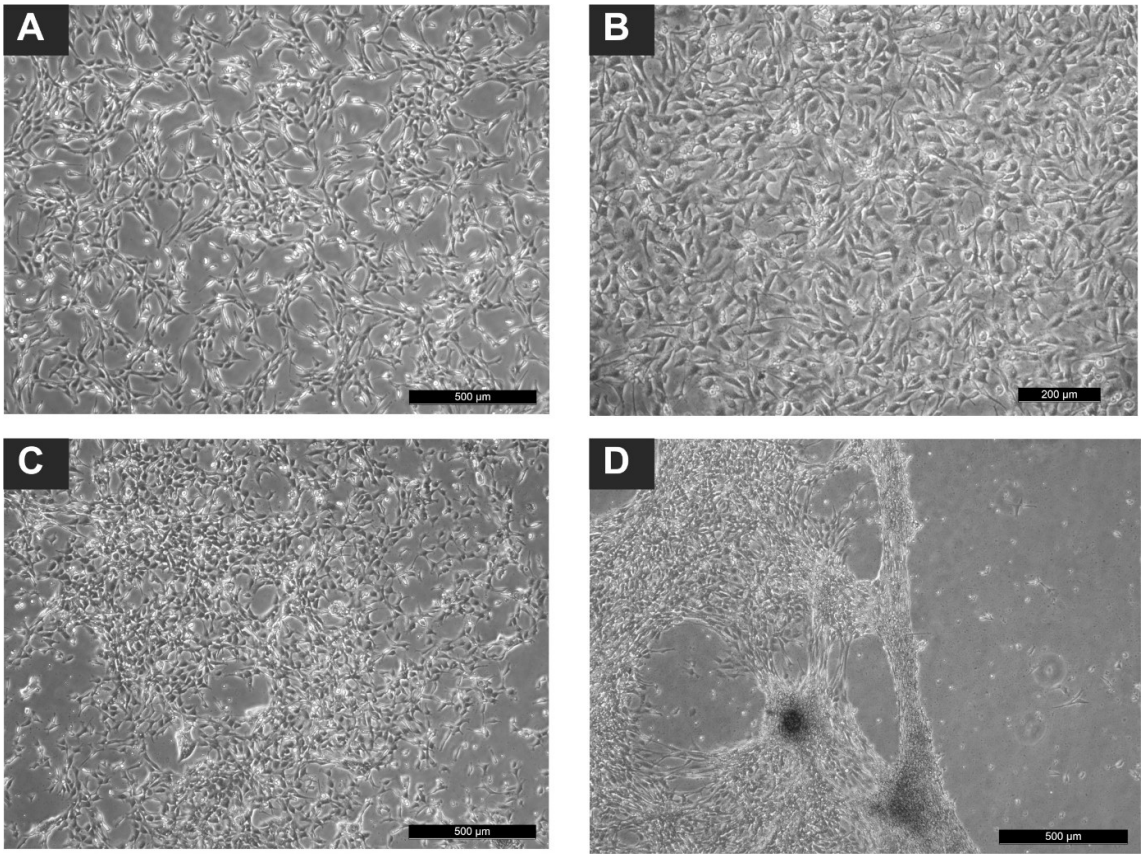

Fig. 11. *Cell growth on different PANI surfaces (after various times of proliferation). A) Reference (24 hrs), B) PANI salt (24 hrs), C) PANI modified with PAMPSA in the molar ratio of aniline hydrochloride to PAMPSA adjusted to 1:1 (144 hrs), D) PANI modified with PAMPSA in the molar ratio of aniline hydrochloride to PAMPSA adjusted to 2:1 (144 hrs). Adapted from article I.*[67].

PANI films modified with PAMPSA were also tested for cell compatibility. These tests are described in **Article I.** Besides PAMPSA, the PANI films were also doped with sulfamic and phosphotungstic acids. Cell adhesion, proliferation, and migration were determined on the modified surfaces. Unfortunately, in this study, the samples of PANI doped with PAMPSA did not show good cytocompatibility, as the cell attachment on this surface was weak (Fig. 11). Moreover, the cells also migrated more slowly on the PANI surface with PAMPSA compared to the other tested surfaces. The doping with sulfamic and phosphotungstic acids resulted in good cytocompatibility. Hence, these surfaces could possibly be utilized in tissue engineering.

3.4 PANI – films prepared in colloidal mode

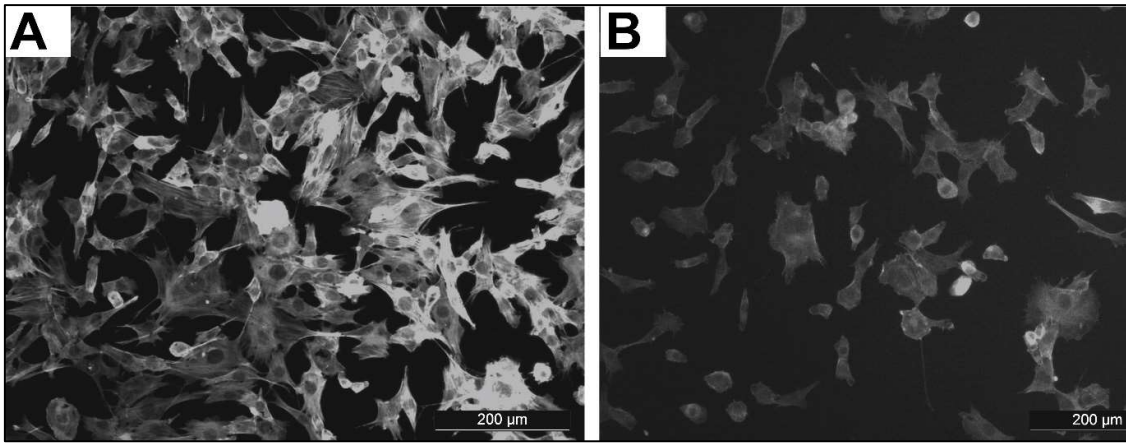

Fig 12. *The cytoskeleton organization of NIH/3T3 cells cultivated for 24 hours on the PANI films prepared in the presence of A) SDS, B) HCl. Adapted from article VII.*[79].

As shown in **article VI.**, the biocompatibility of colloidal PANI can be modified by using suitable stabilizers. Correspondingly, the properties of PANI films, such as surface morphology and electrical properties, can be controlled by using different stabilizers. In an effort to prepare conducting films with low cytotoxicity, PANI colloidal dispersions were prepared in the presence of four stabilizers and films were made thereof, as described in **article VII.** Poly-N-vinylpyrrolidone (PVP), sodium dodecylsulfate (SDS), Tween 20, and Pluronic F108 were chosen for this purpose. In addition, two types of reaction media were used during synthesis, water and 1M HCl, the latter to increase the acidity of the reaction mixture. The material properties of the films, such as surface energy, conductivity, and spectroscopic characteristics, were determined. Biological testing was conducted to determine cell adhesion, proliferation, morphology and migration. Regarding conductivity, higher values were achieved for films where HCl was used as a reaction medium during synthesis. In contrast, the reaction medium did not influence the surface energy. Cells were able to adhere on all surfaces, but their further growth and proliferation were not so uniform. Cells were not able to proliferate on surfaces modified with Pluronic F108 and Tween 20. The morphology of cells growing on films modified with PVP was significantly changed; the cytoskeleton did not form filopodia and the cells did not spread. On the other hand, samples modified with SDS showed good cytocompatibility, which can also be seen in the Fig. 12, where cells are spreading and have their typical triangular shape. Overall, the PANI-SDS films emerged as the samples with the best properties. Because of the presence of SDS, which is a known irritant, a skin irritation test was performed on a 3D reconstructed human tissue model. Surprisingly, the sample scored as a non-irritant material. In fact, it was shown that it had lower irritant potential than the reference, i.e., PANI

prepared without stabilizer. It can be concluded, therefore, that the preparation of PANI films in colloidal dispersion mode can lead to notable improvements in their biological properties.

3.5 PANI and PPy – comparison of biocompatibility

All of the above described papers deal only with PANI. The main reason for this lies mainly in the limited information about its biocompatibility available at the start of my career. Regarding the second investigated conducting polymer PPy, research dealing with its biocompatibility was sparse but some interesting data and studies could be found. For example, polyesters coated with PPy were tested for tissue reactions[80], the cellular response of PPy/biomolecule blends on silicone electrodes was investigated[81], and the doping of PPy was examined[82]. However, no study investigating the properties of pristine PPy was available. Consequently, it was not possible to compare the biocompatibility of PPy and PANI with respect to existing studies. Nevertheless, the general opinion among researchers was that PPy exhibited better biocompatibility with a lower cytotoxic effect than PANI[83,84]. This generally accepted view gave rise to **article VIII**., which compared these two polymers with respect to their biocompatibility. Both polymers were prepared according to standard procedures and their biological characteristics were determined in one laboratory to eliminate differences in testing. The cytotoxicities of the polymer extracts were determined using embryonic fibroblasts and embryonic stem cells. Embryotoxicity was also tested through the impact of the extracts on erythropoiesis and cardiomyogenesis within embryonic bodies. In addition, sample morphologies were captured by SEM and the extracts were analysed by mass spectroscopy. Both forms of PANI and PPy were tested, i.e., protonated salts and deprotonated bases. Observations showed that the differences in cytotoxicity were between the forms of the polymers (salt vs base) rather than between the individual polymers *per se* (PANI vs PPy) (Fig. 13). That is, while the same forms (salt or base) of each polymer showed almost identical cytotoxic and embryotoxic effects, it was the base forms of both polymers that exhibited better cytocompatibility than the protonated salts. Overall, therefore, direct comparison of both polymers using the same methodology evidenced that PPy and PANI exhibit similar degrees of biocompatibility. Hence, the generally accepted opinion that PPy is a less cytotoxic polymer was disputed.

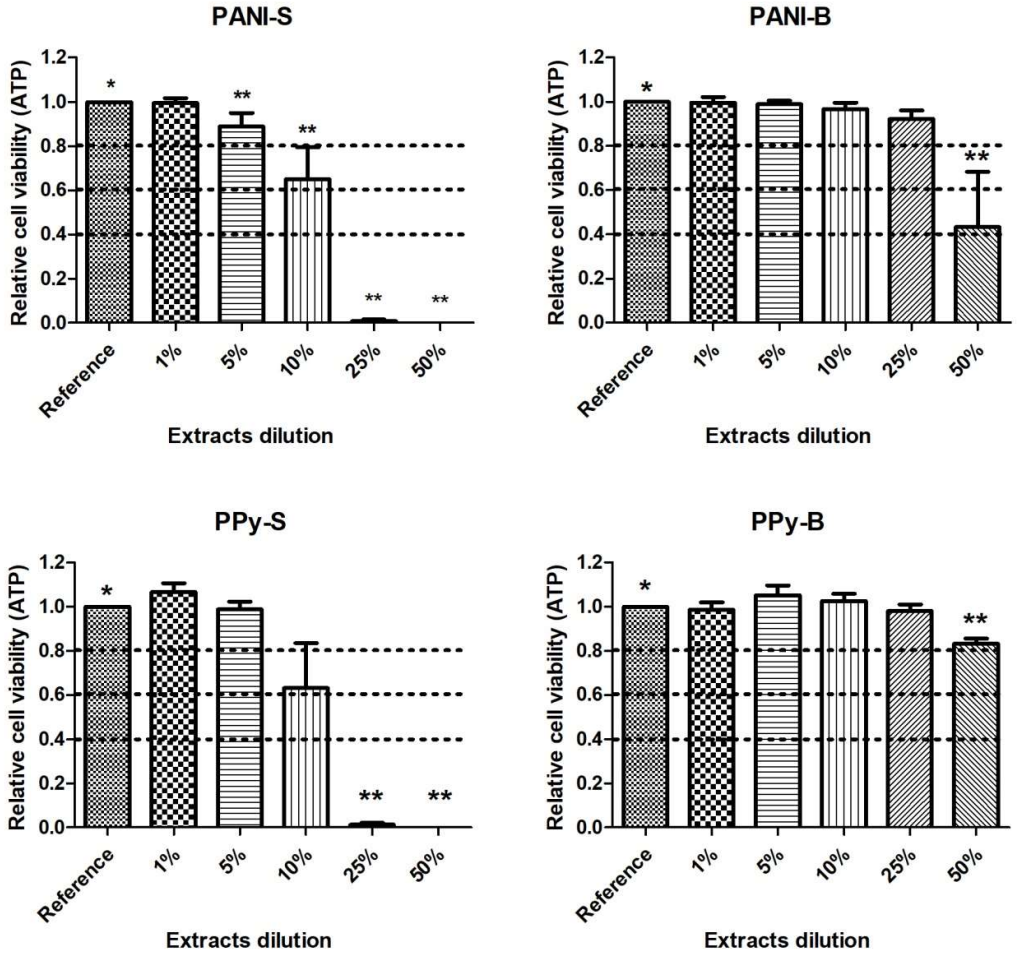

Fig. 13. *Cytotoxicity of extracts of PANI and PPy on ESc determined by the relative level of ATP compared to the reference. The different superscripts correspond to significant differences ($P \leq 0.05$) compared to the reference. The dashed lines highlight the limits of viability according to EN ISO 10993-5: viability > 0.8 corresponds to no cytotoxicity, > 0.6 – 0.8 mild cytotoxicity, > 0.4 – 0.6 moderate cytotoxicity and < 0.4 severe cytotoxicity. Adapted from article VIII.*[85].

The study published in **Article VIII.** was later extended by further work in which PANI and PPy were compared after doping with the same doping agents. This work is outlined in **Article IX**. As already mentioned, the use of conducting polymers in biomedical applications is complicated by the fact that their conductivity rapidly decreases at physiological pH. Researchers worldwide are currently engaged in efforts to increase the pH stability of such polymers by various methods [64,70,86]. In our work, "re-doping" with four types of organic phosphonates was employed. Dimethyl phosphonate (DMPH), diethyl phosphonate (DEPH), dibutyl phosphonate (DBPH), and diphenyl phosphonate

(DPPH) were used. The following properties of such "re-doped" samples were studied: their conductivity and their embryotoxicity and cytotoxicity towards embryonic stem cells. The results showed that the pH stability of phosphonate-doped PANI samples improved in comparison with pristine PANI salt (Fig. 14). In particular, PANI doped with DPPH exhibited significant improvement. Unfortunately, this sample also showed high cytotoxicity. On the other hand, the cytotoxicity of PANI doped with DBPH and DMPH was low compare to pristine PANI. In contrast to PANI, all types of phosphonates improved the cytotoxicity of PPy. It can be concluded, therefore, that PPy doped with phosphonates could serve as a conducting biomaterial.

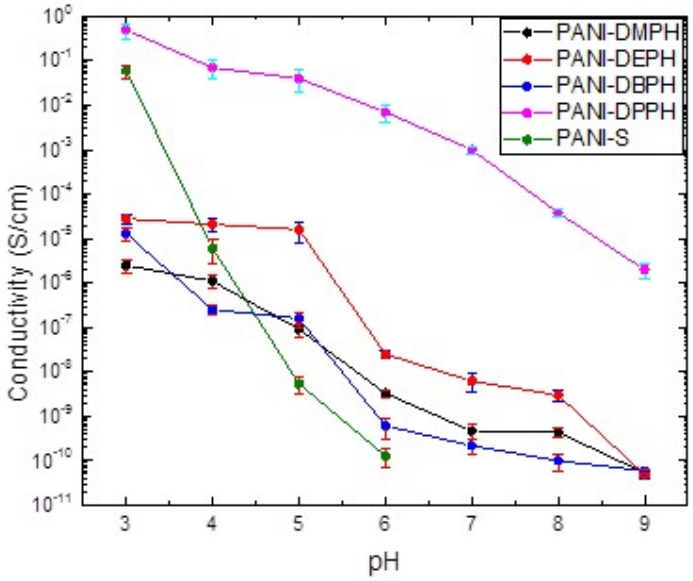

Fig. 14. *The conductivity of PANI doped with organic phosphonates under various pH and the comparison with pristine PANI-S. Adapted from article IX.*[87].

4 SCAFFOLDS – PREPARATION AND CYTOCOMPATIBILITY

Following our evaluation of the basic biological properties of conducting polymers, my attention was directed towards studying more advanced 3D systems, i.e., scaffolds. The aim of the work was to prepare scaffolds for tissue regeneration supported by conductivity. First, it was important to determine the optimal conditions for scaffold preparation, including the choice of a crosslinking agent or matrix. **Articles X., XI, and XII.** deal with this topic. PVA was the common denominator for these studies, being used either as matrix or as crosslinker. PVA is known for its physical properties suitable for biological applications[88], for its good water solubility[89], and for its biocompatibility;[90] therefore, it was chosen as a suitable candidate for scaffold preparation in our laboratory.

In **article X.** scaffolds based on PVA and fractionated kraft lignin were prepared and their material and biological properties evaluated. Kraft lignin was used to improve the stiffness of PVA hydrogels and to increase their antibacterial activity. The following properties were determined to characterise the material: the mechanical and thermal stability, the hydrogel network architecture, and the swelling ratio. Cytocompatibility studies determined the cytotoxicity of hydrogels in direct contact, the cytotoxicity of extracts, and cell ingrowth through the scaffolds in bioreactors. In addition, antibacterial properties were also studied. The mechanical stability was sufficient for all scaffolds with a concentration of kraft lignin up to 10 wt%. The tested cell line was able to grow with unchanged morphology in direct contact with all samples. However, the cytotoxicity of extracts was highly dependent on the concentration of kraft lignin in the scaffolds, with cell viability decreasing as the concentration of kraft lignin increased. Moreover, cell ingrowth in the bioreactor showed that only the scaffold with 1 wt% kraft lignin was suitable for applications in tissue engineering.

Another study on PVA-based hydrogels was published in **Article XI**. Here, PVA was used as a matrix for hydrogel, which was crosslinked by a modified polysaccharide – specifically, dialdehyde cellulose (DAC). DAC represents a less toxic, sustainable, and more effective alternative to highly toxic synthetic crosslinkers such as glutaraldehyde, which is widely used for the preparation of PVA hydrogels [91,92]. Three different concentrations of DAC were used to crosslink PVA and to prepare hydrogels with various mechanical and rheological properties, porosities, and surface areas. The material characteristics of PVA/DAC hydrogels were tunable, resulting in a range of hydrogels from stiff gels suitable for cartilage replacement to soft and highly porous viscoelastic hydrogels ideal for drug-delivery applications. They were also found to be superior to those of analogical material prepared using glutaraldehyde. The *in vitro* biological evaluation of hydrogel extracts using keratinocytes and fibroblasts revealed no cytoxicity on the part of the prepared materials. Similarly,

no negative effects of hydrogels on the cell growth or morphology of fibroblasts were observed. Hydrogels were subsequently loaded with biologically active substances, including the anticancer drug phenanthriplatin (PhPt). In addition to drug release, the cytotoxicity of PhPt-loaded hydrogel toward fibroblasts and malignant lung cells (A549) was evaluated. Notably, a synergistic effect of the loaded drug and hydrogel was observed in cytotoxicity towards the A549 cell line, though no such behavior was visible for fibroblasts. Thus, overall, PVA/DAC hydrogels represent tunable and biocompatible materials ideal for further study.

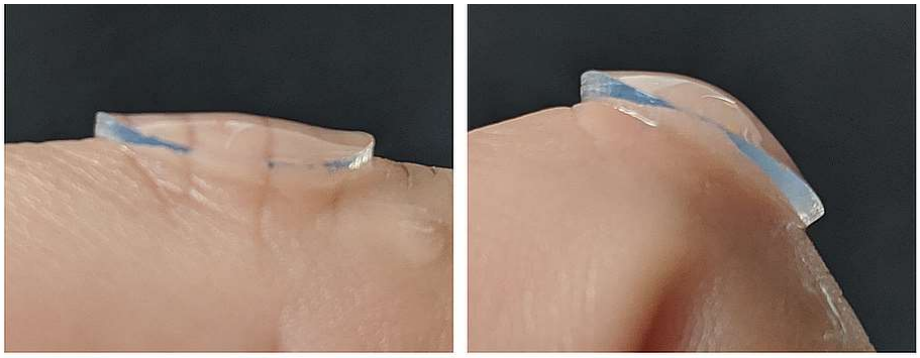

Fig. 15. *Bioadhesivity of PVA/DAC hydrogels. Adapted from article XII.*[93].

In **Article XII,** knowing the influence of DAC on the properties of PVA-based hydrogels, we focused on investigating the role of PVA in the scaffold. Hence, in addition to two different concentrations of DAC crosslinker, PVA of different molecular weights (M_w =1 and 130 kDa) was used for the preparation of PVA/DAC hydrogels. The hydrogels were fabricated in the form of thin films, which are more suitable for coating by conducting polymers. The prepared samples were biocompatible, showed no observable cytotoxicity against fibroblasts, and had no negative impact on cell growth. The hydrogels prepared with a combination of a low amount of crosslinker and high-molecular weight PVA were found to be particularly suitable for topical applications, such as wound dressings or patches. They exhibited high porosity and a high content of water, good bioadhesivity (Fig. 15), and transdermal drug-delivery. This material is thus an ideal candidate for the development of conducting patches capable of further improving wound healing.

My research effort has also been directed towards preparing stimuli-responsive scaffolds with properties mimicking native tissues. The combination of PVA based matrices with conducting polymers was the subject of our research in **articles XIII.** and **XIV**. Both manuscripts are targeted at the synthesis of porous conducting PVA-based cryogels, one in combination with PANI, the other with PPy. Cryogels are gel matrices formed at sub-zero temperatures, and their preparation is carried out under freezing during polymerization[94,95]. Usually, they exhibit a macroporous structure, good elasticity and good flexibility[96–98].

Article XIII describes the preparation of a novel macroporous material in the form of a PANI cryogel exhibiting good mechanical properties. This study reports the material characteristics of the gels, such as thermal conductivity, surface energy, pore-size distribution, and elasticity expressed by Young's modulus. The biocompatibility of these macroporous polyaniline cryogels was demonstrated by evaluating their cytotoxicity towards mouse embryonic fibroblasts and *via* the testing of embryotoxicity based on the formation of beating foci within spontaneously differentiating embryonic stem cells. In addition, the results of biological testing were related to impurities leached from the cryogel, which were characterized by liquid chromatography. The macroporous structure of the PANI cryogel is depicted in Fig. 16.

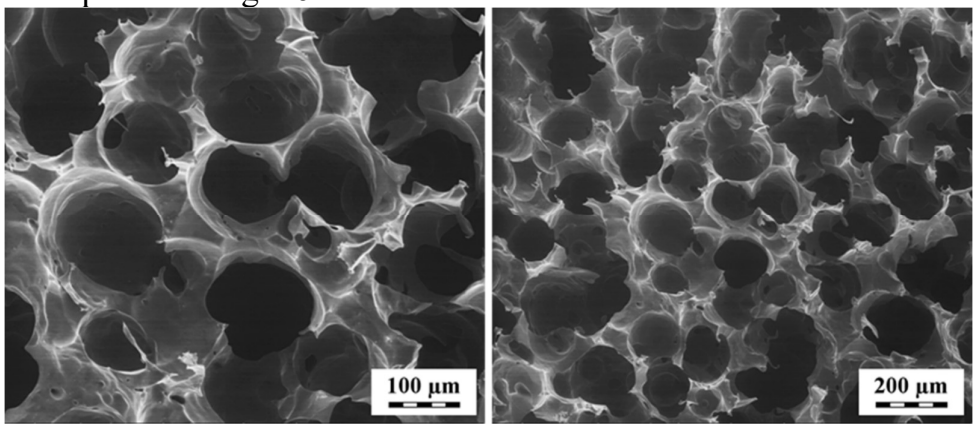

Fig. 16. *The SEM images of the cryogel structure. Adapted from article XIII.*[99].

The mean pore size was assessed to be 159 µm and the specific surface area to be 0.020 m^2 cm^{-3}. The mechanical properties were described by Young's modulus, which reached a mean value of 9.7 ± 0.5 kPa, meaning that the PANI cryogel was an elastic material, what can be seen in Fig. 17.

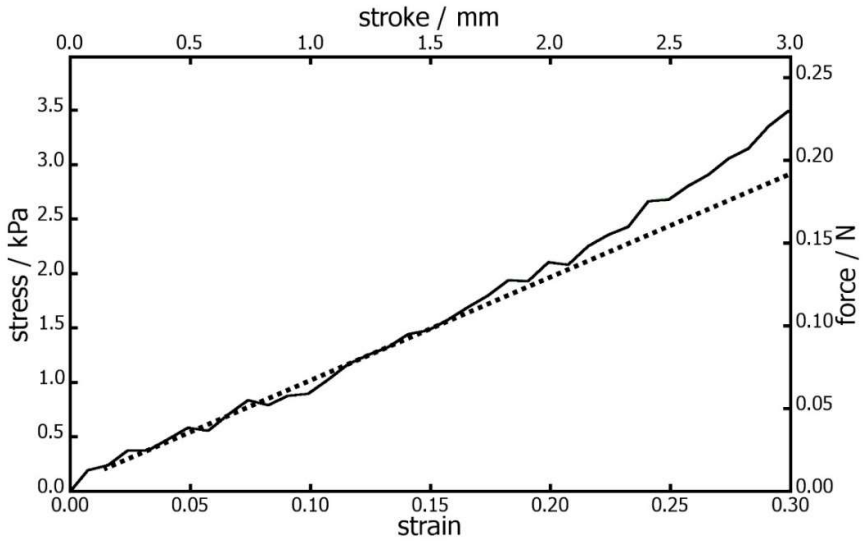

Fig. 17*. Stress-strain curve used for calculation of Young modulus of PVA-PANI cryogel measured under confined conditions. Adapted from article XIII.*[99].

The PANI cryogel also showed low levels of cytotoxicity and embryotoxicity. The embryonic stem cells and embryoid bodies were able both to adhere and grow well on its surfaces. This good biocompatibility can also be related to the low content of low-molecular-weight impurities in the cryogel. Unfortunately, a poor level of interaction of cardiomyocytes and neural progenitors with the PANI cryogel was detected. Cardiomyocytes were not able to attach to the cryogel and only a few neural progenitors, which failed to spread, were observed on its surface. However, the fact that the PANI cryogel mimicked the properties of native tissues and exhibited reasonable biocompatibility opens up possibilities for its utilization in regenerative medicine, given that suitable modifications or purifications can be developed.

Further research on conducting macroporous cryogel is presented in **article XIV.** Here, PPy was used as the conducting component and different concentrations of PVA (ranging from 5 to 8 wt.%) were used for sample preparation. The cryogels were characterized with respect to their morphology, mechanical properties, electrical conductivity, specific surface area, and cytotoxicity. According to SEM images, it can be concluded that cryogels with interconnected pores with macropore diameters ranging from 5 to 100 μm were successfully prepared. The mechanical properties of the PPy expressed by Young's moduli achieved a value of approximately 20 kPa. The stiffness as well as the pore diameters were independent of the concentration of PVA in the cryogel. The conductivity was enhanced compared to standard PPy and reached a value of 18 S cm^{-1}. This value remained unchanged after long-term treatment with water, i.e. close to physiological pH. These findings taken together with comparatively good results from cytotoxicity testing suggest that these cryogels are potentially suitable for use in biomedical applications.

5 CONTRIBUTION TO SCIENCE AND PRACTICE

The main goal of my scientific career so far has been to prepare biocompatible stimuli-responsive scaffolds. These scaffolds should allow the modification of cell behaviour in desired ways through the application of external electrical fields. The conductivity of the scaffold can be achieved by using conducting polymers, such as polyaniline (PANI) or polypyrrole (PPY). However; crucial information about the biocompatibility of conducting polymers was lacking. Therefore, the first aim of my work was to increase our understanding of the factors which influence the impact of conducting polymers on cells. The importance of this research is evidenced by the publications summarized and described in this work. At the beginning, knowledge about thebiocompatibility of PANI was in the centre of attention, and this polymer was studied in the form of powders, colloidal dispersions and thin films. **New information about the influence of the PANI preparation procedure on various parameters of cytocompatibility was revealed.**

Before my work on conducting polymers began, it was generally accepted that pristine PANI powder exhibited a high level of cytotoxicity. Therefore, one of my works dealt with the detection of impurities leached from PANI and the methods of PANI purifications. It was shown that the cytotoxic potential of PANI is mainly induced by low-molecular-weight fractions of the polymer. As was shown by our team**, these cytotoxic fractions can be efficiently removed by purification – specifically, by extraction with organic solvents, a step which resulted in a significant decrease in PANI cytotoxicity.**

One important contribution of this dissertation thesis to science is related to the research of colloidal PANI dispersions, whose biological properties were hitherto unknown. One of my initial studies focused on this topic, and the biological activity of colloidal PANI was reported for the first time. This knowledge about colloidal PANI was further extended with respect to its cytotoxicity, the type of cell death, oxidative burst in neutrophils and whole blood, and its antibacterial activity. **This study significantly improved knowledge about the potential application of colloidal conducting dispersions in tissue engineering.** In addition, the new knowledge acquired through this study along with increased methodological experience and laboratory acumen with respect to the preparation and characterization of PANI led to **an innovative approach to the preparation of cytocompatible electro-conducting substrates, characterised as polymerization in colloidal dispersion mode.** Such prepared substrates have unique properties in terms of their cytocompatibility, as they combine the properties of both synthetic PANI and the biopolymers used as colloidal stabilizers.

Research on PANI continued with the aim of preparing conducting materials with even better cytocompatibility. As a result, various advanced modifications of this polymer were successfully developed. Here, the **incorporation of**

biomacromolecules into PANI films should be mentioned, a procedure which leads not only to the better biological response of these composite films but also to their improved pH stability in physiological environment. This improvement is especially important, as the decrease in the electrical conductivity of PANI at physiological pH is one of the crucial challenges.

When talking about the utilization of conducting polymer films as biomaterials, their surface properties should not be forgotten. Hence, some of the studies also dealt with this topic. One aspect of biocompatibility, important especially for scaffolds which can come in the contact with blood, is blood compatibility. In this resepct, important progress was achieved by the reprotonation of PANI film with polymeric acid, poly(2-acrylamido-2-methyl-1-propanesulfonic acid) PAMPSA. This modification led to a **significant reduction in platelet adhesion on the PANI/PAMPSA surface and significantly influenced blood coagulation.** Moreover, this modification also improved pH stability in comparison with pristine PANI film. One subsequent and exciting study concentrated on the preparation of PANI films in colloidal mode in the presence of surfactants used as stabilizers, e.g. sodium dodecylsulfate. The films thus prepared exhibited not only good cytocompatibility but were also shown to be non-irritant towards skin.

The biological response of PANI was also compared with that of another conducting polymer, PPy. In scientific literature, PPy was previously considered as a material with better biocompatibility than PANI. However, these findings were based on studies in which PPy was examined in the form of various composites and blends, not in its pristine form. These two polymers were therefore prepared in their pristine forms using standard procedures and studied using identical methods. It was shown that the **cytocompatibilities of PANI and PPy were very similar and depended mainly on the form of the polymer (salt or base).** As their differences in biological response were negligible, neither of them should be preferred for biomedical applications.

All the above investigated parameters relating to conducting polymers, such as their surface characteristics, the procedure for their preparation, and the cytocompatibility of their various forms and composites were summarised to collect knowledge crucial for the preparation of scaffolds. In particular, the focus of my research was stimuli-responsive scaffolds in which the responsivity is triggered by electrical conductivity. To determine optimal conditions for the preparation of such conducting scaffolds, several types of non-conducting PVA-based analogues were first prepared. These scaffolds exhibited good mechanical as well as biological properties. Therefore, the synthesis of conducting PVA-based scaffolds followed. As conducting components, both PANI and PPy were used. **These prepared scaffolds also showed good mechanical properties and were able to mimic the properties of native tissues. Even their cytocompatibility was good, which opens up the possibility to use such conducting scaffolds in biomedical applications.**

Besides the topics discussed above, my research also focused on investigating the influence of conducting polymers on the formation of bacterial biofilms. This is also a very important aspect of utilizing materials in the medical area, where bacterial contamination must be avoided. In addition to PANI and PPY, carbon quantum dots as antibacterial and antibiofouling coatings were also investigated and several studies dealing with this topic were conducted, these leading to the development of some promising materials. Another part of my research was also dedicated to drug delivery systems, especially related to cancer therapy and the controlled release of drugs.

6 FUTURE PERSPECTIVE

At present, also thanks to my work, knowledge about the biological properties of conducting polymers has expanded significantly and improved methods to enhance their biological properties have been developed. These two prerequisites open up new possibilities for the utilization of such polymers as conducting components in stimuli-responsive materials for medicine. Although various scaffolds with incorporated conducting polymers that exhibit good biocompatibility and mechanical properties have already been prepared by our team, they are still not perfect materials with the excellent properties required for targeted clinical use. For example, the level of biocompatibility is still not sufficient for general biomedical applications. Another parameter which needs additional improvement is the degree of porosity, which is also quite challenging, because we often encounter materials in which the interconnection of pores in the bulk material is missing or the pore size or shape are not adequate for cell ingrowth. Pore interconnection is an essential characteristic, as it supports cell migration and proliferation and also the penetration of the extracellular matrix into the scaffold. Besides this, it affects the diffusion and exchange of nutrients throughout the scaffold.

Therefore, the preparation of conducting scaffolds with interconnected pores, excellent biocompatibility, and mechanical properties mimicking those of native tissue is the goal of future research. In addition, the incorporation of various bioactive molecules into scaffolds will be undertaken. For example, growth factors which will influence cell differentiation will be used. Another aspect which must be taken into consideration is the real cell environment with its continuously changing mechanical, chemical, and biochemical gradients. Hence, respective gradients will be established to effectively mimic this real environment. All these aspects will be tested in bioreactors to resemble *in vivo* systems in the most consistent way.

LIST OF FIGURES

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LIST OF SYMBOLS AND ABBREVIATIONS

A549	Human cells from lung carcinoma
AH	Aniline hydrochloride
APS	Ammonium peroxydisulfate
CH	Chitosan
CSA	Camphorsulfonic acid
CP	Conducting polymer
DAC	Dialdehyde cellulose
DBPH	Dibutyl phosphonate
DEPH	Diethyl phosphonate
DMPH	Dimethyl phosphonate
DPPH	Diphenyl phosphonate
DBSA	Dodecylbenzenesulfonic acid
HA	Sodium hyaluronate
HaCaT	Human immortalized keratinocytes
HepG2	Hepatocellular carcinoma cell line
NIH/3T3	Mouse embryonic fibroblast
PAC	Polyacetylene
PAMPSA	Poly(2-acrylamido-2-methyl-1-propanesulfonic acid)
PANI	Polyaniline
PANI-B	Polyaniline base
PANI-S	Polyaniline salt
PBS	Phosphate buffered saline
PEDOT	Poly(3,4-ethylenedioxythiophene)
P4APA	Poly(4-aminodiphenylaniline)
PPy	Polypyrrole
PTh	Polythiophene (PTh)
PVA	Poly(vinyl alcohol)
PVP	N-poly(vinylpyrrolidone)
SA	Salycilic acid
SEM	Scanning electron microscopy
SDS	Sodium dodecyl sulfate
γ_{AB}	Acid-base (polar) part of surface energy
γ_{LW}	Disperse part of surface energy
γ_{tot}	Total surface energy

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