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DOCTORAL THESIS

Drawing: Fabrication of Scaffolds for Neural Tissue Engineering

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Abstract

Materials based on orientated fibers have great potential for use in tissue engineering for tissues, where the arrangement of extracellular matrix is fundamental for tissue functionality. The drawing method is extremely suitable method for such applications. It is based on mechanical pulling of a polymer solution out of its base droplet, resulting in a single solidified fiber of determined geometrical characteristics. A new machine designed for lab scale drawing was invented enabling a repeatable quality of drawing conditions. The results demonstrate that by changing the speed of drawing and polymer solution concentration it is possible to influence and define the fiber diameter and its distribution. From the *in vitro* experiments, it is evident that the aligned fibers guide the cell growth in the direction of the fibers. Moreover, the prepared fibers were functionalized with polypyrrole as an example of their versatility. The results from *in vitro* experiments show, that polypyrrole enhanced the biocompatibility of the fibers. These fibers were further used for the preparation of the novel composite spinal cord bridges, which were tested *in vitro* and *in vivo*. The results from *in vitro* experiments show that the oriented fibers support the guidance of neurite outgrowth and narrow the axonal spread which is more focused around the fibers. This phenomenon is even more pronounced, if the fibers are coated with polypyrrole. The new spinal cord bridges were successfully implanted into mice' spinal cord for *in vivo* experiments. The results suggest the non-immunogenicity of the fibrous bridge samples. Moreover, the results show the activation of the pro-healing immune response in the both fibrous bridges. Also, the bridges with PCL fibers show higher axon infiltration compared to control. About 20 % of these axons are myelinated, 75 % of this myelin is derived from the Schwann cells. On the other hand, the axonal infiltration into the bridges with PPy-coated fibers is lower compared to bridges with PCL fibers or compared to control. Nevertheless, the obtained results show, that the oriented fibers enhance the axon infiltration into the spinal cord bridges and that the combination of the currently available approaches with new functionalization methods will be the method of choice for neural tissue engineering.

Keywords:

drawing, fibers, neural tissue engineering, spinal cord, *in vitro*, *in vivo*

Abstrakt

Materiály založené na orientovaných vláknech mají velký potenciál pro využití v tkáňovém inženýrství a to zejména u tkání, kde je organizace mezibuněčné hmoty zcela zásadní pro funkčnost tkáně. Velmi vhodnou metodou pro tyto aplikace je drawing. Ten je založen na mechanickém tažení polymerního roztoku z kapky polymeru umístěné na podložce, což vede k vytvoření vlákna o definovaných geometrických charakteristikách. Pro tyto účely byl navržen a vyvinut laboratorní přístroj, který umožňuje tažení vláken za stálých podmínek. Výsledky ukazují, že změnou rychlostí tažení vláken a změnou koncentrací polymerních roztoků lze ovlivnit a regulovat průměr vláken a zároveň i distribuci jejich průměrů. Z *in vitro* experimentů je patrné, že orientovaná vlákna ovlivňují směr růstu buněk ve směru orientace vláken. Na základě těchto experimentů byly tyto scaffoldy dále funkcionalizovány polypyrrolem, čímž se prokázala jejich univerzálnost pro použití v tkáňovém inženýrství. Výsledky z *in vitro* experimentů s popyrrolovanými vlákny ukazují, že polypyrrole zvyšuje biokompatibilitu vláken. Popyrrolovaná vlákna byla dále použita pro přípravu nových kompozitních míšních můstků, které byly testovány *in vitro* a *in vivo*. Výsledky z *in vitro* experimentů ukazují, že orientovaná vlákna podporují růst a orientaci axonů okolo vláken. Tento jev je ještě více patrný, pokud jsou vlákna potažená polypyrrolem. *In vivo* experimenty byly provedeny na myších, jimž byly můstky implantovány do mích. Hodnocení prozánětlivých a apoptotických markerů naznačuje, že vlákenné míšní můstky jsou neimunogenní. Dokonce tyto výsledky ukazují aktivaci imunitní reakce, zodpovědné za procesy hojení v těle, a to u obou vlákenných míšních můstků. Míšní můstky obsahující PCL orientovaná vlákna dokonce vykazují vyšší infiltraci axonů do můstků oproti kontrole bez vláken. Okolo 20 % těchto axonů je myelinizovaných, 75 % z tohoto myelinu je odvozeno od Schwanových buněk. Na druhou stranu, u míšních můstků s popyrrolovanými vlákny je infiltrace axonů do můstků oproti kontrole nižší. Nicméně výsledky ukazují, že orientovaná vlákna zvyšují infiltraci axonů do míšních můstků a že kombinace dostupných přístupů a metod funkcionalizace materiálů budou volbou do budoucna pro tkáňové inženýrství nervové tkáně.

Klíčová slova:

drawing, vlákna, tkáňové inženýrství nervové tkáně, mícha, *in vitro*, *in vivo*

Contents

1 Introduction

The first successful organ transplanted to human was kidney in 1954 by Joseph Murray and his team [34]. This breakthrough gave rise to new fields of reconstructive medicine, the transplantation biology and immunology. Over the next 25 years, transplantations of kidneys together with transplantations of bone marrow evolved rapidly. Transplantations of other organs remained experimental until the late 1970s. Short after the boom of organ transplantations at the late 1970s it started to be evident that even though the organ transplantations were promising and successful way to replace the non-functional organs, the supply of donor organs highly exceeds the demand [59]. It was clear that the inoculation of the cells only will not be sufficient to repair or replace the whole tissue. Even then, scientists knew that there is a need to immobilize the cells within a scaffold. The scaffold should serve as and resemble a native extracellular matrix, provide the cells suitable environment for proliferation, harbor the cells from the hostile environment and help the cells to survive after implantation. Probably the first experiment carried out with scaffolds seeded with cells and implanted into an animal model was carried out by Vacanti and co-workers in 1988 [59]. They prepared different types of scaffolds (from different polymers and by different techniques), which were seeded with cells isolated either from liver, pancreas or intestine. Seeded scaffolds were implanted into mice 3 to 4 days after cell seeding. These experiments can be definitely considered to be the first evidence of modern tissue engineering. Nevertheless, it took several years to establish this new discipline as an independent scientific field.

Even though tissue engineering is young developing discipline interconnecting a huge variety of sometimes very unrelated scientific disciplines, their goal is the same - to develop and replace nonfunctional tissue.

In the case of healing the spinal cord, tissue engineering might be one of the key approaches. Each year 250,000 to 500,000 people in the world suffer from spinal cord injuries [66], with the annual incidence of 17 thousand people in the USA. In the Czech Republic, the annual incidence of acute spinal cord injury ranges between 250 and 300 cases [20]. These pathologies represent a great health risk for patients. Itaffects their quality of life, the incorporation of the patients back into society and increases the costs of living. And yet, there is no effective treatment for spinal cord injuries. Current treatment options are the use of high dose methylprednisolone to suppress the immune system and decrease inflammation, surgical intervention to stabilize and decompress the spinal cord and rehabilitative care [33]. However, none of these approaches does support active regeneration of neural tissue. Therefore, enormous efforts are being made to enable neural regeneration by various approaches. Neural tissue engineering offers a promising approach to treating nervous system injuries. But scaffolds alone are not sufficient for the regeneration of neural tissue. Neural cells proliferate

through the injury with difficulties and do not extend their neurites enough, the cells forming glial scars instead [23]. In the literature, there are studies into the implementation of stem cell therapy and the combination of stem cells with various types of scaffolds [25]. Although, hydrogels (from synthetic or natural polymers) are very favored materials because they are able to mimic the mechanical properties of natural tissues due to their high water content [13], fabrication of fibrous scaffolds for neural tissue engineering is also highly pronounced. Nowadays, electrospinning is mainly used to prepare fibrous scaffolds for neural tissue engineering due to its versatility to use different polymers [69], [39], [21]. Scaffolds are made from synthetic (poly-β-hydroxybutyrate - PHB, poly(lactic-co-glycolic acid) - PLGA) [69], [39], [21] as well as bio-polymers (collagen) [25], but the results of these studies of neural regeneration are often inconsistent and neither of which has been successfully introduced into practice so far. The latest studies show that the future for neural scaffolds is in the combination of methods for scaffold fabrication with surface functionalization and drug release [5], [9], [48].

It has been shown that the neural cells prefer aligned scaffolds that guide their neurite outgrowth [6]. They also prefer microfibers instead of nanofibers; microfibers promote the neurite extension better than nanofibers. The aligned microfibers also promote the migration of Schwann cells [62]. And there are also many other factors that can influence neuron proliferation and neurite extension, such as the use of conductive polymers [15], [21], and the addition of growth factors and other signal molecules [5]. The electrospun scaffolds can be functionalized by polypyrrole or polyaniline or others [15], [21].

2 Purpose and the aim of the thesis

The aim of this work is to design, develop and optimize novel functional spinal cord bridges, which will foster the growth of neural cells and neurite outgrowth enough to bypass the injury and which will promote the recovery of neurotransmission. To achieve this goal various techniques of scaffold fabrication and surface modification were combined. Drawing was used to prepare oriented microfibers. The surface of these microfibers was further functionalized with conductive polypyrrole. These aligned fibers were incorporated into the complex composite spinal cord implant to enhance the infiltration of neural cells into the inner structure.

3 Overview of the current state of the problem

3.1 Tissue engineering

Tissue engineering is a multidisciplinary field combining diverse scientific disciplines ranging from engineering, material sciences to cell biology, immunology, medicine and many others. And even thought the idea of tissue replacement accompanies the humankind from time immemorial, the term “tissue engineering” was first, but loosely used since the mid 1980’. However, by that time it was mostly used for the manipulation with tissues, organs

or when using prosthetics. The beginning of the independent scientific discipline called tissue engineering, as we know it today, is dated to 1988 and attributed to J. Vacanti [58], [32]. Since that time the number of publications related to tissue engineering grows exponentially every year reaching over 85 thousand titles in 2020 (total) [64], out of which, over 3,000 publications are related to neural tissue engineering.

Tissue engineering is using scaffolds to replace the impaired tissue. Scaffolds should mimic the extracellular matrix (ECM) of the native tissue. They support the cells and guide the cells' growth to form the desired tissue. They provide mechanical stability of the nascent tissue [53]. The requirements for the scaffold vary according to the targeted tissue. Biocompatibility is the key factor of each scaffold. Other requirements as mechanical properties and scaffold architecture consistent with the targeted tissue are no less important. Additionally, bioactivity or in some cases even biodegradability of the scaffold can be beneficial. Depending on the tissue being replaced, its architecture and properties, the appropriate combination of method of scaffold fabrication and material is chosen to address the structural, mechanical, biochemical, physical and other properties of the tissue [40].

3.2 Neural tissue engineering with emphasis on spinal cord injuries

Neural tissue is highly organized structure. It is divided in to the central and peripheral nervous system (CNS and PNS, respectively). It is built from many types of neural and supporting cells, which form various structures as nerves, spinal cord to the brain tissue. The neural cells, which are responsible for the transmission of the action potential (neural signal), have many cytoplasmic projections called dendrites (conduct impulses towards the cell body) and axons (conduct impulses away from cell body) which can be up to tens of centimeters long. Depending on the architecture of dendrites and axons we distinguish between the anaxonic, bipolar, unipolar and multipolar neurons. They are usually site specific for different neural structures and function. The supporting cells called neuroglia are also specific for the CNS (microglia, astrocytes, oligodendrocytes, ependymal cells) and for the PNS (Schwann cells, satelite cells). They preserve the physical and biochemical structure of neural tissue.

There are studies using the cell therapy for spinal cord injuries by implanting stem cells into the injury site *in vivo* [60]. Some researchers use the stem cells in the combination of carrier scaffold for the cells [25], [9]. There are also ongoing clinical trials implanting stem cells (clinicaltrials.gov). Yet, non of such research was translated into the practice. One of the main reasons is the poor regeneration capability of the neural tissue. The neural cells have slow proliferation rate, the axons do not extend enough to reconnect and the injury site is filled with fibroblasts instead, forming a glial scar [23]. Once the glial scar is formed

it acts as physical and chemical barrier which prevents repair and regeneration of the damaged neural tissue.

Because the neural tissue suffers from poor regeneration potential, it is desirable in neural tissue engineering to design complex scaffolds, which will support the cell attachment, proliferation, axon elongation and myelination. This will be achieved by a combination of various scaffold fabrication techniques and functionalization.

The spinal cord is a highly organized structure with predominantly rostral-caudal alignment of axons and myelin. Thus, the aligned spinal cord implants represent ideal microenvironment for directed axonal growth [43]. Moreover, those polymer spinal cord bridges are characterized by high degree of porosity, allowing for infiltration of progenitors that differentiate into myelinating oligodendrocytes resulting axon re-growth and also myelination of these axons [54]. On the other hand, such polymeric spinal cord implants do not meet other biophysical parameters of the spinal cord, as modulus and viscoelastic properties [54]. One possibility is to inject the hydrogels directly into the injury site [27]. The big advantage of such *in situ*-forming scaffold is that there is no need for surgical invasive procedures, the hydrogels can conform specifically to the shape of the defect and can create an integrative implant–tissue interface. On the other hand, these injectable materials do not have a high degree of control over the porosity or alignment and do not provide good axonal guidance [47]. To overcome the problem with the cell guidance and directional growth, hydrogels can be mixed e.g. with magnetically responsive additives such as superparamagnetic iron oxide nanoparticles [47].

Alternatively, the hydrogels can be mold in specific shapes prior to implantation. The hydrogel implants can thus provide an orientation to guide axon regeneration following spinal cord injury. For instance, photosensitive hydrogels can utilized by stereolithography to form specific 3D implants [1]. Eventually, the primary hydrogel beads are annealed into a tubular structure, which is later assembled into a larger implant filling the injury [10]. The advantage of this approach is that the hydrogel microspheres control the porosity of the structure and facilitate regenerative support cells.

Other group of spinal cord implants are nonwoven fibrous scaffolds, out of which the aligned fibrous scaffolds had been shown as the most suitable ones *in vitro* [6]. The oriented structure guides the cell growth and orientation of the cytoplasmic projections in the direction of the fiber alignment. This phenomenon is especially important in the axon orientation. The aligned fibrous scaffold could enhance the axon elongation and could improve the axon guidance in the required direction. This would lead to reconnection of the interrupted axons and to the functional recovery.

Oriented structures in tissue engineering are most often prepared by electrospinning using different polymers [69], [39], [21]. Eventually, forcespinnig can be used to prepare oriented fibers as well [22], [24]. Different studies used synthetic (poly-β-hydroxybutyrate - PHB, poly(lactic-co-glycolic acid) - PLGA) [69], [39], [21] as well as natural polymers

(collagen) [25]. These methods produce mainly nanofibers and are reasonably productive. On the other hand, the degree of orderliness of the fibers is often not very high, but can be sufficient depending on the application. Nevertheless, it was shown that the neural cells prefer microfibers to nanofibers [62]. The axons elongate along the microfibers compared to nanofibers where the cells spread their axons in all directions. Also, the oriented fibers support the migration of Schwann cells [62], which are crucial for the tissue regeneration and proper function of the tissue.

Nevertheless, the fibrous scaffolds alone are not sufficient enough to support the full regeneration of the injured tissue and further functionalization or combination of several methods is needed. Some approaches use growth factors or other signaling molecules to functionalize the surface of the scaffolds [5]. Furthermore, it is possible to use advanced molecular techniques such as lentivirus delivery into the implant to deliver specific anti-inflammatory molecules [42] or molecules supporting the neural cells' regeneration [57]. It is also possible to modify the surface by conducting polymers such as polypyrrole, polyaniline and others [15], [21]. It had been shown that the conducting polymers promote the axon elongation and they also promote the propagation of action potencial along the neural cell. Transmission of the action potential is a crucial property of the neural cells which leads to functional recovery of the whole system [76], [68]. Due to the enormous complexity and functional specificity of the neural tissue it is necessary to combine various techniques of scaffold fabrication and its funtionalization [5], [9], [42].

3.3 Nonwovens in tissue engineering

In general, very promising methods currently used in tissue engineering are nonwoven technologies. These technologies produce micro- and nanofibers, which resemble the fibrous extracellular matrix of native organs (Fig. 3.1).

Depending on the targeted tissue, its architecture and physical and mechanical properties, the method of scaffold fabrication and the polymer (polymers) are chosen.

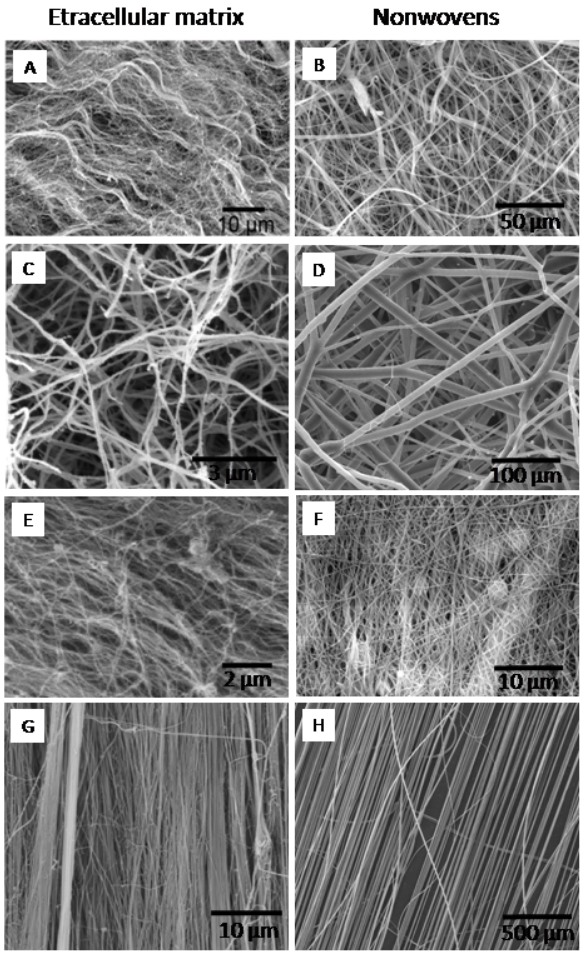

FIGURE 3.1: Comparison of SEM images of the extracellular matrixes and nonwoven textiles. (A) ECM of the decellularized porcine aortic valve (scale bar: 10 µm) [73], (B) Force-spun PCL fibers (scale bar: 50 µm), (C) Fibrin clots (scale bar: 3 µm) [38], (D) Melt-blown PCL fibers (scale bar: 100 µm), (E) ECM of the decellularized bovine corneal stroma (scale bar: 2 µm) [8], (F) Electrospun PCL fibers (scale bar: 10 µm), (G) ECM of the decellularized tendon (scale bar: 10 µm) [74], (H) Drawn PCL fibers (scale bar: 500 µm).

3.4 Drawing

Drawing is one of the many non-woven techniques, which can be used for scaffold preparation. Drawing is a method of pulling a single fiber from a droplet of polymer solution without using an electrical field (Fig. 3.2) [4], [41], and it is possible to do it even by hand. The fibers can be made from various types of polymers, varying in diameter from nano- to micro-scale. Also, different structures can be obtained; it depends on the direction of the fiber-pulling, the combination of nano- and microfibers, and the combination of polymers. With additional processing we can also obtain yarns.

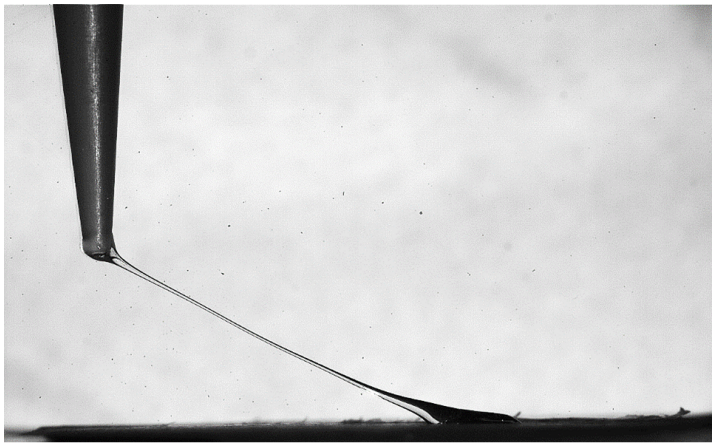

FIGURE 3.2: Drawing a polymer fiber from a droplet of polymer solution.

Mechanical drawing of fibers has been known for centuries. It can be divided into two categories: drawing from a melt [67] or a solution [35], [36], [37], [70]. The first attempt to draw a fiber was already realized in the 19th century. The fiber was prepared by shooting an arrow from a droplet of a melted polymer by a crossbow [4]. The resulting fiber was several hundreds of meters long. By that time there was no imaging technique available to examine the fiber diameter. Nevertheless, by using the existing instruments they estimated the fiber diameter to be under 2,5 μm [4]. In 1988, Vacanti et al. [59] used drawn fibers (drawn from molten polymers, 30 μm in diameter) for one of the first experiments in the tissue engineering field. Since then, nobody showed any interest into this method. In 1998 a modern experiment on a microscopic scale was conducted by Ondarcuhu and coworkers. They were able to draw a single nanofiber from a droplet of a polymer solution using a retracting tip of STM (Scanning Tunneling Microscope). The fiber was tens of nanometers in diameter and was hundreds of micrometers long [41]. Nowadays, drawing is used to produce fibers mainly for optical devices: optical sensing, nanophotonic fibers in the range of tens of nanometers in diameter and up to tens of centimeters in length [14], [67], [70], [31]. Drawing is not common method when talking about tissue engineering. One of the reasons can be the low productivity of fibers compared to other nonwoven technologies, e. g. electrospinning, forcespinning, melt-blown and others, which are often used for fabrication of micro- or nanofibers from various types of polymers [72]. They are reasonably productive, nevertheless, they can produce the oriented structures just in layers, and often the orderliness is not so accurate as well. The other reason, why drawing is not widely used, might be the lack of information about the fiber production as well. It is know that the fiber drawing is influenced by various extrinsic parameters as humidity, temperature, solvent evaporation, trajectory and the speed of drawing [35], [36], [37]. Also the concentration of the polymer solution as well as the molecular weight of the polymer play very important role in drawing [30]. Often, these correlations are described just theoretically, occasionally by basic experiments, but the description of the physical principles is missing.

On the other hand, even Vacanti in 1988 used drawn fibers as one of the scaffolds in his experiments [59]. He had drawn the fibers from a polymer melt, the fibers were 30 μm

in diameter and formed a tufts. He seeded the tufts with cells and implanted them into rats and mice to examine the biocompatibility, angiogenesis and immune response [59]. Recently, drawing was used to fabricate various random and oriented polymers structures by S. Minko and colleagues [56], or by R. S. Keynton and co-workers [75].

In contrast, drawing has lower fiber productivity, but is able to produce highly oriented structures and patterns. This is enabled due to the manipulation of a single fiber. Every single emerging fiber can be manipulated while drawing. This way we can prepare more complicated structures and patterns. Even though electrospinning with a rotational collector provides aligned fibers as well [22], the fibers can be oriented just in one direction in one layer without the possibility to manipulate the fibers, thus unable to provide more sophisticated fibrous structures. These feature make drawing a suitable method for the fabrication of scaffolds for specific tissues with oriented extracellular matrix and cells. Muscles and tendons are a good example of such tissue. Another use of these scaffold could be in neural tissue engineering. The neural tissue as nerves have highly oriented structure. Even the neural cells alone, specifically their axons need to be extended and oriented in specific direction. It had been shown previously that oriented scaffolds enhance the axon elongation and orientation along the fibers [39], [21], [62], [68]. So this makes drawing the method of choice for such application.

4 Methods used, studied material

4.1 Drawing fibers and their characterization

4.1.1 Preparation of polymer solutions

The polymer solutions of PCL, PVA and PA6 were prepared in various concentrations (% by weight) in corresponding solvent system. Depending on the type of the experiment, different polymer solutions were used. Solutions were prepared the day before use in the volume of 10 ml up to 100 ml for drawing or for measuring the solution properties, respectively. All solutions were mixed on magnetic stirrer over night at room temperature.

4.1.2 Solution characterization

All solutions were analyzed by rotational viscometer (HAAKE RotoVisco 1 viscometer with 35/1 Ti L plate) and by surface tensiometer (PocketDyne). Mean values of all measurements were used for further processing.

4.1.3 Drawing samples

All fibrous samples were drawn by the Manipulator using a plastic tip (OKI International). The polymer solution was dosed once per cycle (on both sides) at a constant pressure and time just before the tip touched the surface. The syringe with the tip was attached to

the moving element perpendicular to the underlay and moved with constant acceleration and velocity. The length of the fiber was set to 0.18 m. Fiber drawing was carried out under ambient conditions: room temperature 20 - 25 °C, relative humidity 30 – 35 %.

The fibers were either collected on plastic underlay (for mechanical testing) or fixed within the fixation ring (*in vitro* experiments, WCA measurement) or within the Teflon frame (*in vivo* experiments and conductivity measurement).

4.1.4 Fiber characterization and image analysis

The fiber morphology was observed by SEM. The cross-section of the fibers was done either in the liquid nitrogen with a scalpel or by the SEM (FEI Nova 200 Nanolab), where a thin layer of platinum was deposited on the side of the cut and the fibers were subsequently cut by a laser. Fiber diameter was measured by NIS Elements software. Other image analyses were done in ImageJ.

4.1.5 Mechanical testing of drawn fibers

The mechanical properties were measured using the tensile testing machine LabTest 2.010 with the 1N sensor [16] and the measurement was controlled using the LabTest software. The tensile strength measurement was done according to the standard ČSN EN ISO 5079 (Textiles - Fibers - Determination of breaking force and elongation at break of individual fibers; identical with EN ISO 5079:1995). Each sample was measured until failure. 15 to 20 measurements were done for each sample. 200 fibers were drawn into a bundle of 0.2 m of length on a plastic underlay, from which the fibers were collected and the whole bundle of fibers was used for measurement.

4.1.6 Functionalization of the fibers by polypyrrole

The prepared PCL scaffolds were subsequently functionalized by a thin layer of conductive polypyrrole (PPy). The polymerization reaction of pyrrole (10 mM) was maintained for three days at room temperature in the presence of FeCl3 (23 mM) and p-toluenesulfonic acid (11 mM) under constant stirring. After the polymerization, samples were washed and sonicated in methanol and dH_2O. Samples were dried and stored in vacuum desiccator at RT. The fibers' morphology was evaluated using scanning electron microscope.

4.1.7 Water contact angle (WCA) measurement

Samples for the WCA analysis were prepared by drawing 300 fibers from 12% solution of PCL, M_n 80,000. The fibers were fixed within the fixation ring. The 3 μl droplet of dH_2O was pipetted on the sample and the image of the droplet was captured using Advex Instruments See System camera right after pipetting the water on the sample. The WCA was

measured using See System software. The WCA was measured on five samples per each condition, measuring two or more droplets per sample.

4.1.8 Conductivity measurements

Conductivity was measured by different approaches. The fibers were drawn with Manipulator and fixed within a Teflon frame (Fig. 4.1) and coated with polypyrrole.

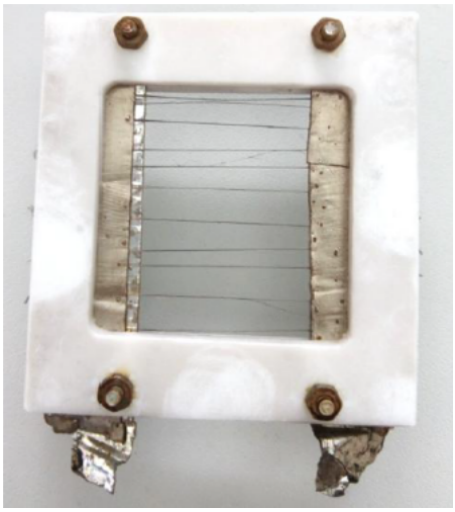

FIGURE 4.1: Polypyrrole-coated PCL fibers made by drawing, fixed within a Teflon frame in between an aluminum foil [19]

Three different measurements were carried out using different multimeters, different number of fibers, or even different setup of the experiment (where the fibers were fixed in between an aluminum foil and the aluminum foil was connected to a multimeter).

4.2 Biological testing

4.2.1 *In vitro* experiments - oriented fibers

The prepared samples were fixed within a supporting ring. The size of the fixation ring is designed to fit in 24-well cultivation plate and allows better manipulation with the fibers as well as it keeps the scaffold in the designed pattern.

First experiments were focused on the direction of cell growth and so the fibers were drawn in two directions (Fib I, Fib II). The second set of experiments was carried out with the Fib I samples and was focused on the evaluation of biocompatibility of the functionalized fibers with polypyrrole (PCL-PPy fibers). The PCL-PPy fibers were compared to plain PCL fibers used as control.

The samples were seeded with 3T3 mice fibroblasts and were cultured in an incubator (37°C, 5 % CO_2). Cell viability and proliferation was measured by MTT assay. The samples were also analyzed by scanning electron microscopy (VEGA3 SB easy probe) and by fluorescent microscopy (Nikon Eclipse Ti-e).

4.2.2 *In vitro* and *in vivo* experiments - spinal cord bridges

4.2.2.1 Bridges for *in vitro* testing / *in vivo* implantations

New composite scaffold (spinal cord bridge) was designed for the *in vivo* experiments. The well established method of spinal cord bridge production was used [54] and modified to place the oriented fibers along the channels. The bridges are sorted under the microscope and the resulting bridges are porous with 6 to 7 longitudinal channels (Fig. 4.2 A, 4.2 B). Three different types of scaffolds were made, one with no fibers (plain PLGA bridge; NoF), one with PCL fibers (PCL) and one with PCL-PPy fibers (PCL coated with polypyrrole; PCL-PPy).

To be able to test this scaffolds *in vitro*, the shape of the bridge had to be modified. The *in vitro* scaffolds were flat from both sides (top and bottom) and had a block shape with just one or two channels with or without fibers (Fig. 4.2 C, 4.2 D).

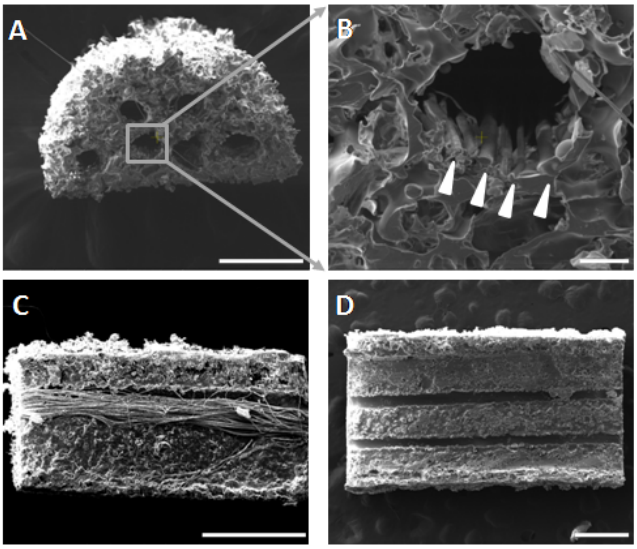

FIGURE 4.2: SEM images of the *in vivo* (A, B) and *in vitro* (C, D) scaffolds. (A) A transversal section of multiple-channel bridge (scale bar 400 μm). (B) Magnification of one particular bridge channel (arrows pointing at the aligned fibers; scale bar 50 μm). (C) *in vitro* sample with oriented PCL-PPy fibers (scale bar 1 mm). (D) *In vitro* samples wit channels only (scale bar 500 μm).

4.2.2.2 *In vitro* testing of the spinal cord bridges

The dorsal root ganglia (DRGs) were isolated from two days old mice pups (C57Bl6). The DRGs were washed and kept in the HBSS buffer (Hanks´ Balanced Salt Solution) before use. The DRGs were placed on the scaffold specifically under the microscope so that the DRG covered the channel. The DRGs were cultured in the complete Neurobasal medium. After 7 days of incubation the samples were fixed in 4% paraformaldehyde and they were stained using the primary antibody against β-tubulin and AF555 goat anti-mouse IgG as secondary antibody. Nuclei were stained with Hoechst (Hoechst 33342, 1:2000). The samples were observed under the inverted fluorescent microscope (AxioObserver) using a 10x dry objective and analyzed by ImageJ.

4.2.2.3 *In vivo* experiments

All animals housing conditions, surgical procedures, and postoperative care was conducted according to IACUC guidelines at the University of Michigan, Michigan, USA. Four weeks old female mice aged 6 - 8 weeks (C57Bl6) received a T9 laminectomy, followed by a left-sided double lateral hemisection and removal of a unilateral 1 - 1.1 mm segment of the spinal cord to enable bridge implantation into the resulting gap. After the bridge implantation, the dorsal surface of the spinal cord above the injury site was covered with gelfoam, the muscle sutured with 5/0 chromic gut, and the skin closed using wound clips. Postoperative care included administration of Baytril, buprenorphine, and lactate ringer solution. Bladders were expressed twice daily until function recovered, and mice monitored daily thereafter.

The bridges were explanted after 2 or 8 weeks. The isolated bridges were either used for RNA isolation with subsequent qPCR, or were deep-frozen into a mounting media and sectioned transversally in 12 μm thick slices (cryostat Microm HM525).

4.2.2.4 RNA isolation

Upon retrieval, the samples were deep frozen on dry ice. Samples were homogenized in TRIZOL Reagent and the RNA was isolated using TRIZOL Reagent according to the protocol. After the isolation, the RNA was purified by Direct-ZOLTM RNA MiniPrep Plus. The RNA concentration and purity was checked by NanoDrop. Isolated RNA was stored at -20°C.

4.2.2.5 cDNA Synthesis

The cDNA was synthesized from the isolated RNA using iScriptTM cDNA Synthesis Kit. For each reaction, 1 μg of RNA was used. The reverse transcription was done according to the manufacturer´s protocol using BIO-RAD C1000 Touch Thermal Cycler. The cDNA was stored in -20°C.

4.2.2.6 qPCR

The quantitative PCR (qPCR) was performed in triplicates for all samples using iQTM SYBR® Green Supermix according to the manufacturer´s protocol. The qPCR was performed on the BIO-RAD C1000 Touch Thermal Cycler. All genes were run in triplicates. The 2-ΔΔCT method [26] was used to calculated fold changes in mRNA levels for all genes compared to the negative control (mice without bridge) using 18s rRNA as the internal control gene.

4.2.2.7 Immunohistochemistry

For the immunohistochemistry, one slide from rostral, middle and caudal region was chosen per each animal. Bridge sections after 2 weeks implantations were fixed and stained using the following primary antibodies to detect neutrophils (goat anti-arginase 1, 1:100)

and M1 macrophages (rat anti-F4/80, 1:200) and CF555 donkey anti-goat IgG (1:1000) and AF647/633 goat anti-rat IgG (1:1000) as secondary antibodies. Nuclei were stained with Hoechst (Hoechst 33342, 1:2000).

Bridges retrieved from mice after 8 weeks were fixed and stained using the following primary antibodies to detect neurites (rabbit anti-NF200, 1:200), oligodendrocytes´ myelin (goat anti-MBP, 1:500) and Schwann cells´ myelin (chicken anti-P0, 1:250) and CF555 donkey anti-rabbit IgG (1:1000), CF488 donkey anti-goat IgG (1:1000) and CF633 donkey anti-chicken IgY (1:1000) as secondary antibodies. Nuclei were stained with Hoechst (Hoechst 33342, 1:2000).

Images were captured at 20x by the fluorescent microscope (AxioObserver). Stitched images were created using the Zeiss software (Zen Pro) and used for cell counting. The counting of the 2 weeks long implantations was done manually using ImageJ. The counting of the 8 weeks long implantations was semi-automated using MATLAB according to McCreedy [29].

4.3 Statistics

pngThe obtained data were processed by Microsoft Excel. Statistical analysis was performed using GraphPad Prism 6 software. Data were analyzed either by multiple comparison analysis using one-way analysis of variance (ANOVA) or by t-test and Scheffe post-hoc analysis with a p-value < 0.05 defined as significant depending on the dataset. For the *in vivo* experiments and all conditions, n=6 mice for histological analysis of neurofilament, while n=4 was used for histological analysis of response and qRT-PCR analysis. The fiber diameter was analyzed from n = 100 and higher. All values are reported as mean +/- standard deviation or standard error of the mean, noted at each analysis.

5 Summary of the result achieved

5.1 Drawing technology

Drawing is not particularly the method of choice for scaffold fabrication, therefore any commercially available lab - scale production device is missing. The advantage of this method is that it is possible to draw the fibers just by hand, but the samples are often inhomogeneous, because it is impossible to keep the same conditions during drawing individual fibers. To improve the drawing technology a lab-scale manipulator for drawing fibers from polymer solutions was designed and constructed. The mobile drawing element of the manipulator moves from one side to another in a repeating manner and produces single fibers one by one, laying them separately on an underlay. A basic production scheme can be seen in Fig. 5.1.

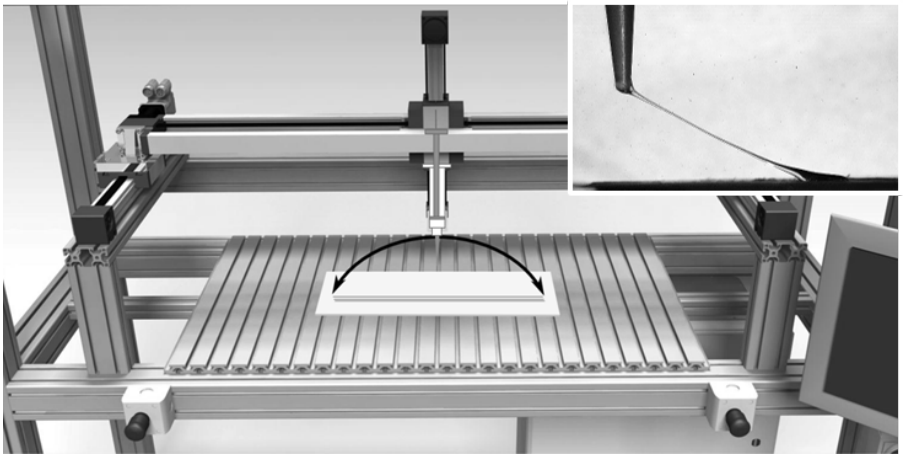

FIGURE 5.1: The manipulator representing the drawing movement [51]

The manipulator is able to move at high speeds (several $m \cdot s^{-1}$) and consists of three axes. The manipulator enables us to program the trajectory of a drawing element and arrangement of fibers, and set the speed in a wide range of velocities. This enables us to keep constant conditions during the fiber spinning, thus being able to study the influence of particular conditions on the fiber formation (solution viscosity, speed and trajectory of drawing, solvent evaporation etc.) on the properties of the fibers.

The Manipulator is a universal machine with an extendable manner of design (Fig. 5.2). It is equipped with a polymer dosing device, professional positioning axes (BAHR Modultechnik GmbH) with a synchronous belt. The machine is controlled by isiMotion system, connected to a panel PC. The repeating accuracy of this system is ± 0.1 mm. A digital dispenser connected to a standard air compressor with an air accumulator is used as a polymer dosing device.

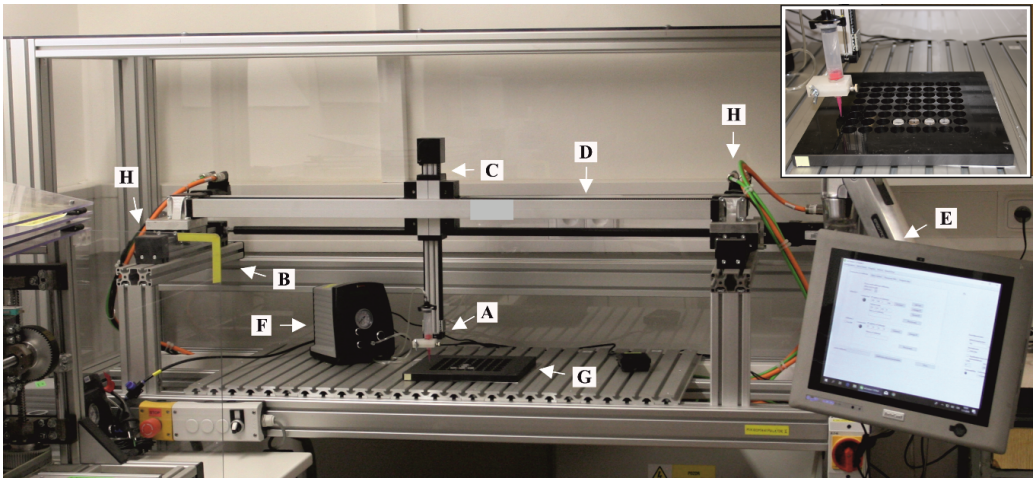

FIGURE 5.2: Image of the Manipulator. (A) the dosing system, (B - D) the positioning axes working in the X, Y and Z coordinates, (E) the computer with controlling software, (F) the dispenser, (G) the workplace, (H) the servomotors. The detail of the workplace is shown in the upper corner of the image.

5.2 Influence of the polymer concentration and drawing speed on the fiber diameter

The influence of extrinsic conditions on the fiber production are indisputable. Changing the extrinsic parameter can influence the final fiber morphology, e.g. the fiber diameter and its distribution within the sample. Therefore, several parameters were followed during fiber drawing. The PCL (M_n 80,000, Sigma Aldrich) fibers were drawn using Manipulator by established velocities (0.1, 0.5, 1, 2 and 3 m·s^{-1}) and polymer concentrations in spinning solutions (4%, 6%, 8%, 12%, 16% and 20% by weight). From our results it is evident that under the ambient conditions the fiber diameter decreases with an increasing speed of drawing and a decreasing polymer solution concentration (Fig. 5.3). Furthermore, we found that the fiber diameter distribution decreases with an increasing speed of drawing. The 6% PCL solution drawn at a speed of 3 m·s^{-1} resulted in the significantly smallest mean value of a fiber diameter (2.51 ± 0.12 μm), whereas the 20% PCL solution drawn with the speed of 0.1 m·s^{-1} resulted in the significantly highest mean value of fiber diameter (9.56 ± 0.49 μm). The lowest fiber diameter measured throughout all the samples was 190 nm for the 6% PCL at the speed of 2 m·s^{-1}. The 6% PCL solution was also the lowest concentration capable of fiber drawing, although only at a drawing speed of 1 m·s^{-1} and higher [51]. Nevertheless, the fibers prepared from 12% solution have the lowest fiber diameters distribution, especially at drawing speeds above 1 m·s^{-1}. The fibers prepared at these conditions were therefore used for cell culturing experiments.

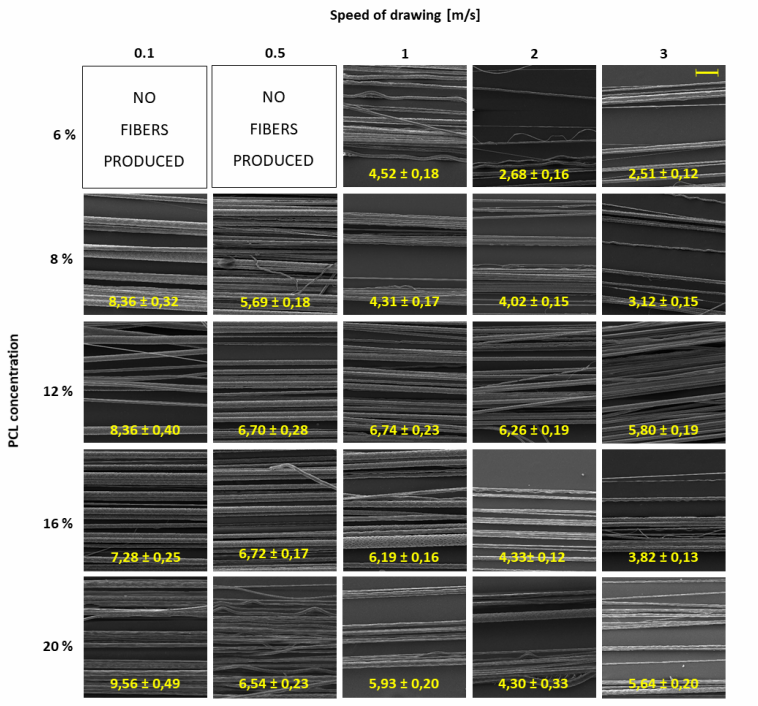

FIGURE 5.3: Diagram of the fibers' morphology depending on the speed of drawing and the polymer concentration. The fiber diameters are presented as mean in μm ± SEM and are marked in yellow. Scale bar: 50 μm

There were two types of defects observed on the prepared fibers from the PCL of M_n 80,000. The fiber narrowing (Fig. 5.4) was one of the phenomena. The formation of thinner regions is significantly less frequent for fibers prepared from polymer solutions at a concentration of 12 % and 16 %. The SEM examinations of fibers reveal that the fibers are prone to necking in some cases, which means the contraction of the fiber diameter from 1,000 nm to nearly 100 nm. The necks are not evenly spaced along the fibers. The necking and crazing was first described in the electrospun nanofibers by Zussman [79] as the failure mode. Therefore, we hypothesize, that the neck structure on a drawn fiber appears as a result of a fast solvent evaporation and strong stretching of solidified fibers during the fiber drawing.

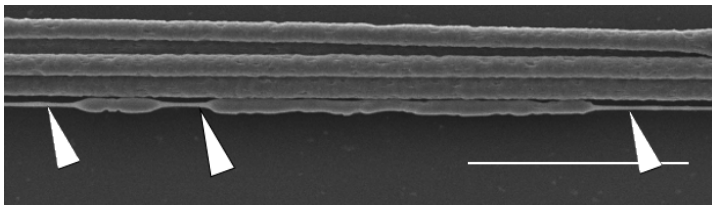

FIGURE 5.4: SEM image of the fibers drawn from the 8% PCL solution at a speed of 1 $m \cdot s^{-1}$,one of the defects (fiber necks) is indicated by arrowheads, Scale bar: 50 μm.

The other type of defects observed during drawing was a nanostructured surface (Fig. 5.5). The pores are hundreds of nanometers wide and deep. The pores are formed by the solvent evaporation and their structure is influenced by the polymer and the solvent system used. Similar structures were described previously on electrospun fibers [79], [7], [68], and are known to be beneficial for the cell-fiber interaction and to enhance the cell adhesion (Megelski et al., 2002).

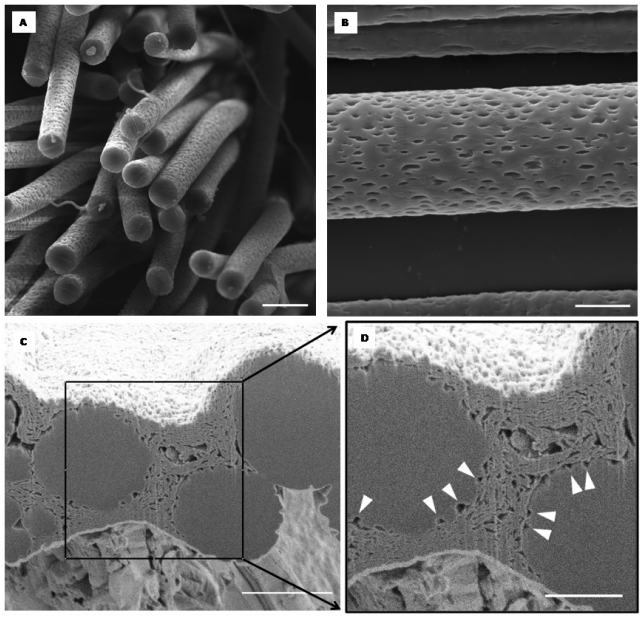

FIGURE 5.5: SEM images showing the morphology of the fibers. (A) A uniform circular cross-section (scale bar: 20 μm), (B) nanostructured porous surface (scale bar: 10 μm) and (C) a cross-section of the fibers (scale bar: 5 μm) and (D) pore depth indicated by arrowheads (scale bar: 3 μm).

5.3 Spinability of different polymers by drawing

The PCL (M_n 45,000), PVA (Mw 130,000) and PA6 at various concentrations were chosen for this experiment. Fiber diameter was typically more than one micrometer. PVA was spinnable from the concentrations above 20 %. Average fiber diameters were 1,056 ± 236 nm for 20 % solution, 1,838 ± 648 nm for 24 % solution and 1,868 ± 763 nm for 28 % solution. In case of PCL (M_n 45,000, Sigma Aldrich), concentration of 28 % and higher led to fiber formation. The diameter of PCL fibers was higher than PVA fibers having the fiber diameter of 5,949 ± 4,188 nm (28 %) and 4,019 ± 1,471 nm (32 %). No fibers were obtained using PA6. Morphology of drawn fibers is depicted in Fig. 5.6.

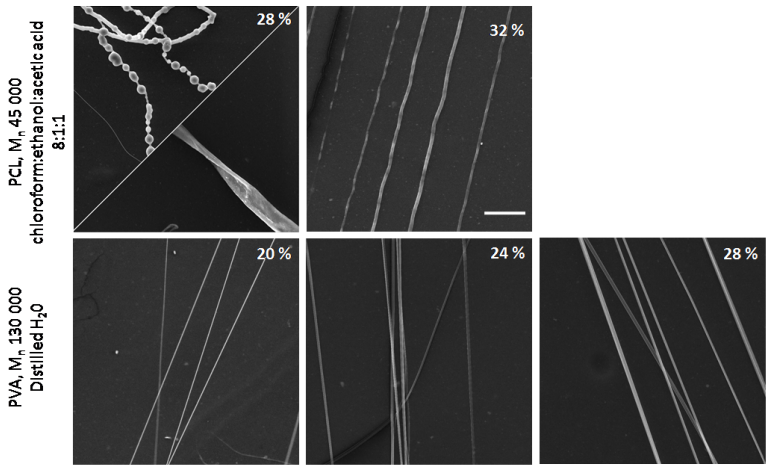

FIGURE 5.6: Images from SEM showing the drawn fibers of PCL (M_n 45,000) and PVA (M_n 130,000) at different concentrations. Scale bar: 50μm.

Compared to the fiber diameter of PCL M_n 80,000 (all used concentrations) drawn at the 1 $m \cdot s^{-1}$ speed, which was 4.31 μm and higher, the PVA had significantly lower fiber diameter (maximum mean value 1.9 μm for the highest concentration). In the case of PCL M_n 45,000, the mean values of the fiber diameter were comparable to PCL M_n 80,000. Both PVA and PCL M_n 45,000 were drawn from higher concentrations then PCL M_n 80,000 in the previous experiment, and below the shown concentrations it was impossible to draw the fibers.

All three polymers (PVA, PCL M_n 45,000, PCL M_n 80,000) were of different molecular weight and were also dissolved in different solvents / solvent system. Both of these parameter influence the solubility of the polymer and the viscoelastic properties of the polymer solution and thus its spinnability and the resultant fiber appearance [78], [18], [46]. For example, the solvent system of chloroform : ethanol : acetic acid (used for PCL M_n 45,000) is mostly used for electrospinning of PCL. Acetic acid and ethanol lowers the surface tension, which enhances the spinnability of the solution via electrospinning by helping with the formation of the polymer jet by electric field. On the other hand, in the case of drawing, this feature can be undesirable. The whole process of fiber formation by drawing is significantly longer compared to electrospinnig and lowering the surface tension of the solution may lead to

breakage of the fluid thread, also called as Rayleigh instability [78]. This leads to lower fiber production and defects forming along the fiber (Fig. 5.6).

The breakup of the polymer jet has been shown to depend on polymer concentration and molecular weight [18]. The destabilization of the jet occurs at low molecular weight polymers, which leads to the beads formation. As the molecular weight increases, the polymer jet is stabilized and typical fibers are formed. As the molecular weight of the polymer grows even higher, the solvent evaporation at the jet surface is fast enough to form a skin, the fibrous structure collapses and flat ribbons instead of fibers are formed [18], [46]. As the solution concentration increases, there is a gradual shift from circular to flat fiber. In low molecular weight polymers, this shift occurs at a higher values of concentrations than in the high molecular weight polymers [18]. These findings are in contrast what was observed during drawing. The beads and ribbons occurred simultaneously at the lower concentration of the low molecular weight PCL. We hypothesize that this discrepancy is caused by different mechanism of fiber formation during electrospinning and drawing. Drawing uses mechanical energy and the fiber is pulled from the polymer droplet. The speed of single fiber formation is incomparably slower during drawing than electrospinning. On the other hand, these defects were seen only in one experiment and more data are needed for final conclusions.

5.4 Viscosity and surface tension

The viscosity and surface tension influence the fiber formation, fiber diameter and distribution. The viscosity and surface tension was measured for the PCL M_n 80,000 of the polymer concentration of 6 %, 8 %, 12 %, 16 % and 20 %. The viscosity grew exponentially depending on the polymer concentration and was ranging from 0.33 Pa·s for the 6% PCL up to 32.91 Pa·s for the 20% PCL (Fig. 5.7). The surface tension was ranging from 35.32 mN/m for the 6% PCL up to 110.64 $mN \cdot m^{-1}$ for the 20% PCL (Fig. 5.7).

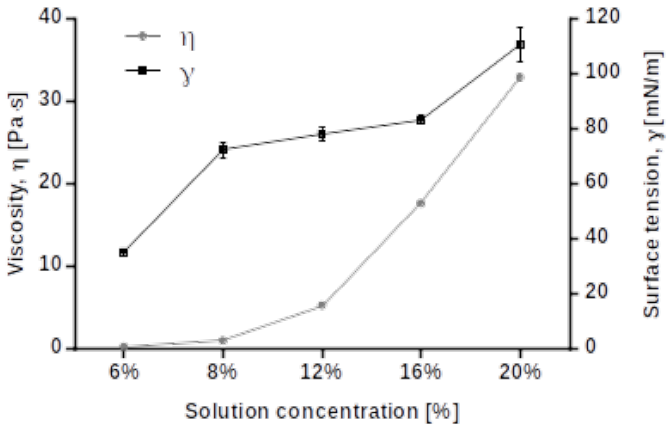

FIGURE 5.7: Comparison of viscosities and surface tensions of the polymer solutions in the dependence on solution concentration

The values of the surface tension are also not linearly arranged, but that could be caused by the inaccuracy in the measurements, as well as by the varying humidity conditions in the lab, since the measurements were not taken at the same time.

This experiment revealed the limit values for drawing under the set parameters. The lowest concentration tested within this experiment, i.e. 6% polymer solution, which refers to value of 0.33 Pa·s for viscosity and 35.32 mN/m for surface tension, was the lowest concentration of the polymer which was possible to draw, but the limit speed of drawing was 1 $m \cdot s^{-1}$. Whereas at higher concentrations (and viscosity), the speed limit of drawing was below 0.1 $m \cdot s^{-1}$. With increasing viscosity and surface tension, the fiber diameter increases and so grows the fiber diameter distribution.

The solution viscosity and surface tension influences the spinnability of the solution. These parameters are influenced mainly by the solution concentration and its composition (polymer and its molecular weight and the solvent system) and each spinning method requires different parameters. In general, e.g. electrospinning requires lower surface tension for the fibers to be formed, whereas e.g. bubble-spinning requires higher surface tension, because first the polymer bubble needs to be formed prior to spinning.

5.5 Mechanical testing

The mechanical properties were measured using the bundles of 200 drawn fibers made from PCL M_n 80,000. The test was done according to the standard ČSN EN ISO 5079 and each sample was measured 15 to 20 times. The diagrams show the measured force [N], which is a function of the gauge length [mm] (Fig. 5.8). The Young's modulus of the 200-fiber bundle was 240.73 MPa. The force, tension and relative elongation at break were 0.1 N, 41.08 MPa and 527.89 %, respectively. All values represent the mean value of all the measurements.

It is evident from the results and also from the shape of the curve that the setup of this method of mechanical testing is not suitable. In neither case have the curves smooth shape, which is typical for testing the fibers. The instability of the tensile curve and the fluctuating shape is given by the non-homogeneity of the particular fiber bundles (Fig. 5.8).

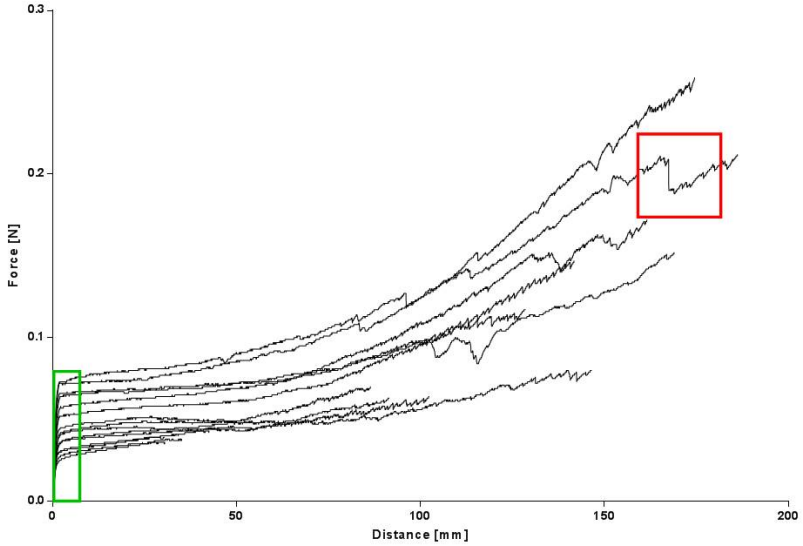

FIGURE 5.8: Diagram of the tensile curves of the individual measurements. The red rectangle shows the abnormality in the tensile measurement - the drop of the force value, which starts growing immediately again.

Also, the fiber diameter of individual fibers in the bundle is not homogeneous, both along the entire fiber length and in between the fibers in the bundle. This is not different from the electrospun fibers. But while electrospun fibers form a tissue-like structure with countless number of fibers and the whole structure is measured as one sample, the drawn samples are bundles of individual 200 fibers. That means that the mechanical properties of individual fibers in the bundle differ. This phenomenon can be observed from the shape of the curve. If there is a drop in the force value and immediately the force starts growing, that means that one or more fibers broke, but there are still many other fibers that can elongate (Fig. 5.8).

Another factor affecting the measurement is the attachment of the sample alone. The fibers are drawn in the bundle, that means that not all of the fibers are attached to the grip evenly and that increases the probability of fiber slipping between the neighboring fibers. That affects the behavior of individual fibers and of the whole bundle during the measurement.

5.6 Functionalization of drawn scaffolds by polypyrrole

The drawn samples with fibers oriented in one direction were coated with thin layer of polypyrrole for further biological experiments, since PPy is widely used conducting polymer in tissue engineering and has very good stability and promotes the adhesion and proliferation of various cell types [76], [49], [68].

The PPy layer was investigated under SEM (Fig. 5.9, 5.10). It is homogeneous and 50 - 100 nm thick (Fig. 5.10). The PPy layer comparably thick and smooth to other studies reported in the literature [21], [68], [55]. Moreover, drawing produces highly porous structure, which remains preserved even after the PPy coating (Fig. 5.9, 5.13).

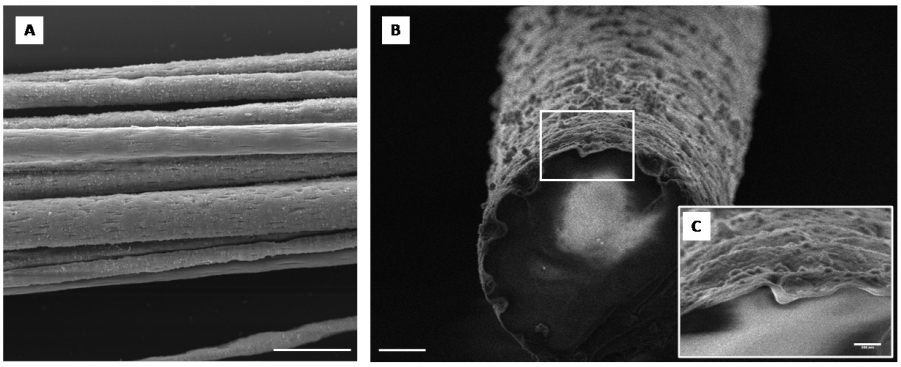

FIGURE 5.9: SEM images showing the PPy modified PCL fibers. (A) The coating of PPy is uniform and retains porous structure of the fibers. (B) The cross-section of the fiber with detailed image of the PPy layer thickness (C).

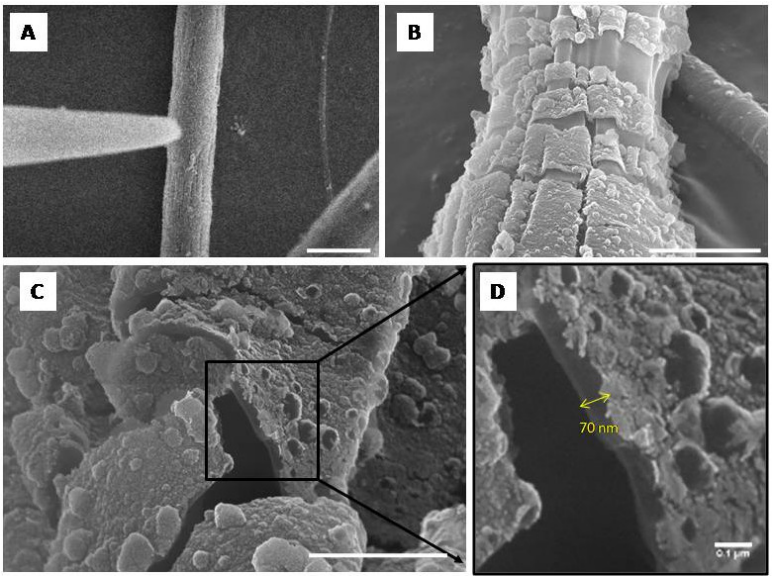

FIGURE 5.10: SEM images of PPy coated PCL fibres. The PPy layer was disrupted using the SEM (FEI Helios 650 Nanolab) retracting tip (A). (B) The disrupted PPy layer. (C, D) represent higher magnifications of the cracked PPy layer.

5.7 Conductivity measurement

The conductivity was measured by three different approaches, which differed in the used multimeter, number of fibers and the experiment setup, but used the measurement of resistivity for all approaches. All of the experiments were unsuccessful (the multimeters were not sensitive enough to measure the resistivity) and all our samples showed no conductivity value. On the other hand, if we use planar samples made by electrospinning from PCL and

cover those by polypyrrole in the same chemical reaction, the resistance on those samples is measurable (approximately $3{\cdot}10^4$ Ω/square) [28].

Probably, the impossibility to measure the conductivity of the drawn PPy-coated fibers is due to the density of the fibers and overall appearance of the sample. Electrospinning produces fibrous mats with thousands of fibers layered over each other, whereas drawing produces separate fibers which never really form a layer even if they are drawn dense.

It is visible from Fig. 5.11 that after the fiber stretching, the homogeneous coherent layer of PPy breaks. This can be caused by inappropriate sample manipulation. Such sample disruption would cause big complications and inaccuracy in the conductivity measurement. On the other hand, such samples should not represent a big problem for the *in vitro* and *in vivo* system, because we expect the cells to elongate along the fibers, thus superimposing and connecting the gaps, which should overcome the problem of the layer conductivity.

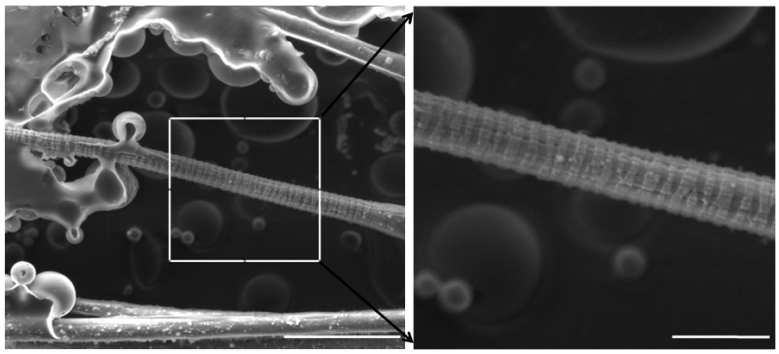

FIGURE 5.11: The SEM image of the micro fractures of the PPy layer on PCL fibers after the fiber stretching (scale bar: 30 μm) with the detailed image of the same (scale bar: 10 μm).

5.8 Water contact angle

Samples for the analysis were prepared by drawing. 300 fibers from PCL M_n 80,000 were fixed within the fixation ring for the analysis and the drops were placed on the fibers as shown in Fig. 5.12 right before the measurement.

The WCA was measured on the plain PCL fibers and the PCL fibers coated with PPy. Measured values of the WCA for both samples were above 120°. The values of WCA for plain PCL and PCL-PPy were 122.5° (±3.54) and 121.5° (±3.74), respectively (Fig. 5.12). The WCA values of both samples show no significant difference. The value of WCA for PCL is consistent with other authors [44], [17]. The WCA values for PPy surfaces vary according to the synthesis conditions [61], [11], [45], [50].

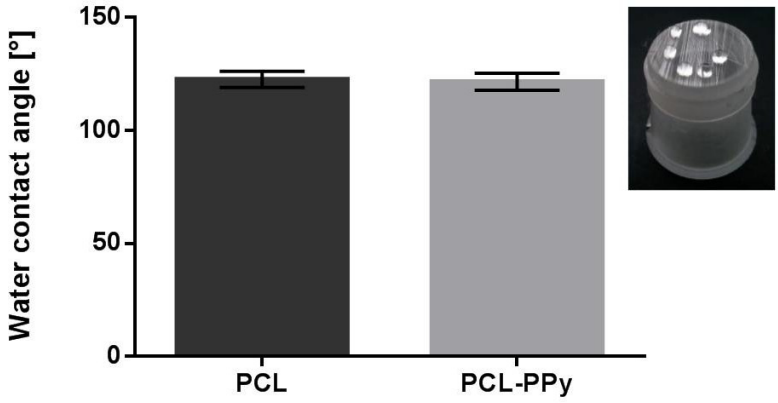

FIGURE 5.12: The diagram of water contact angles for PCL and PCL-PPy samples.

It is known that the surface roughness / porosity influences the surface properties. As the roughness increases, the hydrophobic substrate becomes more hydrophilic. For instance, a drop placed on a porous medium does not merely spread on the surface but also penetrates the depth of the support, thereby modifying its wetting properties [12], [52]. According to our porous fibers, which keep its porosity even after the PPy-coating (Fig. 5.13), it is likely that this porosity contributes to the hydrophilicity of our samples.

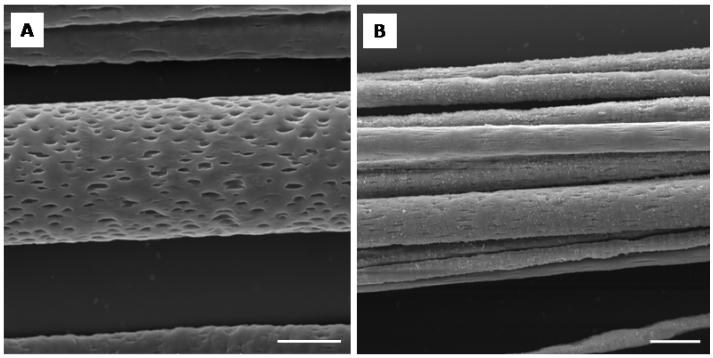

FIGURE 5.13: Comparison of the surface structure of (A) the plain PCL fibers (scale bar: 10 μm) and (B) the PPy coated fibers (scale bar: 20 μm).

5.9 *In vitro* assessment of the biocompatibility of drawn samples

The scaffolds made of fibers ordered either in one or in two directions (Fib I, Fib II) (Fig. 5.14) were tested *in vitro* and should confirm that the oriented microfibers support the oriented cell growth. The biocompatibility of these scaffolds was tested by MTT assay (showing the cell adhesion and proliferation), and by fluorescent and scanning electron microscopy. The results from the MTT assay as well as from the microscopy analysis show, that the scaffolds are capable of supporting cellular attachment and the proliferation during our *in vitro* tests (Fig. 5.15, 5.16, 5.17).

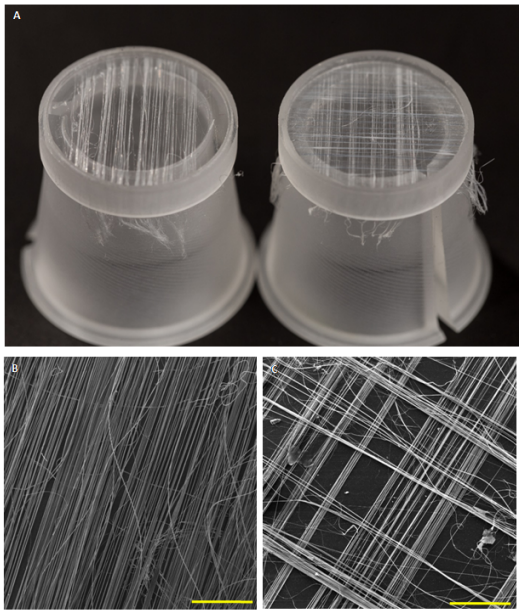

FIGURE 5.14: Images of the *in vitro* samples. (A) Macroscopic image of the fibers fixed within the fixation ring (the ring fits the well in 24-well plate), (B) and (C) SEM images of the fibers ordered in one (Fib I) or two (Fib II) directions (scale bar: 1 mm).

The data from the MTT assay reveal similar rates of cell adhesion (day 1) in both types of scaffolds (Fig. 5.15). Also during the following testing days the proliferation rate of Fib I and Fib II is comparable. This was an expected result, since the number of seeded cells was the same for both scaffolds and the cell adhesion was similar as well.

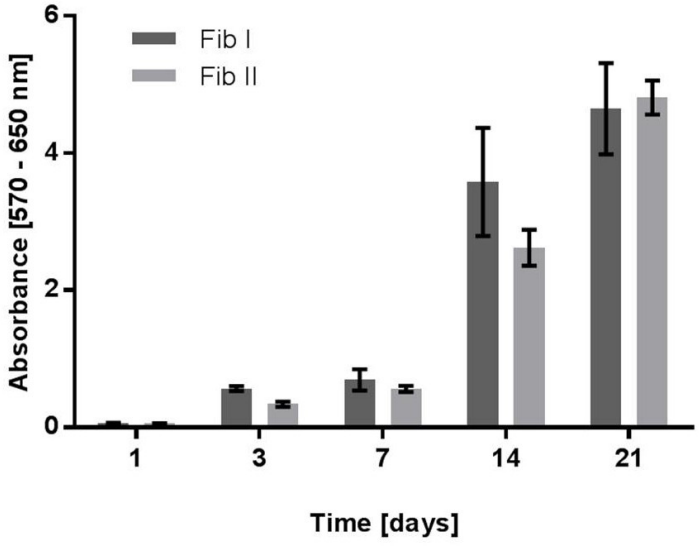

FIGURE 5.15: Results from the MTT assay showing the cell proliferation on two different scaffolds (Fib I, Fib II). Error bars representing SEM.

Fluorescent microscopy reveals very important phenomenon that the cells prefer the growth in the direction of fibers (Fig. 5.16), which can be seen from the orientation of the oval shape of cell nuclei. In addition, this experiment showed that our samples have fibers ordered in two different directions, and the cells follow the fibers in both of them.

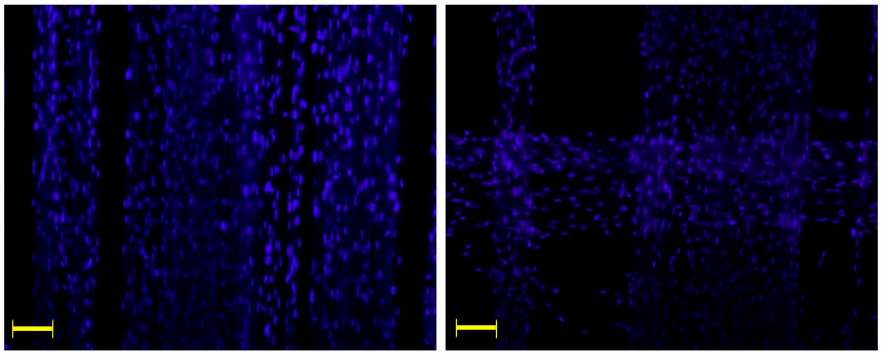

FIGURE 5.16: Images from the fluorescent microscope representing the samples Fib I and Fib II 14 days after cell seeding with mice fibroblasts. Scale bar: 100 μm.

Further, the SEM images show, that the cells are capable of overgrow the holes between the fibers of tens of micrometers in diameter (Fig. 5.17).

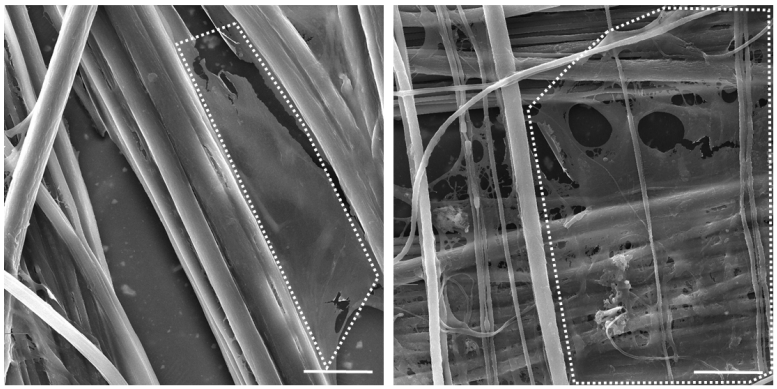

FIGURE 5.17: SEM images of the samples Fib I and Fib II 21 days after cell seeding with mice fibroblasts. Scale bar: 50 μm.

5.10 *In vitro* biocompatibility assessment of the oriented PPy fibers

The images from fluorescent microscope show very good cell adhesion on the PPy-coated fibers. The cells also proliferate within the testing days (Fig. 5.18, 5.19). The viability (Fig. 5.18) tested by MTT assay also shows higher values for the PPy-coated samples. These results are consistent with other studies [21], [68] and show, that the PPy samples are not cytotoxic and support the cell adhesion and proliferation. Moreover, according to the oval shape of the nuclei and their orientation (Fig. 5.19) it is evident, that the cells grow and spread along the fibers, copying the orientation of the fibers. Thus, the PPy-coated PCL fibers are suitable for the further experiments with neural cells.

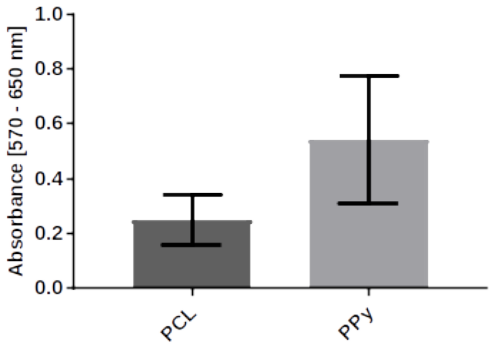

FIGURE 5.18: Diagram of the viability measured by MTT assay at day 14 after cell seeding.

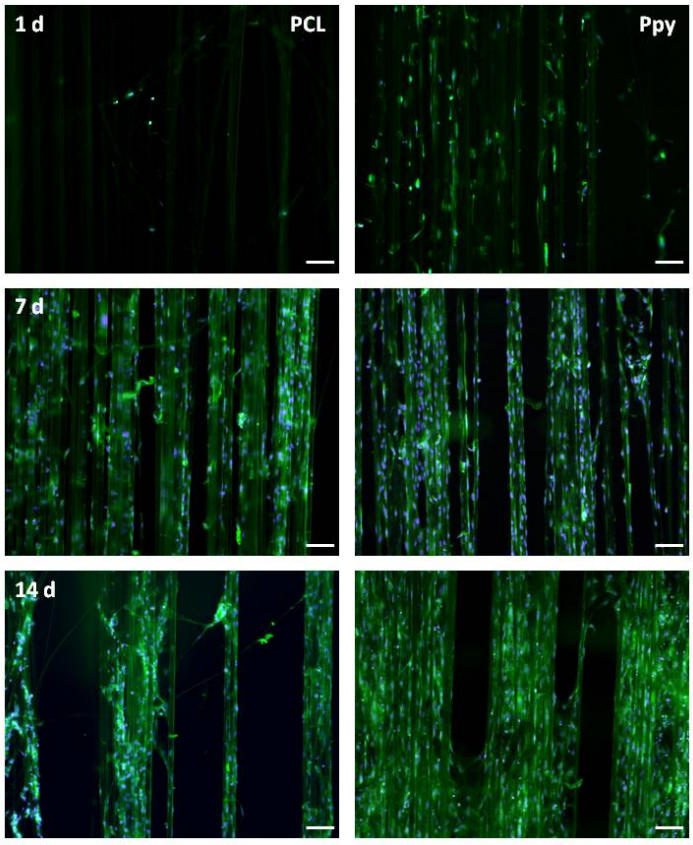

FIGURE 5.19: Images from fluorescent microscope showing the cells seeded on the plain PCL fibers (PCL) and on the PPy-coated fibers (PPy) 1, 7 and 14 days after cell seeding. Nuclei stained with DAPI (blue), cytoskeleton stained with Phalloidin-FITC (green). Scale bar: 100 μm.

5.11 Development, construction and characterization of the composite scaffold for *in vitro* / *in vivo* experiments

It had been shown previously that the aligned PPy fibers support the growth and orientation of the cells [62], [68], [51]. But the problem of such scaffolds is their integrity and the implantation of such fibrous scaffolds *in vivo*. That is why new composite scaffold harboring

the aligned PPy fibers was developed. This scaffold keeps the aligned structure of the fibers even after implantation.

The previously developed and tested spinal cord bridge from PLGA [71] was used as the fiber carrier. The channels within the bridges, are around 150 μm in diameter, allow the cell infiltration through a specific region of the implant, however, the channels itself do not actively guide the cells since the size of the channels is larger compared to the cells and their axons. The channels lined with the fibers were prepared to increase the neural cell infiltration as well as the infiltration of axons into the bridge. The microfibers were placed along the channels (Fig. 5.20). The aligned PCL and PCL-PPy fibers were incorporated into the bridges. Each channel was lined with a bundle of 100 fibers. The size of the *in vivo* sample was approximately 1.1 mm in length, 1.5 mm in width and 0.75 mm in height. The overall porosity is about 70 % [54]. However, some of the PCL-PPy bridges were bigger (higher) than the average size of the bridge, since it very much depends on the manual fabrication of the bridges. Also, it was often observed that the PCL-PPy fibers did not align along the whole channel, but stayed gathered in the bundle on one side of the channel. This in-homogeneity of the samples definitely influences the final *in vivo* results, since the size of the bridge influences the side of the injury *in vivo* (can cause much severe injury after implantation). The distribution of the fibers along the channel influences the contact area of the fibers with the cells, and the bundles of PCL-PPy fibers thus have smaller area to be in contact with the cells.

In the case of the *in vitro* experiments, the samples were designed to be flat-bottomed. The plain PLGA sample had 2 plain grooves, whereas the PCL a PCL-PPy samples had one bundle of 100 fibers incorporated into the groove. The size of the *in vitro* sample was approximately 2.6 mm in length to 1.5 mm in width.

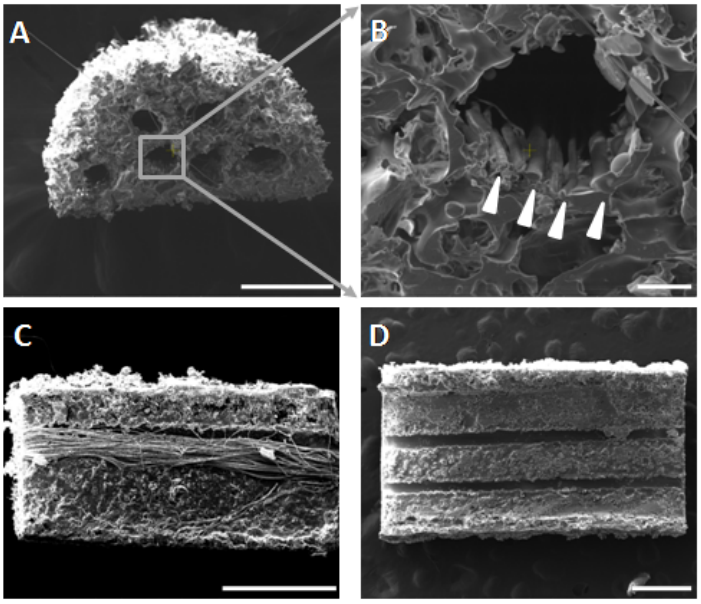

FIGURE 5.20: A – D: The SEM images of the fibers aligned along the channels. (A) Transversal cut of the bridge (scale bar: 400 μm). (B) Magnification of the particular channel (scale bar: 50 μm). (C, D) Longitudinal cut of the bridge (scale bar: 200 μm (C) and 100 μm (D)).

5.12 *In vitro* testing of the novel spinal cord bridges

The *in vitro* samples were seeded with DRGs isolated from two days old mice pups. The DRGs were placed on the scaffold specifically under the microscope so that the DRG covered the groove / fibers. After 7 days of incubation the samples were fixed in 4% paraformaldehyde and they were stained against β-tubulin (nuclei were stained with Hoechst). The samples were observed under the inverted fluorescent microscope. The axonal spread of neural cells was evaluated using the ImageJ.

The results show statistically significant decrease in the axonal length to width ration (axonal spread) on the samples without fibers, with PCL fibers and with PPy-coated fibers. That means, that the axons of the neural cells follow rather the fibers, if available, than the plain channels. The effect is stronger if the fibers are coated with PPy (Fig. 5.21).

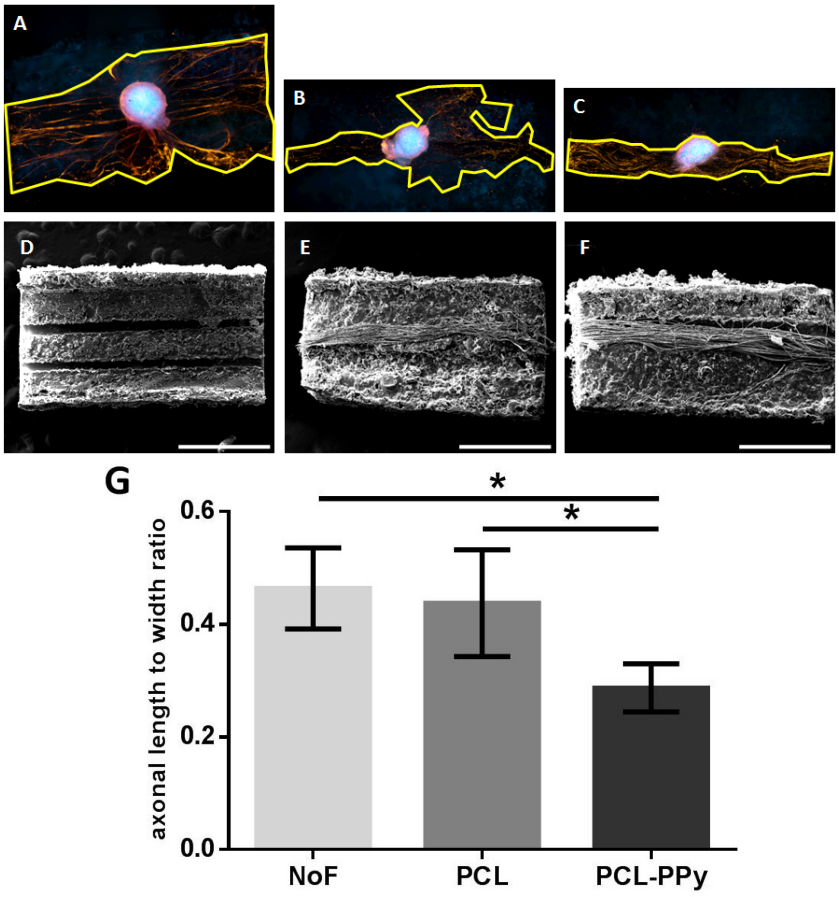

FIGURE 5.21: Axonal spread and axon elongation *in vitro*. A – C: Fluorescent images of DRGs 7 days after seeding on scaffolds. *In vitro* scaffolds with (A) no fibers, (B) PCL fibers and (C) Ppy-coated fibers. Yellow line represents the axonal spread. D - F: SEM images of the *in vitro* samples (scale bar: 1 mm). (D) sample without fibers (NoF), (E) sample with PCL fibers, (F) sample with PCL-PPy fibers. (G) Axonal length to width ratio. Data presented as mean ± SD. * denotes $p < 0{,}05$.

5.13 *In vivo* experiments with the novel spinal cord bridges

According to the *in vitro* results the prepared spinal cord bridges were implanted into the mice's spinal cord after lateral hemisection at T9 (Fig. 5.22). Four weeks old female mice aged 6 - 8 weeks (C57Bl6) were used for this experiment. The bridges were explanted after 2 or 8 weeks. Bridges isolated after 2 weeks were either used for RNA isolation with subsequent qPCR, or were deep-frozen into a mounting media and sectioned transversally in 12 μm thick slices and used for immunohistochemistry (immune response to the bridges). The bridges isolated after 8 weeks were only collected for sectioning. The sections were fixed and subjected to immunohistochemistry (cell infiltration into the bridges).

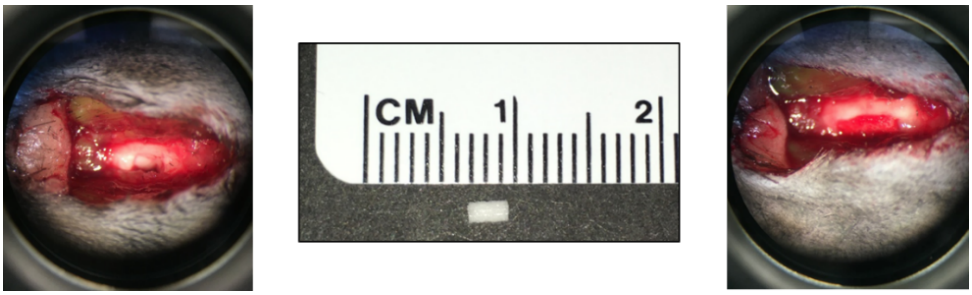

FIGURE 5.22: Lateral hemisection at T9 spinal cord region (left) and hemisection after the bridge implantation (right). The bridge implanted into the hemisection is depicted in the middle. The images are illustrative. The samples used for the *in vivo* study were half the size of the depicted bridge.

The 2-week timepoint samples were tested for the immune response by immunostaining followed by fluorescent microscopy (Fig. 5.23) and by RT-PCR (Fig. 5.24). The images from the fluorescent microscope show similar immune response in all tested samples (bridges without fibers, with PCL fibers and with PPy-coated fibers). The immune response evaluated with immunostaining primarily evaluated pro-inflammatory M1 macrophages (F4/80+ cells) and anti-inflammatory M2 macrophages (F4/80+Arginase1+ cells), as both are necessary this time point for clearing cellular debris and laying down matrix for attachment of infiltrating cells. Together, the absence of qualitative differences in macrophage populations suggests that the inclusion of the PCL fibers or PPy-coated fibers do not exacerbate the immune reaction to the bridges making them suitable for further evaluation.

The results from the RT-PCR show the expression of immunogenic markers compared to the control SHAM mice (mice with laminectomy only). There is significant increase in CD86 in non-fibrous samples compared to SHAM samples, and moderate increase in MHC II and iNOS, which suggests the activation of pro-inflammatory M1 immune response. The CD86 marker is significantly lower in the PPy-coated samples compared to the non-fibrous samples, but it is comparable to SHAM samples, with the iNOS expression lower than SHAM mice. Also, the Arginase 1 marker is significantly higher in the both fibrous samples (PCL and PPy-coated fibers) compared to SHAM and non-fibrous sample, which suggests the activation of the pro-healing M2 immune response. Other pro-inflammatory markers as MHC II, iNOS, CD206 and apoptotic markers cytochrome C and caspase 3 from all samples are

comparable to the SHAM samples, suggesting the non-immunogenicity of the bridge samples.

These findings support our immunostaining results in demonstrating that the addition of the PCL fiber or PPy-coated fibers do not lead to increase immunogenicity, but rather they increase pro-healing immune cell infiltration compared to the bridge alone. PPy is considered as the least immunogenic from the all conducting polymers used in tissue engineering [15], [3], still there are some reports of its immunogenicity, mostly after electrical stimulation [65], [63]. Also it had been shown that the biocompatibility is dependent on the synthesis method, the conditions and used dopants. The biocompatibility is dependent on the washing step of the PPy layers before cell seeding [2], [11].

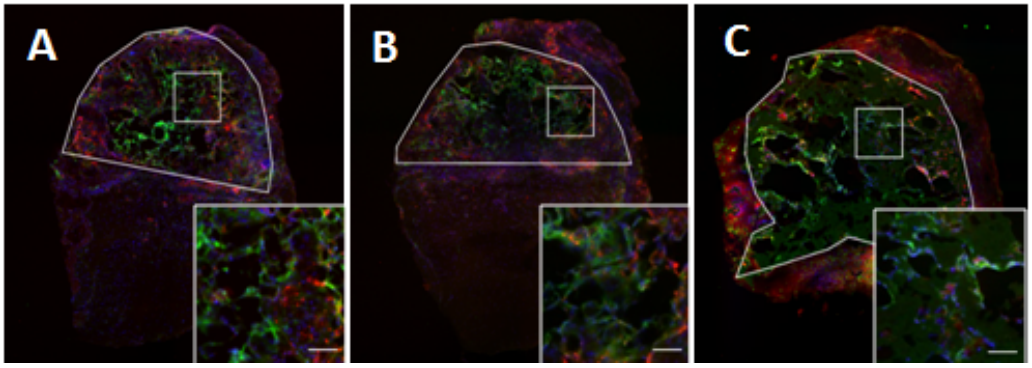

FIGURE 5.23: Immune response to the bridges with PCL / PPy fibers. F4/80 and Arginase1 fluorescence (Arginase 1 in red, F4/80 in green, nuclei in blue) at 2 weeks after bridge implantation. (A) Plain bridge, (B) bridge with PCL fibers, (C) bridge with PPy fibers. The bridges with PCL / PPy fibers do not reveal any excessive immune response compared to the plain bridge. Scale bar: 50 μm.

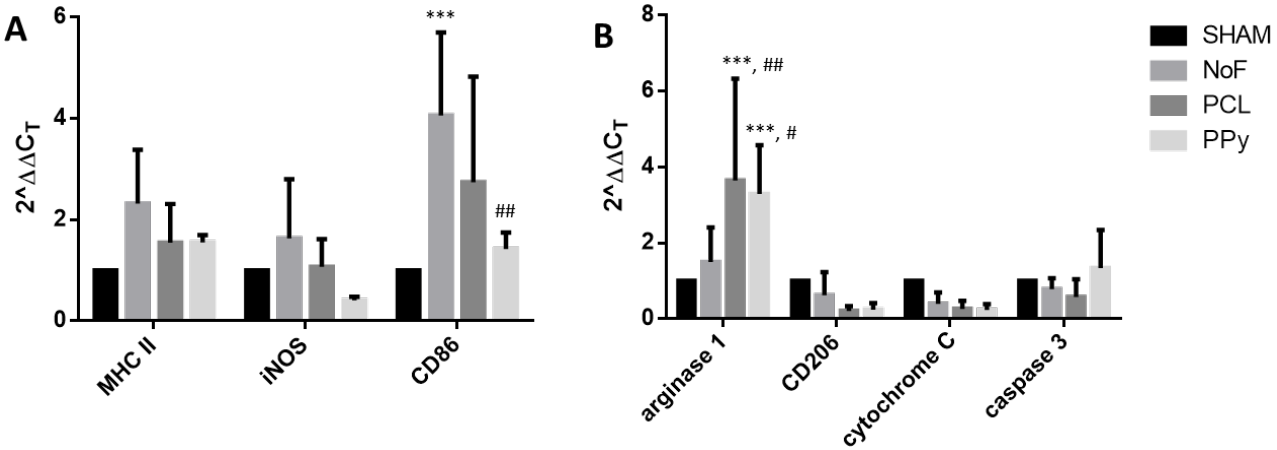

FIGURE 5.24: Quantification of immune response using qPCR. Samples collected 2 weeks after implantation. (A) qPCR data for M1 (A) and M2 and inflammation markers (B). *** denotes $p < 0{,}001$ vs. SHAM, ## denotes $p < 0{,}01$ vs. NoF, # denotes $p < 0{,}05$ vs. NoF.

The 8-week timepoint samples were tested for the axonal infiltration into the spinal cord bridge and for the axonal myelination by immunostaining (Fig. 5.25).

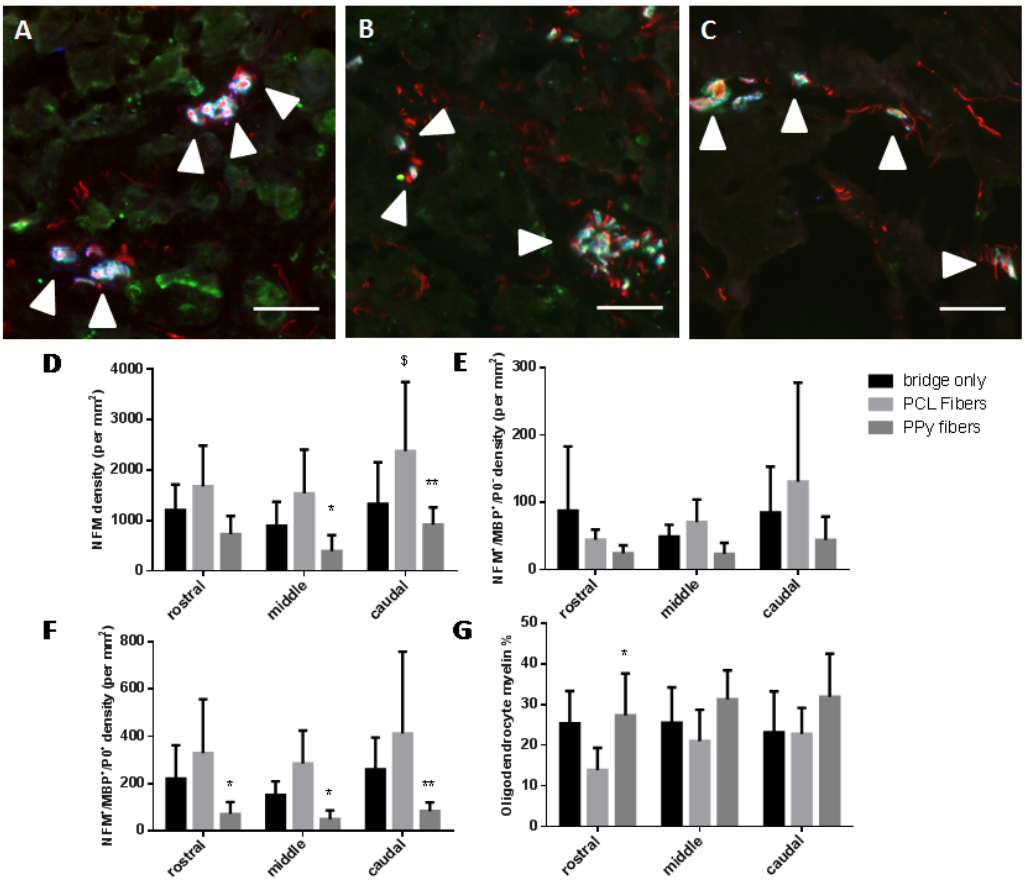

FIGURE 5.25: Axon infiltration and myelination 8 weeks after injury. Immunofluorescence from bridges of Schwann cell (NFM+/MBP+/P0+: red/green/blue, respectively) and oligodendrocyte (NFM+/MBP+/P0-) derived myelin fibers from bridge with no fibers (A), PCL (B) and PPy fibers (C). White arrow show fibers wrapped by Schwann cell-derived myelin. Brightness and contrast were adjusted for clarity. Density of axons (D), oligodendrocytes (E), Schwann cells (F), and percentage of oligodendrocyte derived myelinated axons (G) in bridge with no fibers, PCL and PPy fibers conditions (mean +/- SD). Scale bar: 50 um. ** denotes $p < 0.01$ vs. PCL bridge, * denotes $p < 0.05$ vs. PCL bridge, $ denotes $p < 0.05$ vs. bridge only.

Axons (NFM density) were present throughout the bridges (Fig. 5.25 D) 8 weeks after SCI under all experimental conditions. NFM-positive axons were typically observed in small groups or bundles as previously reported for multichannel PLG bridges [57], [29]. The plain bridges had a mean of axons approximately 1,100 axons/mm2. The bridges with PCL fibers show higher axon infiltration compared to control (plain bridges) with the mean of approximately 2,000 axons/mm2, but the difference was statistically significant only in the caudal region. The PPy-PCL laden brides had the mean of axon infiltration approximately 600 axons/mm2. However, these findings are statistically significant only for the middle and caudal region compared to PCL bridge. About 20% of these axons are myelinated, 75% of this myelin is derived from the Schwann cells (Fig. 5.25 G). The myelination of axons is important, because it allows the neural cells to conduct the nervous impulses. In the peripheral nervous system it is the Schwann cells who are responsible for the myelination of axons. Nevertheless, it is expected that Schwann cell - derived myelin is less effective for

CNS function [77]. The number of infiltrating axons as well as the percentage of myelination is comparable to other studies [9], [48].

The lower axonal infiltration into the bridges with PPy-coated fibers compared to bridges with PCL fibers or compared to control is not consistent with the *in vitro* experiment. That could be caused by several factors. First, the PPy-coated fibers tend to form bundles. That results in the non-homogeneous distribution of the PPy-coated fibers around the channel perimeter, thus the surface area and therefore the contact surface of the PPy-coated fibers is much lower than in the case of PCL fibers. Second, the bridges with PPy-coated fibers were visibly bigger (Fig. 5.23) in perimeter than the control bridges and the bridges with PCL fibers. That resulted in the compression of the remaining half of the spinal cord, causing more severe injury, which is harder to heal. More experiments with more precise samples with emphasis on the fiber distribution would be needed, however, these results suggest that the inclusion of fibers within the bridge channels can improve regenerative outcomes following spinal cord injury.

On the other hand, despite these shortcomings, the bridges with PCL-PPy fibers do not reveal any excessive immune response compared to other two types of samples and the cell infiltration is comparable to bridges without fibers.

6 Evaluation of the results and new findings

Drawing is a unique method to produce precise fibrous structures. To ensure stable drawing conditions, a new drawing machine called Manipulator was used. Several polymers were used to for drawing to show the versatility of this spinning method. Finally, drawing was used for the fabrication of scaffolds for tissue engineering and these scaffolds were tested *in vitro* and *in vivo*.

- Fiber production by drawing is dependent on the polymer concentration, molecular weight and speed of drawing. These parameters also influence the fiber diameter. It had been shown using the PCL M_n 80,000, that under ambient conditions the fiber diameter decreases with an increasing speed of drawing and a decreasing polymer solution concentration. Furthermore, the fiber diameter distribution decreases with increasing the speed of drawing. With decreasing polymer concentration, higher speed of drawing is necessary to prepare the fibers.
- Also high molecular weight PVA and low molecular weight PCL was possible to spun with drawing, although much higher concentrations had to be used. That is due to the solubility of these polymers in their solvents and also due to the chosen molecular weight.
- During drawing formation of some common defects was observed. Almost all of the drawn fibers are porous. The pores are hundreds of nanometers wide and deep. The pores are formed by the solvent evaporation and their structure is influenced by the polymer and the solvent system used. Similar structures were described previously

on electrospun fibers, and are known to be beneficial for the cell-fiber interaction and to enhance the cell adhesion. Other defects found were necking as a result of a fast solvent evaporation and strong stretching of solidified fibers during the fiber drawing, and formation of beads and ribbons, which are influenced by the molecular weight of the polymer and the polymer solution concentration.

- Mechanical testing revealed that the tension values of the drawn samples are comparable to some results obtained from the measurement of electrospun fibers or fibers obtained by gravity spinning, which have the fiber diameter higher than 1 micrometer. The results also revealed that more suitable method for measuring the mechanical properties of drawn fiber would be beneficial.
- WCA measurement shows that both samples have the WCA higher than 120°. The value of WCA for PCL is consistent with other authors. The WCA values for PPy surfaces vary according to the synthesis conditions.
- The conductivity was measured by three different approaches, but all of the experiments were unsuccessful and all our samples showed no conductivity value. The failure of those experiments is probably given by the overall appearance of our samples. First, the PPy layer is very thin (70 nm), second, the samples contained only 100 or 200 fibers which is in this case very little number.
- The *in vitro* experiments of the oriented fibers show that all the samples are capable of supporting cell adhesion and proliferation during our experiment. Also, the results confirm that the cells prefer the growth in the direction of fibers.
- The *in vitro* experiment of the PPy-coated fibers very good biocompatibility of the PPy-coated fibers. These fibers were subsequently used for the preparation of the novel composite spinal cord bridges.
- New composite spinal cord bridge was developed using previously tested PLGA bridges as matrix for harboring aligned PCL and PPy-coated fibers.
- The new spinal cord bridges were tested *in vitro* for the axonal spread of neural cells. The results show that the oriented fibers support the guidance of neurite outgrowth. This phenomenon is even more pronounced, if the fibers are coated with PPy.
- The new spinal cord bridges were successfully implanted into mice for *in vivo* experiments. The bridges were implanted into mice spinal cord after lateral hemisection. After two and eight weeks the samples were explanted and subjected to further analysis. The assessment of the pro-inflammatory and apoptotic markers after two weeks of implantations were comparable to the SHAM samples, suggesting the non-immunogenicity of the fibrous bridge samples.Moreover, the expression levels of Arginase 1 was significantly higher in the both fibrous bridges compared to SHAM and non-fibrous sample, which suggests the activation of the pro-healing M2 immune response.

- Samples explanted 8 weeks post surgery were evaluated for the axon infiltration and axonal myelination. The bridges with PCL fibers show higher axon infiltration compared to control. About 20 % of these axons are myelinated, 75 % of this myelin is derived from the Schwann cells. The axonal infiltration into the bridges with PPy-coated fibers is lower compared to bridges with PCL fibers or compared to control. This result is not consistent with the *in vitro* experiment. That could be caused by several factors. First, the PPy-coated fibers tend to form bundles. That results in the non-homogeneous distribution of the PPy-coated fibers around the channel perimeter, thus the surface area and therefore the contact surface of the PPy-coated fibers is much lower than in the case of PCL fibers. Second, the bridges with PPy-coated fibers were visibly bigger in perimeter than the control bridges and the bridges with PCL fibers. That resulted in the compression of the remaining half of the spinal cord, causing more severe injury, which is harder to heal. The obtained results show, that the oriented fibers enhance the axon infiltration and that the combination of the currently available approaches with new functionalization methods will be the method of choice for neural tissue engineering.
- Drawing appears to be a suitable tool also for other applications such as fabrication of hernia meshes or development of new and unique method for the biocompatibility evaluation of polymer scaffolds. This work proved that drawing is very unique and suitable method for fabrication of specific fibrous scaffolds for tissue engineering. The obtained results will help us to precisely control the fiber morphology in the future, design different patterns of scaffolds and fulfill the needs of tissue engineering, where other nonwoven methods are often inadequate. Moreover, this technique can be used to study the real-time dynamics of the population and the cell - material interactions (cytocompatibility), for which other spinning techniques are inadequate.

7 List of papers published by the author

7.1 Publications in journals

1. **Strnadová, K**, Stanislav, L, Krabicová, I, Sabol, F, Lukášek, J, Řezanka, M, Lukáš, D, Jenčová, V, **(2020)** Drawn aligned polymer microfibres for tissue engineering. Journal of Industrial Textiles 50 (3), 263 - 277. DOI: 10.1177/1528083718825318
2. Lukasek, J, Hauzerova, S, Havlickova, K, **Strnadova, K**, Masek, K, Stuchlik, M, Stibor, I, Jencova, V, Rezanka, M **(2019)** Cyclodextrin-Polypyrrole Coatings of Scaffolds for Tissue Engineering. Polymers 11 (459), 1 - 11. DOI: 10.3390/polym11030459
3. Dumont, CM, Carlson, MA, Munsell, MK, Andrew, J, **Strnadova, K**, Park, J, Cummings, BJ, Aileen, J, Shea, LD **(2019)** Aligned hydrogel tubes guide regeneration following spinal cord injury. Acta Biomaterialia 86, 312 - 322. DOI: 10.1016/j.actbio.2018.12.052

4. Erben, J., Jencova, V., Chvojka, J., Blazkova, L., **Strnadova, K.**, Modrak, M., Kuzelova Kostakova, E. **(2016)** The combination of meltblown technology and electrospinning – The influence of the ratio of micro and nanofibers on cell viability. Materials Letters 173, 153 - 157, In Press, DOI: 10.1016/j.matlet.2016.02.147

5. Erben, J., **Pilarova, K.**, Sanetrnik, F., Chvojka, J., Jencova, V., Blazkova, L., Havlicek, J., Novák, O., Mikeš, P., Prosecka, E., Lukas, D., Kuzelova Kostakova, E. **(2015)** The combination of meltblown and electrospinning for bone tissue engineering. Materials Letters 143, 172 – 176, DOI: 10.1016/j.matlet.2014.12.100

6. Krchová, S., Dzan, L., Lukáš, D., Mikeš, P., Jenčová, V., Horáková J., **Pilařová K. (2014)** Nanovlákna v hojení kožních ran. Česká Dermatovenerologie 4, 234 – 240, ISSN: 1805-0611

7.2 Patents

1. Stanislav L., Bajáková J., Lukáš, D., Chaloupek J., **Pilařová K.**, Jenčová V., Horáková J. (2014) Způsob výroby polymerních vláken o průměru 100 nm až 10 μm, a způsob výroby lineárního, plošného nebo prostorového útvaru obsahujícího tato polymerní vlákna, submitted patent 2014-920.

2. Lukáš D., Mikeš P.,Kuželová-Košťáková E., Pokorný P., Novák O.,Sanetrník F., Chvojka J., Havlíček J., Jenčová V., Horáková J., Blažková L., **Pilařová K.**, Erben J., Kovačičin J. (2014) Zařízení pro výrobu kompozitního textilního materiálu obsahujícího polymerní nanovlákna, textilní kompozitní materiál obsahující polymerní nanovlákna, patent 306018, 2014-947, utility model 28190, 2014-30498.

3. Dzan L., Krchová S., Chvojka J., Lukáš D., Kuželová-Košťáková E., Mikeš P., Pokorný P., Zálešáková D., Jirsák O., Sanetrník F., Jenčová V., Novák O., **Pilařová K.**, Horáková J. (2015) Způsob výroby plošného útvaru z biodegradabilních a biokompatabilních nanovláken, především pro kryt kožních ran, a zařízení k provádění tohoto způsobu, submitted patent 2015-117.

7.3 Contributions in conference proceedings

1. **Biomateriály a jejich povrchy X. 2017**, Herbertov, presentation: Aligned Drawn Fibers Improve the Axon Infiltration into the Spinal Cord Bridges, **2nd place in the student competition**

2. **TheFiber Society'sFall 2017 Technical Meeting and Conference** and 2nd International Symposium on MaterialsfromRenewables, Athens, Georgia, USA, poster: *Aligned Drawn Fibers Improve the Axon Infiltration into the Spinal Cord Bridges*

3. **Biomateriály a jejich povrchyX. 2017**, Herbertov, presentation: *Aligned Drawn Fibers Improve the Axon Infiltration into the Spinal Cord Bridges*
4. **TheFiber's Society Spring 2016 Conference** – Textile Innovations – Opportunities and Challeges, presentation: *Functionalization and Biocompatibility Evaluation of Drawn Fibers for Neural Tissue Implants*
5. **Bioimplantologie 2016**, presentation: *Příprava orientovaných scaffoldů pro tkáňové inženýrství metodou drawing*
6. **Summer School 2016 – Regenerative Nano-Medicine: From Advanced Delivery Systems to Electronic-Based Devices**, poster: *Functionalization and Biocompatibility Evaluation of Drawn Fibers for Neural Tissue Implants*
7. **Workshop pro doktorandy Technické univerzity v Liberci 2015**, presentation: *Drawing: Fabrication of scaffolds for tissue engineering*
8. **Bioimplantologie 2015**, presentation: *Drawing: Příprava scaffoldů pro tkáňové inženýrství*
9. **27th European Conference on Biomaterials 2015**, poster: *The Impact of Thrombocytes on the Cell Proliferation within the 3D Scaffolds*
10. **TheFiber's Society Spring 2015 Conference** in conjunction with the 2015 International Conference on Advanced Fibers and Polymer Materials – Functional Fibers and Textiles, presentation: *Nanofibrous Materials from Polycaprolactone as Wound Dressings*
11. **NanoCon 2014**, presentation: *Drawing Nanofibrous Scaffolds with Precise Structure as a Tool for Tissue Engineering*

7.4 Citations

Strnadová, K, Stanislav, L, Krabicová, I, Sabol, F, Lukášek, J, Řezanka, M, Lukáš, D, Jenčová, V, (2020) Drawn aligned polymer microfibres for tissue engineering. Journal of Industrial Textiles 50 (3), 263 - 277. DOI: 10.1177/1528083718825318 **- cited 2-times (from WOS)**

Dumont, CM, Carlson, MA, Munsell, MK, Andrew, J, **Strnadova, K**, Park, J, Cummings, BJ, Aileen, J, Shea, LD (2019) Aligned hydrogel tubes guide regeneration following spinal cord injury. Acta Biomaterialia 86, 312 - 322. DOI: 10.1016/j.actbio.2018.12.052 **- cited 8-times (from PubMed NCBI)**

Lukasek, J, Hauzerova, S, Havlickova, K, **Strnadova, K**, Masek, K, Stuchlik, M, Stibor, I, Jencova, V, Rezanka, M (2019) Cyclodextrin-Polypyrrole Coatings of Scaffolds for Tissue Engineering. Polymers 11 (459), 1 - 11. DOI: 10.3390/polym11030459 **- cited 4-times (from Scopus)**

Erben, J., Jencova, V., Chvojka, J., Blazkova, L., **Strnadova, K.**, Modrak, M., Kuzelova Kostakova, E. (2016) The combination of meltblown technology and electrospinning – The influence of the ratio of micro and nanofibers on cell viability. Matterials Letters 173, 153 - 157, In Press, DOI: 10.1016/j.matlet.2016.02.147 **- cited 11-times (from Scopus)**

Erben, J., **Pilarova, K.**, Sanetrnik, F., Chvojka, J., Jencova, V., Blazkova, L., Havlicek, J., Novák, O., Mikeš, P., Prosecka, E., Lukas, D., Kuzelova Kostakova, E. (2015) The combination of meltblown and electrospinning for bone tissue engineering. Matterials Letters 143, 172 – 176, DOI: 10.1016/j.matlet.2014.12.100 **- cited 27-times (from Scopus)**

8 Brief description of the current expertise, research and scientific activities

8.1 Doctoral Studies

Doctoral studies	Full time student at the Faculty of Textile
	Department of Nonwovens and Nanofibrous Materials
	Specialization: Textile Technics and Materials Engineering
List of Exams Passed	Proteomics
	Tissue Engineering
	Polymers
	Statistics
	Experimental Technique of Textile
State Doctoral Exam	Passed on 4. 12. 2017

8.2 Teaching Activities

Teaching

- Winter semestr - practical courses of Materiály pro tkáňové inženýrství (KNT / MTI) (2014 - 2018) and Polymery (KNT / PPO) (2016, 2017)
- Summer semestr - practical courses of Molekulární biofyzika (KNT / MBI) (2015 - 2017), participation on the lectures - usualy one lecture per semestr (2015 - 2017)
- Preparing new subject "Tissue Engineering" under new study program Bioingeneering

Leading Bachelors/ Master students

- Leading of the bachelor thesis of Andrea Šamanová (Buněčná migrace na vlákenných materiálech, 2015)
- Consultant of the bachelor thesis of Jan Gavura (Výroba vodivých vláken pomocí technologie drawing, 2015)

- Consultant of the bachelor thesis of Filip Sabol (Příprava a charakteristika orientovaných vláken metodou drawing, 2017).
- Consultant of the bachelor and master thesis of Ilona Krabicová (Příprava tkáňového nosiče pro náhradu nervové tkáně (míšní léze), 2015; Příprava a charakteristika vlákenného nosiče pro náhradu nervové tkáně, 2017).
- Consultant of the master thesis of Šárka Hauzerová (Přípravava a charakteristika polyesterových orientovaných vláken metodou drawing, 2018)

8.3 Research projects

2019 - 2021

Improving Education Quality at the TUL and its Relevance to Labor Market Needs (ESF II, CZ.02.2.69/0.0/0.0/$18_0$56/0013333)

Procurement infrastructure of TUL new study programs
(ERDF II, CZ.02.2.67/0.0/0.0/$18_0$57/0013357)

2018 - 2021

Léčba diabetických ran nanovlákennými kryty uvolňujícími složky lyzátu krevních destiček (AZV: MZČR NV18-01-00332)

2016 - 2018

Aligned Conductive Nanofibers for Tissue Engineering (Czech Science Foundation, 16-02316Y)

2015 - 2016

Nanofibrous biodegradable small diameter vascular grafts – (NV15-21241A)

2015

Centrifugal spinning from a melt and development of scaffold (Student Grant Project no. 21091) Incorporation of active molecules into nanofibrous tissue scaffolds (Student Grant Project no. 21092)

2014 – 2015

Nanofibrous materials for tissue engineering (reg.no. CZ.1.05/3.1.00/14.0308) VaVpI Preseed – feasibility study - 7.3; 3.1 VaVpI MŠMT CR, 2014)

Bibliography

[1] Karina Arcaute et al. In: *Tissue Engineering - Part C: Methods* 17.1 (2010), pp. 27–38.
[2] D D Ateh et al. In: *Journal of the Royal Society Interface* 3 (2006), pp. 741–752.
[3] Anca-Dana Bendrea et al. In: *Journal of Biomaterials Applications* 26 (2011), pp. 3–84.
[4] Charles Vernon Boys. In: *Proceedings of the Physical Society of London* 9.8 (1887), pp. 8–19.
[5] Milada Chudickova et al. In: *Journal of tissue engineering and regenerative medicine* (2015).
[6] Joseph M Corey et al. In: *Journal of Biomedical Materials Research Part A* 83.3 (2007), pp. 636 –654.
[7] Wenguo Cui et al. In: *Science and Technology of Advanced Materials* 11.1 (2010), pp. 1–11.
[8] Ying Dai et al. In: *Plos One* 7.11 (2012), pp. 1–10.
[9] Courtney M Dumont et al. In: *Tissue Engineering: Part A* 24 (2018), pp. 1588–1602.
[10] Courtney M Dumont et al. In: *Acta Biomaterialia* 86 (2019), pp. 312–322.
[11] John M Fonner et al. In: *Biomedical M* 3.3 (2008), pp. 1 –25.
[12] Pierre-Gilles de Gennes et al. Springer, New York, NY, 2004, p. 291.
[13] Santiago Grijalvo et al. In: *Biotechnology Journal* 14.12 (2019), pp. 1–8.
[14] Fuxing Gu et al. In: *Nano Letters* 8.9 (2008), pp. 2757–2761.
[15] Nathalie K Guimard et al. In: *Progress in Polymer Science* 32 (2007), pp. 876–921.
[16] Šárka Hauzerová. PhD thesis. Technical University of Liberec, 2018, p. 81.
[17] Hoda Jahani et al. In: *Journal of Biomedical Materials Research Part A* 5 (), pp. 1875–1881.
[18] A. Koski et al. In: *Materials Letters* 58.3-4 (2004), pp. 493–497.
[19] Ilona Krabicová. PhD thesis. Technical University of Liberec, 2017, p. 77.
[20] J Kriz et al. In: *Spinal Cord* 55.9 (2017), pp. 870–874.
[21] Jae Y Lee et al. In: *Biomaterials* 30.26 (2009), pp. 4325–4335.
[22] Dan Li et al. In: *Nano Letters* 3.8 (2003), pp. 1167–1171.
[23] Jun Li and Guilherme Lepski. In: *BioMed Research International* 2013 (2013), pp. 1 –32.
[24] Shu-liang Liu et al. In: *Journal of Nanomaterials* 2013.1 (2013), pp. 1–9.
[25] Ting Liu et al. In: *Tissue Engineering: Part A* 18.9-10 (2012), pp. 1057–1066.
[26] Kenneth J Livak and Thomas D Schmittgen. In: *Methods* 408 (2001), pp. 402–408.
[27] D. Macaya and M. Spector. In: *Biomedical Materials* 7.1 (2012).
[28] Michal Martínek. PhD thesis. 2014, pp. 1–110.
[29] Dylan A McCreedy et al. In: *Journal of Neuroscience Methods* 263 (2016), pp. 15–22.
[30] Gareth H Mckinley and Tamarapu Sridhar. In: *Annual Review of Fluid Mechanics* 34 (2002), pp. 375–415.
[31] Chao Meng et al. In: *Advanced Materials* 23.33 (2011), pp. 3770–3774.
[32] U Meyer. In: *Fundamentals of tissue engineerning and regenerative medicine* (2009), pp. 1–12.
[33] Andrea J Mothe and Charles H Tator. In: *International Journal of Developmental Neuroscience* 31.7 (2013), pp. 701–713.
[34] JE Murray et al. In: *Surgical forum* 6 (1955), pp. 432–436.
[35] Amrinder S Nain et al. In: *Proceedings of 2005 5th IEEE Conference on Nanotechnology* 1 (2005), pp. 366–369.
[36] Amrinder S Nain et al. In: *IEEE Transaction on Nanotechnology* 5.5 (2006), pp. 499–510.
[37] Amrinder S Nain et al. In: *Applied Physics Letters* 89 (2006), pp. 183103–183105.
[38] Søs Neergaard-Petersen et al. In: *Plos One* 8.8 (2013), pp. 1–8.
[39] Liudmila N Novikova et al. In: *Biomaterials* 29 (2008), pp. 1198–1206.
[40] Fergal J O'Brien. In: *Materials Today* 14.3 (2011), pp. 88–95.
[41] Thierry Ondarcuhu and Christian Joachim. In: *Europhysics Letters* 42.2 (1998), pp. 215–220.
[42] Jonghyuck Park et al. In: *Journal of Controlled Release* July (), pp. 88–101.
[43] Kiran Pawar et al. In: *Biomaterials* (), pp. 1–12.
[44] Molamma P Prabhakaran et al. In: *Nanotechnology* 19 (2008), pp. 1 –8.
[45] Rajeswari Ravichandran et al. In: *Journal of the Royal Society Interface* 7 (2010), S559 –S579.
[46] Darrell H. Reneker and Alexander L. Yarin. In: *Polymer* 49.10 (2008), pp. 2387–2425.
[47] Jonas C Rose et al. In: *Biomaterials* 163 (2018), pp. 128–141.
[48] Dominique R Smith et al. In: *Biotechnology and Bioengineering* 1 (), pp. 155–167.
[49] Benjamin Spearman et al. In: *ACTA BIOMATERIALIA* 28 (2015), pp. 109 –120.
[50] Elise Stewatr et al. In: *Tissue Engineering: Part C* 4 (), pp. 385–393.
[51] K. Strnadová et al. In: *Journal of Industrial Textiles* 50.3 (2020).
[52] Piotr K Szewczyk et al. In: *Polymers* 11.1 (2018).
[53] Achilleas D Theocharis et al. In: *Advanced Drug Delivery Reviews* 97 (2016), pp. 4–27.

[54] Aline Thomas et al. In: *Biomaterials* 34.9 (2013), pp. 2213–2220.
[55] Arjun Prasad Tiwari et al. In: *Applied Materials Interfaces* 24 (), pp. 20256 –20270.
[56] Alexander Tokarev et al. In: *Advanced Materials* 27.41 (2015), pp. 6526–6532.
[57] Hannah M Tuinstra et al. In: *Biomaterials* 33.5 (2013), pp. 1618–1626.
[58] Charles A Vacanti. In: *Journal of Cellular and Molecular Medicine* 10.3 (2006), pp. 569–576.
[59] Joseph P Vacanti et al. In: *Journal of Pediatric Surgery* 23.1 (1988), pp. 3–9.
[60] Reaz Vawda et al. In: *Stemm Cells Translational Medicine* (), pp. 1–12.
[61] T V Vernitskaya and O N Efimov. In: *Russian Chemical Reviews* 66.5 (1997), pp. 443–457.
[62] Bing Han Wang et al. In: *Acta Biomaterialia* 6.8 (2010), pp. 2970–2978.
[63] Xioadong Wang et al. In: *Journal of Biomedical Materials Research Part A* 68 (2004), pp. 411 –422.
[64] *Web of Science [v.5.34] - Web of Science Core Collection Result Analysis*. 2020.
[65] R L Williams and P J Doherty. In: *Journal of Materials Science: Materials in Medicine* 5.6 - 7 (1994), pp. 429–433.
[66] World Health Organization. *Spinal Cord Injury*. 2013.
[67] Xiaobo Xing et al. In: *Optics Express* 16.14 (2008), pp. 10815–10822.
[68] Anneng Yang et al. In: *Colloids and Surfaces B: Biointerfaces* 134.24 (2015), pp. 469–474.
[69] F Yang et al. In: *Biomaterials* 25.10 (2004), pp. 1891–1900.
[70] Quing Yang et al. In: *Journal of Appl* 110 (2008), pp. 1080–1084.
[71] Yang Yang et al. In: *Tissue Engineering: Part A* 15.11 (2009), pp. 3283–3295.
[72] Alexander L Yarin et al. 1st. Cambridge: Cambridge University Press, 2014.
[73] Xiaofeng Ye et al. In: *Plos One* 8.1 (2013).
[74] Daniel W Youngstrom et al. In: *Plos One* 8.5 (2013).
[75] Hanwen Yuan et al. In: *Journal of Visualized Experiments* (), pp. 1–10.
[76] Jingwen Zeng et al. In: *Colloids and Surfaces B: Biointerfaces* 110 (2013), pp. 450–457.
[77] Shu Xin Zhang et al. In: *Neural Regeneration Research* 8.2 (2013), pp. 177–185.
[78] Andrzej Ziabicki. 1st. Chichester, United Kingdom: John Wiley and Sons Ltd, 1976, p. 504.
[79] E. Zussman et al. In: *Applied Physics Letters* 82.22 (2003), pp. 3958–3960.

Curriculum Vitae

Name	**Mgr. Kateřina Strnadová**
Address	Holého 936/10, 46001 Liberec
Date of birth	1. 4. 1987
Email	katerina.pilarova@gmail.com
Maiden name	Pilařová

Education and courses

Education

2014 - till now	Technical University of Liberec, Faculty of Textile Engineering, Department of Nonwovens and Nanofibrous Materials (postgraduate study)
2012 - 2013	Charles University in Prague, Faculty of Science, Institute for Environmental Studies, research conducted at the Czech Academy of Science, Institute of Microbiology (postgraduate study – not finished)
2010 - 2012	Charles University in Prague, Faculty of Science, Department of Parasitology (graduate studies)
2006 - 2010	Charles University in Prague, Faculty of Science, Biology (undergraduate studies)

Courses and Internships

2016 - 2017	Fulbright Scholarship – University of Michigan – 10 months (supervisor prof. Lonie Shea, ldshea@umich.edu) (Drawing: A Tool for Fabrication of Scaffolds for Neural Tissue Engineering)
2014	Research internship at Michigan Technological University, Department of Biomedical Engineering, USA, Michigan – 1 month (supervisor Dr. Jeremy Goldman, jgoldman@mtu.edu)
2010	Course of Real – Time PCR, Applied Biosystems, Czech Republic
2006	Deutsches Sprachdiplom – Zweite Stufe

Professional Practice

2014 - till now	Researcher at the Technical University of Liberec, Faculty of Textile Engineering, Department of Nonwovens and Nanofibrous Materials
2013 - 2014	Administration of the register of medicaments in the expert system of veterinary practice "webVet" (Netsystem int. a.s., RNDr. Zdeněk Strnad, zstrnad@netsystemas.com)
2012 - 2013	Researcher in the Laboratory of Environmental Biotechnology, Institute of Microbiology, Czech Academy of Science (supervisor doc. Tomáš Cajthaml, PhD., cajthaml@biomed.cas.cz)
2010 - 2011	Laboratory technician at Jana Evangelisty Purkyně University in Ústí nad Labem, Faculty of Environment, Department of Technical Science (at Ing. Josef Trögl, Ph.D., josef.trogl@ujep.cz)
2006 - 2012	Researcher in the Laboratory of Molecular and Biochemical Parasitology, Department of Parasitology at Charles University, Prague (supervisor doc. RNDr. Ivan Hrdý, Ph.D., hrdy@cesnet.cz)

Personal Skills and Competences

English	advanced
German	intermediate
Professional Programs	NIS Elements, GraphPadPrism, ImageJ, Clustal X Multiple Sequence Alignment, BioEdit Sequence Alignment Editor, programs of DNASTAR (SeqMan, EditSeq)
Laboratory techniques	drawing technology – fabrication of fibrous scaffolds for tissue engineering, SEM, fluorescence microscopy, rheometry, molecular biology and biochemistry (PCR, qRT-PCR, design of primers, isolation of RNA by TRIZOL and reverse tanscription to cDNA, cloning of a gene construct into vector, SDS and NATIVE PAGE, western blotting, 2D PAGE, imunofluorescent dying, affinity purification of enzymes, FPLC, measuring of protein concentration by Lowry and Bradford assay, spectrofotometric measurement of proteins activities, isolation of organelles by gradient centrifugation), practice in tissue culture lab, practice in vivarium and with laboratory animals, histology, histological staining

Recommendation of the supervisor

TECHNICKÁ UNIVERZITA V LIBERCI
Fakulta přírodovědně-humanitní
a pedagogická

Disertační práce: **DRAWING: Fabrication of Scaffolds for Neural Tissue Engineering**

Autorka: **Mgr. Kateřina Strnadová**

Hodnocení školitele

Disertační práce Mgr. Kateřiny Strnadové „DRAWING: příprava materiálů vhodných pro regeneraci nervové tkáně" se zabývá vývojem materiálu vhodného pro léčbu míšních poranění. Regenerace nervové tkáně je velkou výzvou regenerativní medicíny a v současné době není dostupná žádná efektivní léčba. Pro funkčnost nervové tkáně je zásadním faktorem organizace mezibuněčné hmoty a nervových buněk. Podstatou předkládané disertační práce je vývoj materiálu založeného na jedinečných vlastnostech orientovaných mikrovláken, které napodobují morfologii mezibuněčné hmoty míšní tkáně. Pro přípravu vláken s definovanými geometrickými vlastnostmi použila doktorandka technologii drawing. Jedná se o mechanické tažení jednotlivých vláken z kapky polymerního roztoku. Orientovaná vlákna byla připravována zejména z degradabilních polyesterů, konkrétně polykaprolaktonu (PCL) a kopolymeru kaprolaktonu s kyselinou mléčnou (PLCL). Pro přípravu vláken byl použit manipulátor umožňující nastavení parametrů a tažení vláken za stálých podmínek. Připravená vlákna byla dále funkcionalizována pomocí vodivého polypyrrolu (PPY), který má v regeneraci nervové tkáně rovněž potenciál. Výše zmíněné scaffoldy byly dále použity pro přípravu kompozitních míšních můstků. Laboratorně vyrobené materiály byly charakterizovány z hlediska jejich fyzikálně-chemických vlastností. Následné *in-vitro* testy prokázaly stimulaci orientovaného růstu buněk vlivem struktury scaffoldu a také další zvýšení biokompatibility vlivem funkcionalizace PPY. *In vitro* a následné *in vivo* testy kompozitních míšních můstků obsahujících orientovaná mikrovlákna a mikrovlákna funkcionalizovaná PPY prokázaly podporu růstu a orientaci axonů a jejich myelinizaci. Výsledky zároveň ukazují, že materiál je neimunogenní, přičemž ale dochází k aktivaci imunitní reakce zodpovědné za procesy hojení v těle. Práce byla realizována na Technické univerzitě v Liberci a v průběhu 10ti měsíční stáže na University of Michigan.

Téma disertační práce Mgr. Kateřiny Strnadové je multidisciplinární a metodicky velmi náročné. Doktorandka prokázala nevšední schopnost zvládnutí techniky samotné přípravy vláken, jejich charakteristiky vč. hodnocení morfologie, vodivosti a mechanických vlastností a zároveň biologické testování zahrnující *in vitro* experimenty s několika druhy buněčných linií a *in-vivo* experimenty na myších. V rámci své práce hledá souvislosti mezi fyzikálně-chemickými vlastnostmi orientovaných mikrovláken a chováním těchto materiálů v rámci *in-vitro* a *in-vivo* testů. Na disertační práci pracovala velmi svědomitě, oceňuji nevšední pracovní nasazení, nadšení a invenci, stejně jako schopnost vyvozovat logické závěry na základě naměřených dat. V rámci práce nejprve hledala vhodné parametry a polymery pro přípravu orientovaných mikrovláken, vedle zmíněných PCL a PLCL je dále vhodným polymerem např. i vodorozpustný polyvinylalkohol (PVA). Na základě následných testů F-CH vlastností materiálů a spolu s *in-vitro* hodnocením identifikovala parametry vláken vhodné pro stimulaci orientovaného růstu buněk. Pro další zvýšení biokompatibility materiálů zvolila doktorandka funkcionalizaci vodivým polypyrrolem, kdy je modifikován povrch vláken. Pozitivní vliv funkcionalizace následně potvrdila *in-vitro*. Při pobytu v USA měla možnost testovat chování materiálů *in-vitro* s nervovými buňkami. Tyto testy prokázaly, že rovněž nervové buňky preferují růst podél orientovaných vláken, přičemž efekt je patrnější u funkcionalizovaných vláken. Na základě těchto výsledků navrhla kompozitní scaffold obsahující orientovaná vlákna, který následně testovala *in-vivo* na myším modelu. Implantace do oblastí po laterální hemisekci míchy prokázaly stimulaci infiltrace axonů do implantovaných můstků, a to vlivem přítomnosti orientovaných vláken.

Kladně hodnotím i publikační činnost Mgr. Kateřiny Strnadové, která čítá v databázi WOS k dnešnímu datu 5 publikací v impaktovaném časopise a 5 konferenčních příspěvků. Její H-index je 4, počet citací 71. V neposlední řadě také oceňuji vedení bakalářů a diplomantů a podíl na realizaci řady výzkumných projektů.

Doporučuji, aby disertační práce Mgr. Kateřiny Strnadové byla přijata k obhajobě

Školitel: Ing. Věra Jenčová, Ph.D. V Liberci 10.5.2021

Opponents' reviews


UNIVERZITA KARLOVA – LÉKAŘSKÁ FAKULTA V PLZNI

Spectabilis
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Číslo jednací TUL – 21/4814/021445

Oponentní posudek

na dizertační práci paní **Mgr. Kateřiny Strnadové,** doktorandky v oboru Textilní technika a materiálové inženýrství, na téma **„Drawing: příprava scaffoldů pro regenerace nervové tkáně".**

Doktorandka předložila anglicky psanou dizertační práci o 119 stranách, v níž zkoumá složení, přípravu, úpravy a uplatnění tkáňových nosičů založených na tažených vláknech.

a) Význam dizertační práce pro obor: Práce si klade za cíl vyvinout a optimalizovat vlastnosti tkáňového nosiče založeného na vhodném uspořádání tažených vláken tak, aby byl použitelný k překlenutí míšního defektu. Podpora růstu neuronů a jejich výběžků se zdá být velmi perspektivním směrem, který nastupuje po určitém vystřízlivění z nadějí vkládaných v minulosti do terapie poškození nervového systému pomocí kmenových buněk. Jde o práci mezioborovou v příkladném a nejlepším slova smyslu. V oboru textilní techniky a materiálového inženýrství přináší pokrok v přípravě a cílené úpravě nosičů. V oboru experimentální biologie nervové tkáně je přínosem překlenutí zcela zásadní mezery oddělující *in vitro* testy s *in vivo* implantací do zvířecího modelu. Jde o vysoce aktuální a potřebný výzkum cílící do oblasti, kde je nedostatek dobře podložených a interpretovatelných experimentálních studií.

b) Postup řešení problému a použité metody: Po historickém úvodu formuluje uchazečka výše zmíněné cíle. Poté mapuje současný stav poznání v podobě velmi čtivě a srozumitelně zpracované literární rešerše, v níž mapuje uplatnění tkáňového inženýrství u míšních poranění, typy tkáňových nosičů vhodné pro tyto účely, jejich materiálové složení, funkcionalizaci a měřitelné vlastnosti. Metodická část je psána velmi podrobně a styl podání protokolů prozrazuje značnou erudici uchazečky. Spektrum postupů plně pokrývá řetězec od výchozích chemikálií, přes technologii přípravy vláken tažením, uspořádání vláken, popis vláken nástroji obrazové analýzy, mechanické testování vláken, měření smáčivosti a vodivosti, až k *in vitro* testům, *in vivo* implantaci do míšní hemisekce, RNA izolaci, syntézu cDNA, qRT-PCR, imunohistochemii a

mikroskopické techniky. Vysoká metodická úroveň umožňuje opakovatelnost práce a podporuje validitu touto cestou získaných výsledků.

c) Splnění cílů práce: Z výsledkové části a ze závěrů vyplývá, že práce splnila cíle formulované v kapitole 1.

d) Výsledky dizertace, nové poznatky a přínos autorky: V kapitole 5 je formulováno celkem 13 zjištění, která jsou opodstatněna výsledky a diskuzí z kapitol 3-4. Spatřuji tyto hlavní přínosy práce:

- Popis vlivů koncentrace polymeru, molekulární hmotnost a rychlosti tažení na průměr vláken a na vznik porézních defektů v nich.
- Metodická upřesnění při měření mechanických vlastností a vodivosti.
- *In vitro* popis adheze, proliferace, orientace buněk a biokompatibility na orientovaných a potažených vláknech.
- Přípravu a *in vivo* otestování nového typu můstku pro překlenutí míšního defektu u myši s vyhodnocením růstu výběžků, apoptózy, imunotolerance a myelinizace.

Míru přímého podílu autorky nenacházím výslovně uvedenou a mohla by být upřesněna během vlastní obhajoby. Způsob, jakým je práce sepsána, však svědčí o významném zapojení do většiny činností. Doktorandka prokázala svoje znalosti při formulaci vědeckého problému na základě kritického rozboru současné úrovně vědeckého poznání. Prokázala schopnost plánovat, provést a vyhodnotit vhodně navržené experimenty v účelném uspořádání. Doktorandka je schopna vědecké práce se získáním konkrétních výstupů, je schopna výsledky kriticky interpretovat a z těchto výsledků vyvodit odpovídající závěry. Celou práci je schopna shrnout do srozumitelně psaného a logicky provázaného odborného sdělení. Autorka uplatnila v diskusi velmi vyváženou argumentaci a rovněž schopnost pojmenovat nově vyvstalé otázky a navrhnout jejich zodpovězení během další práce v jejím oboru. To vše považuji za součást vizitky zralého a samostatného vědeckého pracovníka.

e) Vyjádření k publikacím uchazečky: Součástí dizertace nejsou plné texty vlastních publikací, ani autorčina úplná bibliografie. Předpokládám doložení těchto informací před komisí pro obhajoby. Z veřejných zdrojů (Web of Science, ResearchGate) lze dohledat tři recenzované publikace, které spadají do tématu práce a kde je uchazečka spoluautorkou – ve všech případech jde o časopisy s vysokým rankingem (Polymers, Q1 dle JCR; dvakrát v Materials Letters, Q2). Dále je k dohledání spoluautorství publikace z jiného oblasti (Antimicrobial Agents and Chemotherapy, Q1).

f) Formální zpracování a jazyková úroveň: Práce je přehledně členěna, jednotlivé oddíly jsou dobře logicky provázány. Vysoce hodnotím srozumitelnost textu, práce se dobře čte. Fotodokumentace je vynikající a velmi informativně popsaná. Grafická úprava je profesionální, nechybí seznamy ilustrací a poznámkový aparát. Překlepy se prakticky nevyskytují (str. 50 „permeabilizedin"). K formálnímu zpracování snad jen drobná doporučení:

- Obvyklé typografické konvence v angličtině nepoužívají mezeru mezi číselnou hodnotou a znakem %.
- V některých obrázcích a grafech zůstaly české desetinné čárky namísto v angličtině používaných teček (Fig. 4.3., 4.4).

g) Připomínky a dotazy: Díky obsažnosti a mezioborovému přesahu práce je otázek, které se nabízejí, velmi mnoho a doktorandka si některých z nich sama všímá v kapitole věnované diskuzi. Kritické připomínky či výhrady nenacházím. K diskuzi při obhajobě si však dovolím položit uchazečce následující dotazy:

- Lze z dat Vašeho *in vivo* experimentu usoudit alespoň přibližně na rychlost regenerace nervových výběžků?
- V některých skupinách Vašeho experimentu je počet jedinců n=4 příliš nízký pro uplatnění konvenčních statistických testů, které většinou vyžadují n>=6. Vidíte v tom překážku pro publikaci výsledků?
- Jaké známky degradace jevila vlákna po 8 týdnech *in vivo*? Nebo byla zcela rozpuštěna při histologickém zpracování? Jaká je očekávaná životnost Vámi připravených vláken *in vivo*? Jaká je optimální životnost vláken pro hojení defektů nervového systému dříve, než degradují?
- Vámi připravované nosiče jsou vhodné k implantaci do poranění s ostrými konci (míšní hemisekce se ztrátou míšního segmentu). Můžete stručně diskutovat uplatnění u poranění s tupým pohmožděním nervových struktur?
- Vidíte nějaké rozdíly v přípravě nosičů pro překlenutí míšních defektů v porovnání s nosičem pro podporu hojení poranění periferních nervů?
- Jak sama uvádíte na str. 6, Schwannovy buňky obalují nervové výběžky, popř. tvoří myelin v periferním nervovém systému. V centrálním nervovém systému, kam patří i Vámi zkoumaná mícha, jsou obaly nervových vláken tvořeny výběžky astrocytů a myelin je tvořen oligodendrocyty. Proto působí překvapivě, když popisujete u Vašeho experimentu v míše „Schwann cell-derived myelin"? Schwannovy buňky se v míše samotné přirozeně nevyskytují, byť se někdy experimentálně transplantují při výzkumu léčby míšních lézí (to ale patrně nebyl případ Vašeho experimentu), popř. se vyskytují v míšních kořenech.
- Anatomická poznámka: Vaše práce se soustředí na podporu regenerace bílé hmoty míšní, tj. míšních drah/traktů. Ke způsobu hojení odstraněné šedé hmoty míšní se nevyjadřujete. Je ke zvážení toto zohlednit a nehovořit o regeneraci míchy jako celku.
- Experimentálním u výzkumu regenerace míšních lézí u zvířecího modelu se na vysoké úrovni věnují na Anatomickém ústavu MU v Brně (doc. Marek Joukal, práce např. Pubmed PMID 30927067, 29740961, 30778286). Vidíte nějaký potenciál spolupráce v možném testování Vašich tkáňových nosičů?

h) Závěr a doporučení: Z předložené práce vyplývá, že doktorandka **prokázala schopnost samostatné a tvůrčí činnosti** v oblasti **výzkumu a vědecké práce**. Dizertační práce **splňuje požadavky kladené na doktorskou dizertaci** zákonem č. 111/1998 Sb. o vysokých školách v platném znění. Proto ve smyslu § 47 uvedeného zákona **doporučuji komisi** její přijetí v předložené podobě jako podklad k obhajobě. Za předpokladu úspěšné obhajoby **doporučuji**, aby paní **Mgr Kateřině Strnadové** byl udělen akademický titul „doktor" (Ph.D.) v příslušném oboru podle § 47 Zákona o vysokých školách č. 111/98 Sb.

V Plzni dne 26.7.2021

prof. MUDr. Mgr. Zbyněk Tonar. Ph.D.

Oponentský posudek disertační práce Mgr. Kateřiny Strnadové „Drawing:Fabrication of Scaffolds forNeural Tissue Engineering“

Doktorská práce **Mgr. Kateřiny Strnadové** patří svým zaměřením do rychle se rozvíjejícího oboru tkáňové inženýrství. Zabývá se přípravou vhodných tkáňových náhrad využitelných zejména pro léčbu poškozených nervových tkání, kde organizace mezibuněčné hmoty má prioritní význam pro jejich funkčnost.
O zodpovědné přístupu disertantky k řešené problematice svědčí nejen přehledně zpravovaná literární část vycházející z dostupných recentních publikací, ale i rozsáhlá experimentální část, která pokrývá laboratorní přípravu cílových vláken a dále jejich úpravu a biologické testování
Vlastní práce je koncipována klasicky tj. bez kombinace textu s publikacemi disertantky, což je dle mého názoru časově náročnější, zato však pro hodnotitele přehlednější.
Úvodní kapitola má přiměřený počet stran, jak již bylo uvedeno, je přehledná a vychází z dosud publikovaných studií týkající se dané problematiky. Disertantka popisuje základy, metody a cíle tkáňového inženýrství s důrazem na léčební postupy nervových tkání. V seznamu literatury je uvedeno kolem 200 literárních zdrojů využitých jak v literárním úvodu, tak v metodice a diskuzi. Tento počet svědčí i tom, že Mgr. Strnadová věnovala náležitou pozornost jak teoretické přípravě, tak i experimentální části a jejímu vyhodnocení.

Experimentální část je poměrně rozsáhlá a jsou v ní uvedeny všechny relevantní informace o použitých chemikálií, buňkách, přístrojovém vybavení a použitých metodických postupech. Pro přípravu vhodně orientovaných vláken využitelných pro vývoj tkáňových nervových náhrad, použila doktorandka nekomerční, méně často používanou drawing metodu (vlákno tažené z kapky). Navrhla a podílela se na konstrukci laboratorního přístroje a optimalizaci podmínek přípravy cílových vláken z roztoku polyméru, včetně měření namáhání připravených vláken v tahu, jejich vodivosti. Vláknová struktura byla po té funkcionalizovaná polypyrrolem zajišťujícím stabilitu připravené tkáňové struktury. Vedle *in vitro* měření byl sledován *in vivo* vývoj, konstrukce a charakterizace takto připraveného nosiče (scafoldu) buněk.
Ve všech částech práce je text doplněn vhodnými obrázky, grafy a názornými fotografiemi pořízenými při hodnocení vzorků SEM.
Výsledky a diskuze jsou spojené do jedné části (35 stran), po níž následuje kapitola Závěry (4 str.), která přehledně sumarizuje významné výsledky. I když já sama preferuji ve vědeckých pojednáních výsledky oddělené od diskuze, musím konstatovat, že v tomto případě je toto spojení plně funkční. Troufám si zařadit disertaci Mgr. Strnadové mezi nejlepší práce z těch, co jsem kdy hodnotila.
Pokud mohu posoudit, práce je psána v anglickém jazyce velmi dobré úrovně. Po formální stránce jsem našla pouze několik málo překlepů a v literatuře např. dvakrát citovaný článek samotné disertantky. Naopak jsem velmi ocenila barevně odlišené údaje roku vydání publikace, které umožňuje rychlou orientaci v použité literatuře v textu. K vlastní odborné práci nemám žádné konkrétní připomínky, ale mám dva příspěvky do diskuze.

V tab. 2.5 a 2.6 jsou uvedeny konduktivní polymery a jejich charakteristika. Proč pro vlastní práci disertantka vybrala právě polypyrrol? Na základě vlastních zkušeností či z literárních údajů?

Dle uvedených výsledků v disertační práci lze usoudit, že vyvinutý postup přípravy tkáňových náhrad má perspektivní využití. Zajímalo by mě, zda bude práce na této problematice pokračovat a jaká je perspektiva zavedení navržených postupů do léčebných procesů.

Při závěrečném hodnocení doktorské disertační práce Mgr. Strnadové bych ráda poukázala i na její aktivní publikační činnost. Ve WOS jsou uvedeny čtyři impaktované publikace (3xQ1, 1xQ2). V jedné z nich je disertantka první autorkou, a u ostatních spoluautorkou. Výsledky byly též prezentovány na vědeckých setkáních, což také svědčí o jejich aktuálnosti v oboru. Disertantka jednoznačně prokázala schopnost vědecky pracovat a dosažené výsledky zpracovat do publikovatelné formy.

Na závěr svého posudku konstatuji, že se Mgr. Kateřina Strnadové zhostila svého úkolu s velkým nasazením a jsem přesvědčena o tom, že její disertační práce splňuje veškeré požadavky vyplývající z *§ 27, odst. 4, zákona č.111/1998 Sb. O vysokých školách*, a proto ji doporučuji přijmout k obhajobě a po jejím úspěšném obhájení doporučuji udělení akademického titulu Ph.D.

V Praze dne 24.8. 2021.

Prof. Ing. Kateřina Demnerová, CSc.