

# We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index  
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?  
Contact [book.department@intechopen.com](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



---

# Nanofibrous Scaffolds as Promising Cell Carriers for Tissue Engineering

---

Lucie Bacakova, Marketa Bacakova, Julia Pajorova,  
Radmila Kudlackova, Lubica Stankova, Elena Filova,  
Jana Musilkova, Stepan Potocky and  
Alexander Kromka

Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/63707>

---

## Abstract

Nanofibers are promising cell carriers for tissue engineering of a variety of tissues and organs in the human organism. They have been experimentally used for reconstruction of tissues of cardiovascular, respiratory, digestive, urinary, nervous and musculoskeletal systems. Nanofibers are also promising for drug and gene delivery, construction of biosensors and biostimulators, and wound dressings. Nanofibers can be created from a wide range of natural polymers or synthetic biostable and biodegradable polymers. For hard tissue engineering, polymeric nanofibers can be reinforced with various ceramic, metal-based or carbon-based nanoparticles, or created directly from hard materials. The nanofibrous scaffolds can be loaded with various bioactive molecules, such as growth, differentiation and angiogenic factors, or functionalized with ligands for the cell adhesion receptors. This review also includes our experience in skin tissue engineering using nanofibers fabricated from polycaprolactone and its copolymer with polylactide, cellulose acetate, and particularly from polylactide nanofibers modified by plasma activation and fibrin coating. In addition, we studied the interaction of human bone-derived cells with nanofibrous scaffolds loaded with hydroxyapatite or diamond nanoparticles. We also created novel nanofibers based on diamond deposition on a SiO<sub>2</sub> template, and tested their effects on the adhesion, viability and growth of human vascular endothelial cells.

**Keywords:** nanofibers, nanoparticles, natural polymers, synthetic polymers, ceramics, carbon, diamond, biomaterial, biocompatibility, tissue engineering, tissue regeneration, nanomedicine, drug delivery, gene delivery, wound healing

## 1. Introduction

In recent years, nanofibrous materials are becoming more and more popular for tissue engineering applications, because they mimic nanofibrous components of the native extracellular matrix (ECM), for example, collagen fibers. Nanofibers are also widely used in other biotechnologies, such as drug and gene delivery [1–5], gene silencing [6], construction of biosensors [7, 8], or preparation of wound dressings absorbing the exudate and protective against microbial infection [9, 10].

Nanofibers are typically prepared from polymeric materials, such as natural and synthetic polymers and their various combinations. Nature-derived polymers comprise a wide range of proteins, peptides, and polysaccharides, for example collagen [11, 12], elastin [13] and elastin-like peptides [14], silk fibroin [15, 16], amyloid [3], chitosan [12, 17], cellulose [10, 18], glycosaminoglycans [11, 12], or hyaluronan [19, 20]. Even demineralized bone matrix (DBM) was used for preparation of nanofibers by electrospinning [21]. In fact, DBM, a natural polymer, is allograft bone with inorganic material removed. DBM contains the protein components of bone, which includes adhesion ligands and osteoinductive signals, such as important growth factors. The DBM nanofiber mats exhibited good cytocompatibility with human dermal fibroblasts [21].

Synthetic polymers include a broad spectrum of biostable polymers, for example polyethylene terephthalate [9], polytetrafluoroethylene [22] and polyurethane, suitable for fabrication of vascular grafts [23], and biodegradable polymers, for example polylactides (PLA) [24, 25] and their copolymers with polyglycolides (PLGA) [1, 26], polycaprolactone (PCL) [2, 4, 6], poly-3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV) [27], polydioxanone [5], polyvinylalcohol (PVA) [28, 29], and synthetic peptides [7, 30].

However, pure polymeric nanofibers are suitable mainly for soft tissue engineering, such as reconstruction and regeneration of blood vessels [13, 22, 23, 31, 32], myocardium [33, 34], heart valves [35, 36], skeletal muscle [37, 38], skin [15, 39–41], tendon and ligament [42, 43], intestine [44, 45], tissues of the respiratory system, such as trachea and bronchi [46, 47], components of urinary tract, such as bladder [48] and urethra [49], visceral organs, such as liver [50, 51] or pancreas (pancreatic islets [52, 53]), central nervous system, such as brain [6, 54, 55], spinal cord [56, 57], optic system, such as optical nerve [58] and retina [59], and peripheral nervous system [17, 60]. Nanofibrous scaffolds can be associated with another advanced technique in recent tissue engineering—controlled delivery of various types of stem cells, such as bone marrow mesenchymal stem cells [51, 61–63], adipose tissue-derived stem cells [64, 65], neural tissue-derived stem cells [57], and induced pluripotent stem cells [20, 34], and their appropriate differentiation into desired cell types.

For hard tissue engineering, that is for reconstruction of bone, teeth, cartilage, and osteochondral interface, it is necessary to improve mechanical properties of the nanofibers. This is feasible by addition of inorganic nanoparticles into nanofibers, such as ceramic nanoparticles, for example hydroxyapatite [16, 27, 28, 30], tricalcium phosphate [63, 66], calcium oxide [67], or calcium silicate [24]; metal-based nanoparticles, for example gold nanoparticles [29], ferro-

magnetic Fe<sub>3</sub>O<sub>4</sub> nanoparticles [25], or antimicrobial silver nanoparticles [26]; and also carbon-based nanoparticles, such as carbon nanotubes, graphene [62], or nanodiamonds [68]. These nanoparticles not only reinforce the polymeric nanofibers but also enhance their bioactivity in terms of increased cell adhesion, growth, osteogenic cell differentiation, bone matrix mineralization and antimicrobial activity. The mineral component can also be added to the nanofibers by biomimetic mineralization in simulated body fluid [69] and other ionic solutions [70]. Nanofibers can also be created exclusively from inorganic or other hard materials, for example hydroxyapatite [71, 72] or carbon and bioactive glass [73], and also from SiO<sub>2</sub> [74, 75] and diamond [76, 77] or their combinations [78, 79].

On the other hand, carbon nanoparticles (mainly carbon nanotubes) have been also added into nanofibers for soft tissue engineering in order to allow electrical stimulation of cells or delivery of drug and other bioactive molecules to the cells. The electrical stimulation is suitable especially for cells of excitable tissues, such as neural tissue [80] (for a review, see [81]), myocardium [82], skeletal muscle [38], and vascular smooth muscle [83]. The addition of carbon nanoparticles also improved the mechanical properties of the nanofibrous scaffolds for engineering of muscular tissues, which are exposed to a relatively high mechanical loading in the organism. For the purpose of electrical stimulation of cells, nanofibers can be also coated with polypyrrole [55] or directly made of this polymer [58].

Nanofibers can be further loaded with various biomolecules in order to achieve their specific effects on cells, for example, with growth factors such as basic fibroblast growth factor and epidermal growth factor [12, 40, 42, 84], with angiogenic factors, such as vascular endothelial growth factor or platelet-derived growth factor [52, 85], with differentiation factors, such as bone morphogenetic protein-2 (BMP-2) [18] or with brain-derived neurotrophic factor [54]. Other bioactive agents include ascorbic acid, promoting the production of collagen by cells [86, 87], glutamate for neural tissue engineering [56], vitamin E and polyphenols (e.g., curcumin and green-tea polyphenols), that are natural compounds with excellent antioxidant, anticancer, antimicrobial, anti-inflammatory and wound-healing properties [88, 89], hormones and their analogues (estradiol [10], dexamethasone [89]), honey [9], and propolis [90]. The cell adhesion and growth on nanofibrous scaffolds can be enhanced by their functionalization with ligands for cell adhesion receptors, for example RGD-containing oligopeptides [20, 30, 39].

Nanofibers can be prepared by various techniques, for example self-assembly (silk-elastin-like protein polymers [14]), interfacial polymerization, suitable for electrically conductive materials [8], melt processing [91], and antisolvent precipitation [92]. The latter two methods are suitable for preparation of porous nanofibers for loading various substances. However, the most effective method for large-scale production of nanofibers is electrospinning, particularly needleless electrospinning [93]. Polymer composites produced via the needleless electrospinning allow a polymer nanofiber to act as a host for nanoparticles, and, in addition, the polymer nanofibers will act as a three-dimensional carrier for cells imitating natural extracellular matrix.

This chapter comprises our experience in using nanofibers for experimental soft and hard tissue engineering in correlation with studies of other authors in recent years. We have focused particularly on *skin tissue engineering* using polymeric nanofibers made of PLA, PCL, PLA/PCL

or cellulose, and further modified with plasma or coated with fibrin and on *bone tissue engineering* using polymeric nanofibers loaded with hydroxyapatite or diamond nanoparticles, or SiO<sub>2</sub> nanofibers coated with nanodiamond.

## 2. Nanofibers in skin tissue engineering

The skin is composed of three main layers—epidermis, dermis, and hypodermis. The epidermis, the outermost layer, consists mainly of keratinocytes (more than 90% of all cell types) but it also contains subpopulations of melanocytes, Langerhans cells, and Merkel cells. The keratinocytes produce many important molecules, such as growth factor and protective immunogenic molecules. These molecules include interleukins, transforming growth factors  $\alpha$  and  $\beta$ , platelet-derived growth factor, fibroblast growth factor, tumor necrosis factor  $\alpha$ , and interferons  $\alpha$  and  $\beta$ . The melanocytes produce the pigment melanin that protects the skin against harmful effect of sunlight. The Langerhans cells, that is a type of leucocytes, are responsible for immune activation. The function of Merkel cells is not yet clearly elucidated yet but they probably occasionally participate in formation of synaptic junction with peripheral nerves, and in low-vertebrates, they participate in slow-adapting touch perception [94].

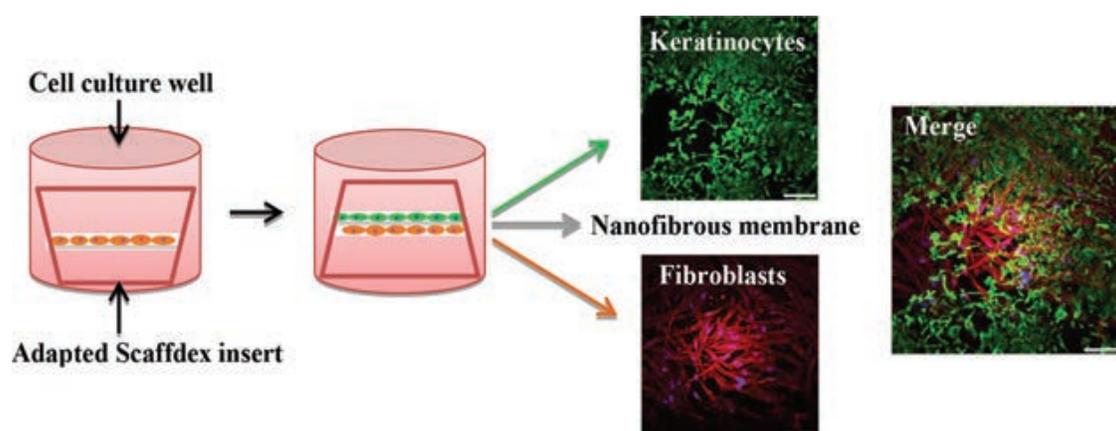
The epidermal cells form five sublayers: *stratum basale*, *stratum spinosum*, *stratum granulosum*, *stratum lucidum*, and *stratum corneum*. New cells are created in the deepest basal layer as stem cells, and then, they differentiate and mature in adult keratinocytes and move towards the skin surface. As keratinocytes mature and ascend toward the epidermal layers, their shapes become flattened, and these cells synthesize structural protein keratin. The outermost cornified layer is created by dead keratinocytes rich in protein keratin. The importance of keratinization is creating a barrier to prevent fluid loss and unwanted entry of potentially harmful molecules and microorganisms.

Dermis, situated below the epidermis, is responsible for elasticity and mechanical integrity of the skin, cutaneous nutrition, immunosurveillance, sensory perception, and temperature regulation. The main cellular type of dermis are fibroblasts that are responsible for synthetic and degradation of dermal proteins. The other cells included in the dermis are endothelial cells, smooth muscle cells and immune cells (dendritic cells, monocytes, and lymphocytes). The dermis also contains nerves, vessels, sweat glands, and hair follicles [94].

Hypodermis, the undermost layer, is mainly composed of adipose tissue and collagen and acts as a fat storage, an energy source, and enables an anchorage of the skin to bone or muscle [94].

Skin tissue engineering is focused mainly on the reconstruction of epidermis and dermis using a biomaterial scaffold (as cell carrier) and two main epidermal and dermal cell types, that are keratinocytes and fibroblasts, respectively. Nanofibrous meshes can be advantageously used for creating a bilayered epidermal–dermal construct containing keratinocytes and fibroblasts located on the opposite sides of the membrane. The keratinocytes and fibroblasts could communicate physically and biochemically through the pores in the membrane, if the membrane is of appropriate thickness and pore size. The crosstalk between the keratinocytes and fibroblasts contributed to epidermal stratification, higher tensile strength of the construct,

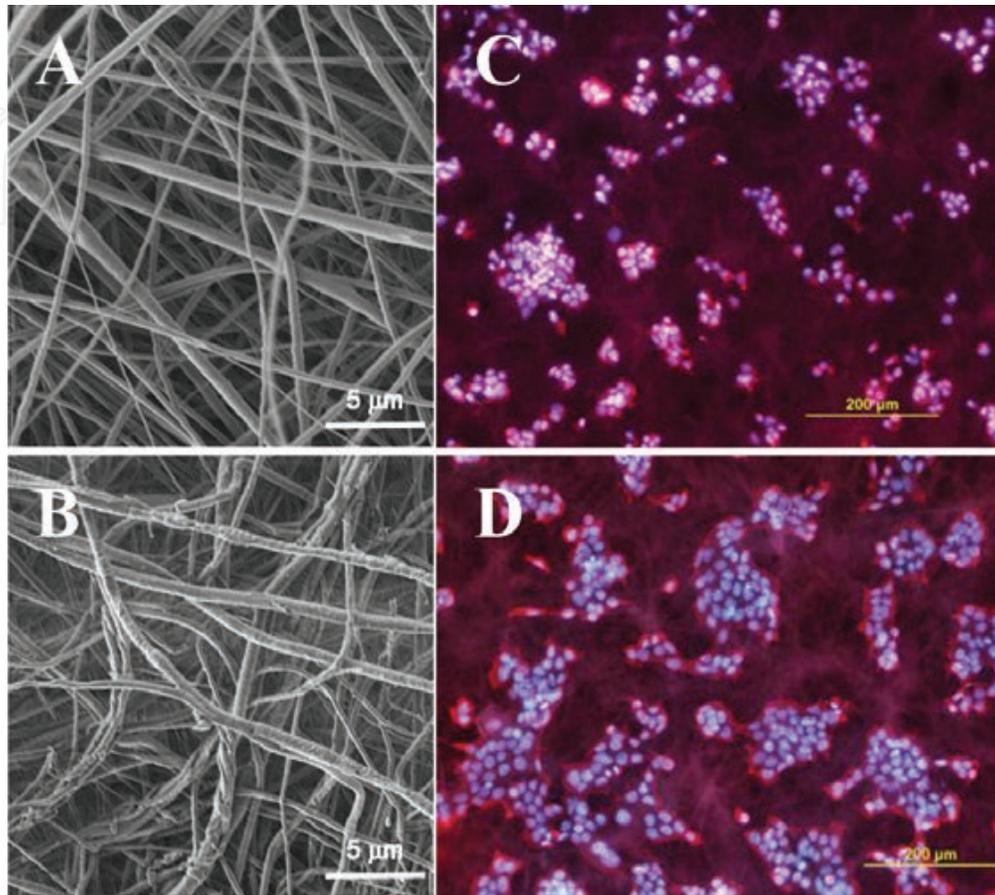
modulation of cytokine and growth factor expression, and increased angiogenic properties compared with constructs containing fibroblasts or keratinocytes alone [95]. Nevertheless, the bilayered epidermal–dermal construct has been rarely developed on nanofibrous membranes. For its creation, other forms of synthetic and natural polymers have been used, for example a knitted PLGA mesh combined with collagen–hyaluronic acid sponge [96], porous scaffolds made of a copolymer of poly(ethylene glycol terephthalate) and poly(butylene terephthalate) [97] matrices containing fibrin, collagen, hyaluronan and their combinations [98–100], and even spider silk woven on steel frames [101]. Thus, we have attempted to develop a bilayer of keratinocytes and skin fibroblasts using nanofibrous PLA membranes, fixed in adapted CellCrown inserts (Scaffdex, Tampere, Finland). The membranes were first seeded with fibroblasts, because these cells served as a feeder layer for keratinocytes, and after reaching confluence of fibroblasts (on day 7 after seeding), the inserts were converted, seeded with keratinocytes, and these cells were cultivated for 4 days (**Figure 1**).



**Figure 1.** Development of a bilayered construct of HaCaT keratinocytes and neonatal human dermal fibroblasts on the opposite sides of a nanofibrous membrane (scale bar = 100  $\mu\text{m}$ ). The keratinocytes were stained by immunofluorescence against cytokeratin 5, an early marker of keratinocyte differentiation. The fibroblasts were stained with Texas Red C<sub>2</sub>-Maleimide and Hoechst #33258.

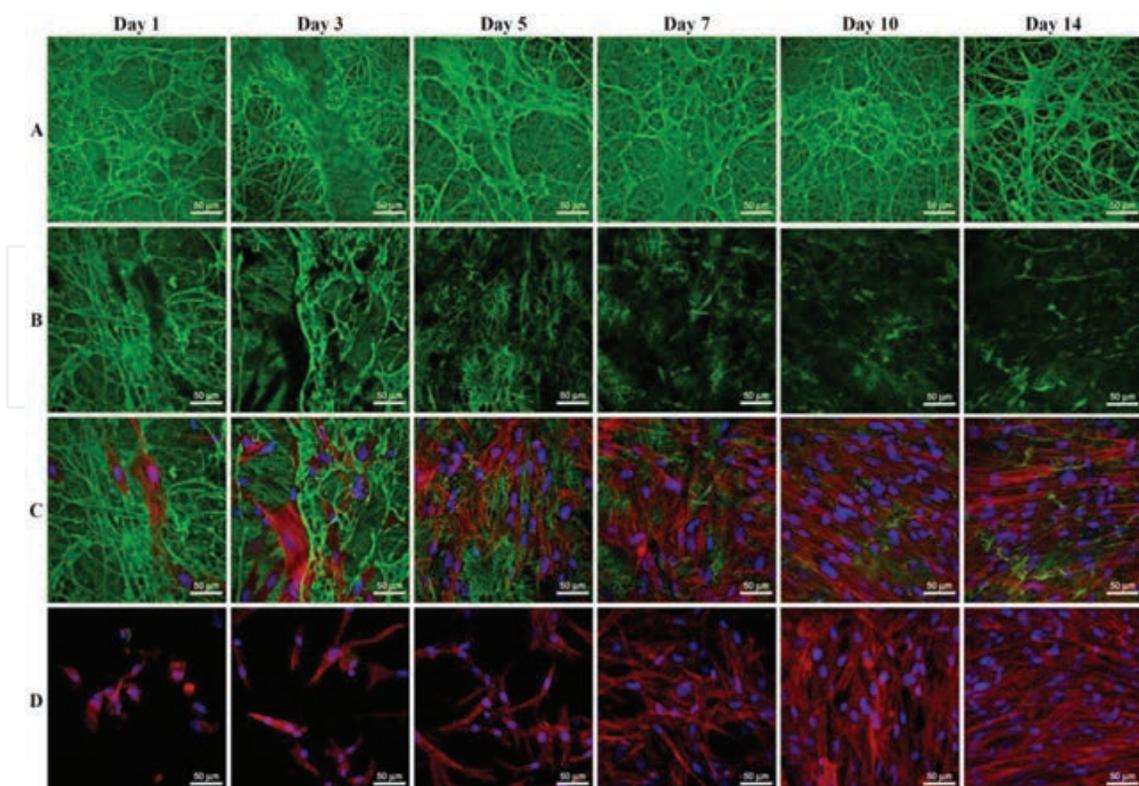
As evident from the **Figure 1**, the keratinocyte layer on the PLA nanofibrous membrane was not continuous. In general, synthetic polymeric materials in their pristine state are rather hydrophobic and may behave as bioinert. Thus, we activated the PLA membranes by treatment with oxygen plasma in order to improve the cell-material interaction. We found that the plasma treatment improved the adhesion and growth of human HaCaT keratinocytes, which was manifested by the formation of larger cell islands (**Figure 2**), and also by an increased activity of mitochondrial enzymes (measured by the XTT assay), and increased DNA content (measured by the Picogreen dsDNA assay kit), which both are indicators of an increased cell number [102]. These beneficial effects of the plasma treatment of the cell behavior could be attributed to the formation of new oxidized structures on the membrane surface, increase in surface wettability, and changes in surface stiffness. Higher plasma power and, in particular, longer exposure times resulted in more pronounced improvement of the cell adhesion and growth. The fiber density of the membranes also played an important role in cell adhesion and growth. The cells preferentially adhered on membranes of lower fiber densities, due to the larger void

spaces between the fibers. Thus, PLA nanofibrous membranes subjected to physical modifications proved as promising materials for the construction of temporary carriers for skin cells [102].



**Figure 2.** The morphology of unmodified nanofibrous poly(lactide) scaffolds (A) and scaffolds treated by oxygen plasma (power 75 W, time 30 min, B), and the morphology of human HaCaT keratinocytes on these scaffolds (C and D). Note larger keratinocyte islands on plasma-treated scaffolds (D) than on untreated scaffolds (C). A, B: FE-SEM Tescan MIRA3 scanning electron microscope, objective magnification 10,000 $\times$ , scale bar 5  $\mu\text{m}$ . C, D: Cells stained with Texas Red C<sub>2</sub>-Maleimide and Hoechst #33342. Olympus IX 51 microscope, obj. 10 $\times$ , DP 70 digital camera, scale bar = 200  $\mu\text{m}$ . Day 3 after seeding.

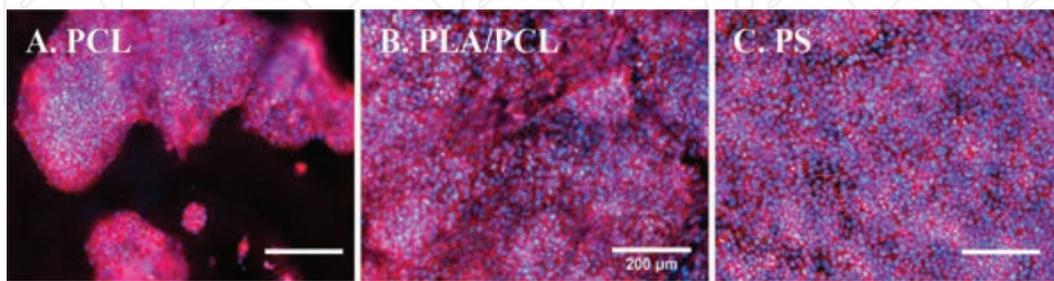
Also the modification of polymeric membranes with fibrin, that is a provisional matrix molecule playing an important role in tissue regeneration, had beneficial effects on the adhesion, growth, and functioning of skin cells, particularly human dermal fibroblasts (**Figure 3**). Fibrin films were developed by *in vitro* simulation of a specific part of physiological hemocoagulation process [87, 103]. Fibrinogen for the preparation of fibrin could be isolated in reasonable quantities from the patient's own blood, that is used in autologous form [103]. Fibrin either enveloped individual fibers or formed additional nanofibrous network on the synthetic polymeric nanofibrous membranes. Fibrin was gradually degraded by the adhering and growing cells and was replaced by their own extracellular matrix, which was manifested by increased production of collagen in these cells. The cell growth and collagen production were further enhanced by the presence of ascorbic acid in the culture medium [87].



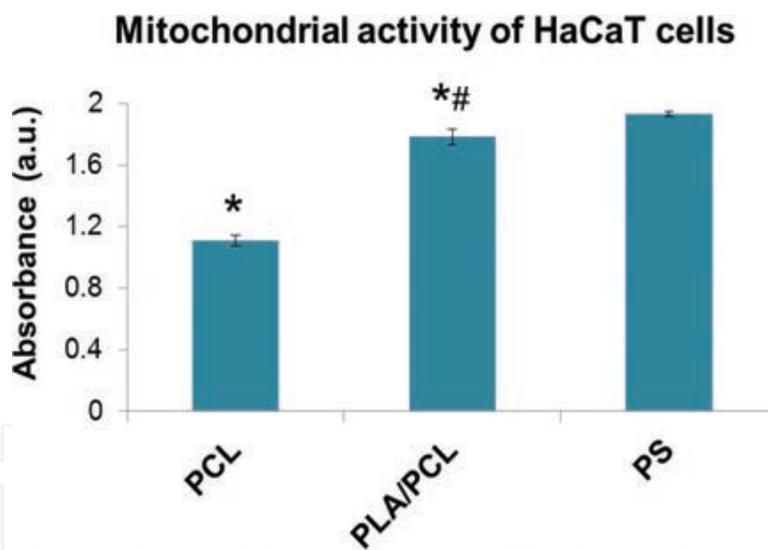
**Figure 3.** Morphology of fibrin coatings (green immunofluorescence) on nanofibrous PLA membranes in six time intervals incubated without cells at 37°C, 5% CO<sub>2</sub> (A), or incubated with human dermal fibroblasts (B—only fibrin, C—fibrin with cells). (D) Cells on non-modified PLA membranes. The cells were stained with phalloidin-TRITC and Hoechst #33258. Leica TCS SPE DM2500 confocal microscope, obj. 40×/1.15 NA oil, scale bar = 50 μm.

In another set of experiments, we studied the adhesion and growth of human keratinocytes on nanofibrous membranes made of poly- $\epsilon$ -caprolactone (PCL) and its copolymer with PLA (PLA/PCL, ratio 70:30). PCL and PLA/PCL copolymers have been experimentally used for vascular tissue engineering, particularly for replacement of small caliber blood vessels [31, 32], neural tissue engineering, specifically for generation of conductive sheaths for neurite outgrowth [104], for substituting the *dura mater* [105], and also for bone tissue engineering in order to mimic hemi-osteons and to control the spatial organization of osteoblasts [106]. These applications of PCL and PLA/PCL were enabled, among others, by suitable mechanical properties of these polymers, particularly in case of PLA/PCL. PLA/PCL also proved as suitable carriers for controlled delivery of drugs, for example antibiotics [107]. However, the potential of PCL and PLA/PCL in skin tissue engineering has not yet been fully explored. Our preliminary results with electrospun aliphatic polyesters, namely PCL and a PLA/PCL copolymer (kindly provided by the Technical University of Liberec, Faculty of Textile Engineering, Liberec, Czech Republic), showed that PCL and particularly PLA/PCL nanofibrous membranes would be suitable scaffolds for the adhesion and growth of skin cells. On PCL, human HaCaT keratinocytes were able to form large islands on day 7 after seeding (cell seeding density of 15,000 cells/cm<sup>2</sup>), and on the PLA/PCL copolymer (ratio 70:30), even a confluent layer similar to that achieved on standard cell culture polystyrene dishes (**Figure 4**). The

activity of mitochondrial enzymes, measured by the WST-1 test, showed a similar trend (**Figure 5**). The beneficial effect of the PLA/PCL copolymer was probably due to its higher hydrophilicity, and also to a greater thickness of the PLA/PCL fibers (diameter 1000 nm compared to 500 nm in PCL fibers), which might provide a better adhesion and growth support for cells. The interaction of skin cells with the PLA/PCL nanofibrous scaffolds can be further improved by loading these scaffolds with ascorbic acid [86], vitamin E, and curcumin [88].



**Figure 4.** Human HaCaT keratinocytes on day 7 after seeding on nanofibrous membranes made (A) of poly- $\epsilon$ -caprolactone (PCL), (B) of a copolymer of PCL and polylactide (PLA/PCL) and (C) on standard cell culture polystyrene dishes (PS). Cells stained with phalloidin conjugated with TRITC (red fluorescence), and the cell nuclei counterstained with DAPI (blue fluorescence). Olympus IX 71 microscope, DP 70 digital camera, scale bar = 200  $\mu\text{m}$ .



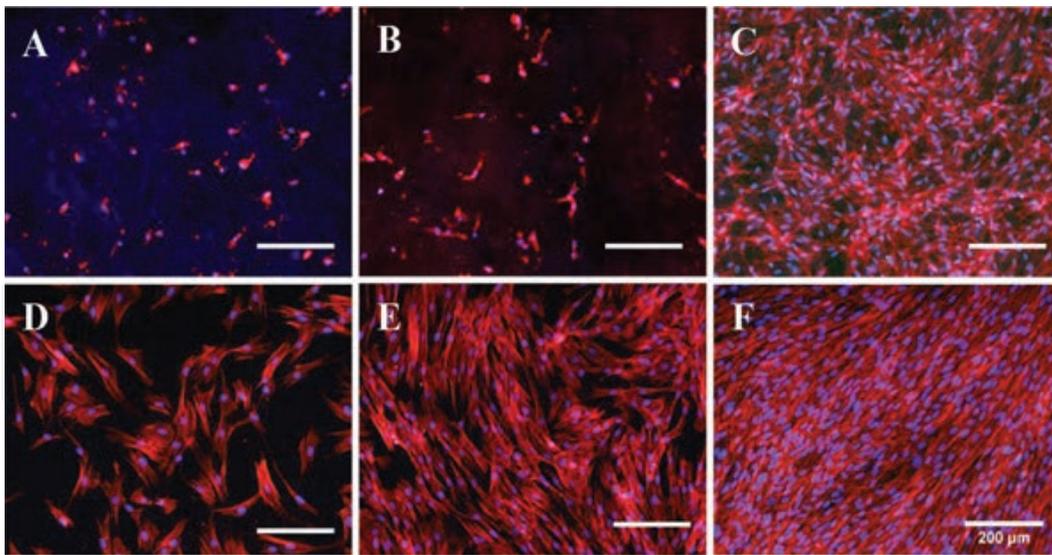
**Figure 5.** Activity of mitochondrial enzymes, measured by the WST-1 test in human HaCaT keratinocytes on day 7 after seeding on nanofibrous meshes made of poly- $\epsilon$ -caprolactone (PCL) and its copolymer with PLA (PLA/PCL), and on standard cell culture polystyrene dishes (PS). Mean  $\pm$  SEM (standard error of mean) from nine measurements for each experimental group. ANOVA, Student–Newman–Keuls method. Statistical significance: \*#:  $p \leq 0.05$  in comparison with PS and PCL, respectively.

Other promising nanofibrous scaffolds for skin tissue engineering are made of cellulose-based materials, which have achieved a remarkably wide range of applications in clinical practice. These materials serve as wound dressings, carriers for drug delivery, preparations for treatment of ophthalmological disorders, membranes for prevention of postoperative adhesions, meshes for hernia repair, materials for hemostasis, membranes for hemodialysis, and

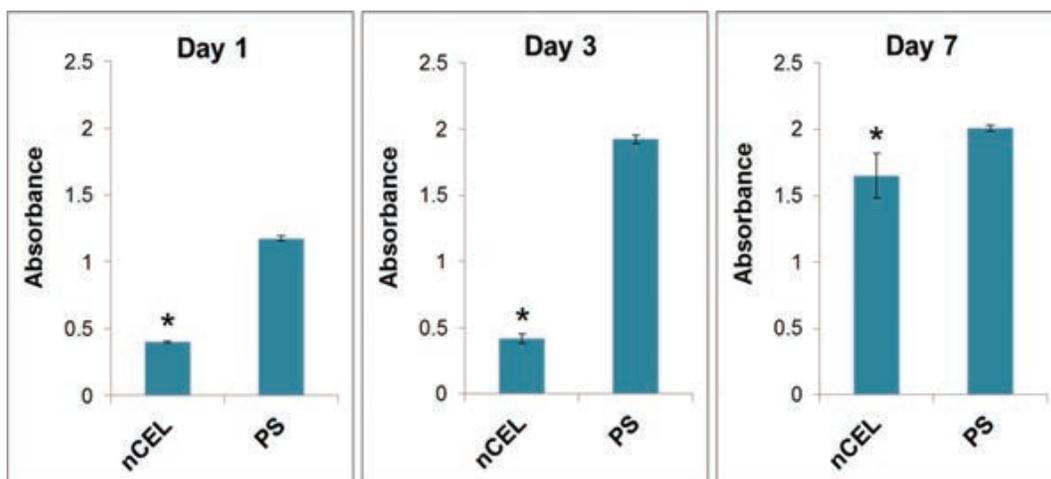
also as materials for plastic, reconstructive, and aesthetic surgery (for a review, see [108–110]). For tissue engineering, including skin tissue engineering, cellulose is promising due to its relatively good mechanical properties, low immunogenic properties, high biocompatibility, and water-holding ability [111, 112] (for a review, see [109, 110]). However, in its pure natural form, cellulose is nondegradable in the mammalian organism, while in tissue engineering, particularly that of skin, degradability of material scaffolds is necessary in order to achieve perfect skin regeneration without scar formation. Cellulose can be specifically degraded by cellulase, which hydrolyzes the 1,4-D-glycosidic linkage in the cellulose molecules and is produced by fungi, bacteria, and protozoans (for a review, see [113]). Thus, in preparation of scaffolds for tissue engineering, these enzymes have been incorporated in bacterial cellulose sheets [114]. Other approaches how to achieve and control the cellulose degradability were its esterification, that is formation of carboxymethylcellulose, which was then susceptible to degradation by esterases [115], acetylation, that is the formation of cellulose acetate, and its further mixing with another polysaccharide, pullulan, in various ratios [116]. Cellulose is also susceptible to hydrolysis by acids and, to a lesser extent, by alkalis (for a review, see [113]). Our earlier study showed that the degradation rate of cellulose can also be adjusted by percentage (wt.%) of COOH groups introduced into the cellulose molecules, but the following degradation of cellulose was accompanied by the release of glucuronic acid into the culture medium, which considerably lowered its pH and hampered the cell growth even at relatively low concentrations of COOH groups in the cellulose molecules (about 6 wt.% [108]).

Our recent experiments revealed that nanofibrous scaffolds made of cellulose acetate, acted as suitable growth support for human dermal fibroblasts *in vitro*. The cellulose acetate was purchased from Sigma–Aldrich (Cat. No. 180955), and the nanofibrous scaffolds were fabricated in Nanopharma Joint-Stock Co., Prague, Czech Republic. Neonatal human dermal fibroblasts (Lonza, Basel, Switzerland) were seeded on the scaffolds at the density of 15,000 cells/cm<sup>2</sup> and cultured in a Dulbecco's modified Eagle's medium (Sigma–Aldrich, Cat. No. D5648) supplemented with 10% of fetal bovine serum (FBS; Sebak GmbH, Aidenbach, Germany) and 40 µg/mL of gentamicin (LEK, Ljubljana, Slovenia). The number of the initially adhering cells on day 1 after seeding was lower on the nanofibrous cellulose scaffolds than on standard cell culture polystyrene dishes, and this number also remained lower on day 3 after seeding. The cell spreading was also lower on the nanofibrous cellulose acetate scaffolds than on polystyrene dishes. However, on day 7 after seeding, the cell numbers on the nanofibrous scaffolds and polystyrene dished almost equaled and reached confluence (**Figure 6**). Corresponding results were also obtained by the WST-1 test measuring the activity of mitochondrial enzymes (**Figure 7**).

Similarly, in a recent study, micro- and nanofibrous scaffolds made of cellulose acetate provided an excellent growth support for dermal fibroblasts *in vitro*, promoting a higher cell adhesion and mitochondrial activity (measured by the MTT test) compared with the control scaffolds made of PCL [117]. Positive influence on the adhesion and metabolic activity of human dermal fibroblasts were also obtained in composite nanofibrous scaffolds made of PCL and with cellulose acetate, cellulose acetate and pullulan [116], and particularly of electrospun cellulose acetate and gelatin in a ratio of 25:75 [118].



**Figure 6.** Human dermal fibroblasts on day 1 (A, D), day 3 (B, E), and day 7 (C, F) after seeding on nanofibrous membranes made of cellulose acetate (A–C) and on standard cell culture polystyrene dishes (D–F). Cells stained with phalloidin conjugated with TRITC (red fluorescence), and the cell nuclei counterstained with DAPI (blue fluorescence). Olympus IX 71 microscope, DP 70 digital camera, scale bar = 200 μm.



**Figure 7.** Activity of mitochondrial enzymes, measured by the WST-1 test in human dermal fibroblasts on days 1, 3, and 7 after seeding on nanofibrous membranes made of cellulose acetate (nCEL) and on standard cell culture polystyrene dishes (PS). Mean ± SEM (standard error of mean) from nine measurements for each experimental group and time ANOVA, Student–Newman–Keuls method. Statistical significance: \*:  $p \leq 0.05$  in comparison with PS.

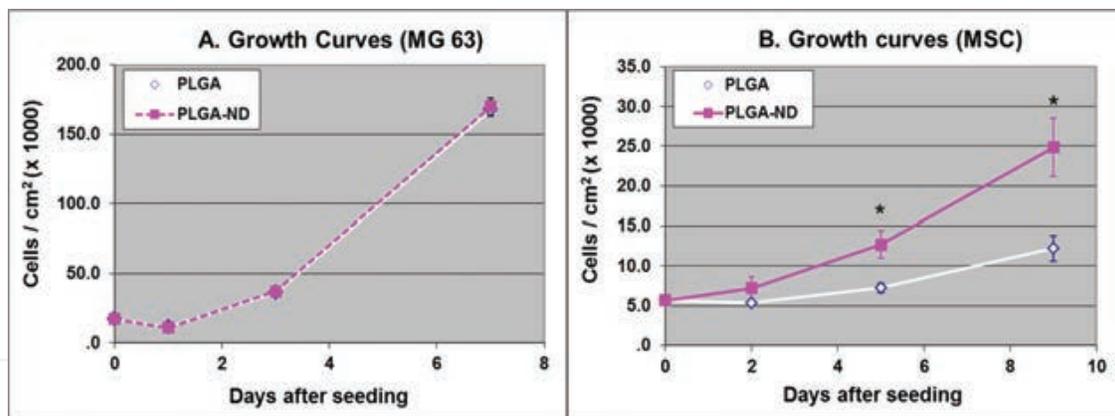
### 3. Nanofibers in bone tissue engineering

Among a wide range of nanofibrous scaffolds used for bone tissue engineering, our studies concentrated on scaffolds reinforced with hydroxyapatite nanoparticles, diamond nanoparticles, and nanofibrous scaffolds created by the deposition of nanodiamond on SiO<sub>2</sub> nanofibers.

Nanofibrous PLA-hydroxyapatite (HAp) composites were created by addition of hydroxyapatite nanoparticles in concentrations of 5 wt.% and 15 wt.% to a PLA matrix before electrospinning. The addition of nanoparticles improved mechanical properties of the scaffolds by suppressing their creep behavior in their dry state. Addition of HAp nanoparticles also increased the proliferation of human osteoblast-like MG 63 cells, and particularly their osteogenic differentiation, manifested by production of osteocalcin, an extracellular matrix glycoprotein binding calcium [119].

Diamond nanoparticles were added either into PLGA or PLA matrix before electrospinning. PLGA nanofibers were enriched with 23 wt.% of diamond nanoparticles, and PLA nanofibers were enriched with several concentrations of diamond nanoparticles, ranging from 0.44 to 12.28 wt.%. To the best of our knowledge, we were the first laboratory creating nanodiamond-loaded polymeric nanofibrous scaffolds for potential bone tissue engineering. Earlier, nanofibrous polymer–nanodiamond composites were prepared only for technical applications, for example protection of various surfaces against scratch and potential damage by the ultraviolet light irradiation [120].

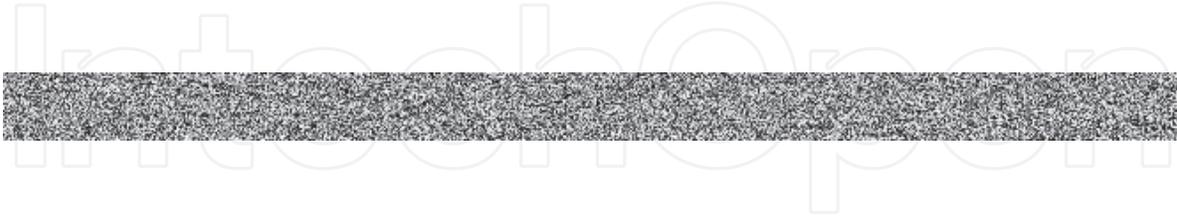
Our PLGA-nanodiamond nanofibrous scaffolds supported the adhesion and growth of human osteoblast-like MG 63 cells to a similar degree as the pure PLGA nanofibrous scaffolds [68] but accelerated the growth of human bone marrow mesenchymal stem cells [61] (**Figure 8**).



**Figure 8.** Growth curves of human osteoblast-like MG 63 cells (A) and human bone marrow mesenchymal stem cells (MSC, B) in cultures on pure poly(lactide-co-glycolide) (PLGA) scaffolds and scaffolds loaded with 23 wt.% of diamond nanoparticles (PLGA-ND). Mean  $\pm$  SEM (standard error of mean) from 8 to 28 measurements for each experimental group and time interval. ANOVA, Student–Newman–Keuls method. Statistical significance: \* $p \leq 0.05$  in comparison with pure PLGA membranes.

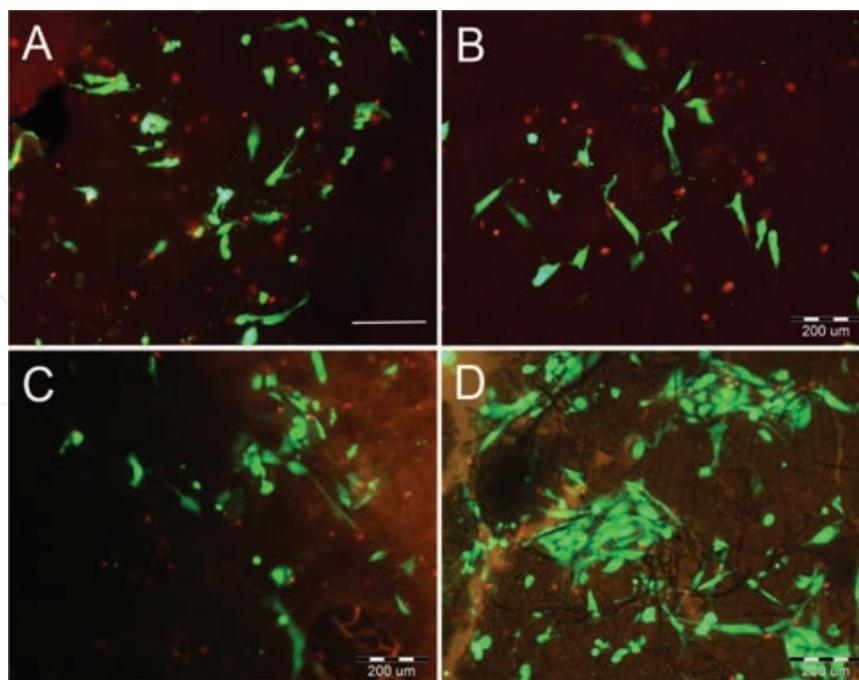
However, when nanodiamond nanoparticles were incorporated into PLA nanofibrous scaffolds, they had rather negative effects on human osteoblast-like MG 63 and Saos-2 cells. The number and mitochondrial activity of cells growing on these scaffolds (**Figure 9**), as well as their expression of alkaline phosphatase and osteocalcin on the mRNA and protein levels decreased with increasing diamond particle concentration. This discrepancy was probably due to the different origin and different physicochemical properties of the diamond nanoparti-

cles used for addition into PLGA and PLA nanofibers. For PLGA nanofibers, diamond nanoparticles were prepared by a radio-frequency PACVD method, while the PLA nanofibers were loaded with detonation nanodiamonds with hydrophobic surface (purchased from the Nano Carbon Research Institute, Japan, under the product name NanoAmando) (for a review, see [121]).



**Figure 9.** The mitochondrial activity of human osteoblast-like MG 63 and Saos-2 cells, measured by XTT test on day 3 after seeding on nanofibrous polylactide membranes loaded with 0–12.28 wt.% of diamond nanoparticles (DNP). Absorbances are given in % of values obtained from pure PLA membranes (sample A). Mean  $\pm$  S.E.M. from 17 to 22 measurements for each experimental group and cell type. ANOVA, Student–Newman–Keuls Student–Newman–Keuls method. Statistical significance: <sup>A</sup><sub>p</sub>  $\leq$  0.05 in comparison with pure PLA membranes and membranes with the lowest DNP concentration, respectively.

Interesting results were obtained with a novel nanofibrous material, that is SiO<sub>2</sub> nanofibers prepared by electrospinning and then coated by a thin diamond film. SiO<sub>2</sub> nanofibers were purchased from the Technical University of Liberec, Czech Republic. Nanodiamond coating was performed by microwave plasma chemical vapor deposition [122]. Finally, both nanodiamond-coated and pure SiO<sub>2</sub> nanofibers were terminated by oxygen in order to enhance their attractiveness for the cell adhesion and growth. The nanofibers were seeded with human umbilical vein endothelial cells (HUVEC, passage 4) purchased from Lonza (Cat. No. C2517A) at the density of approx. 16,000 cells/cm<sup>2</sup>, and the cells were cultured in endothelial growth medium (EGM-2, Lonza, Cat. No. CC-3162) for 1 and 4 days. The cells were then visualized using a Live/Dead Viability/Cytotoxicity assay kit (Life Technologies). The endothelial cells were chosen because they are an important cell type present in the bone, playing a key role in the scaffold vascularization. In addition, primary and low-passaged endothelial cells are a relatively demanding cell type sensitive to the physical and chemical properties of the material and its potential cytotoxicity. We found that the diamond coating on SiO<sub>2</sub> nanofibers markedly improved the growth of HUVEC cells. On day 1 after seeding, the number of viable cells was similar on both pure and diamond-coated SiO<sub>2</sub> nanofibers. However, on day 4, the cell number on pure SiO<sub>2</sub> remained similar as on day 1, while on diamond-coated SiO<sub>2</sub> nanofibers, it increased, and the cells formed islands (**Figure 10**).



**Figure 10.** Human umbilical vein endothelial cells (HUVEC) cells grown on O-terminated SiO<sub>2</sub> nanofibers (A, B) and on O-terminated diamond-SiO<sub>2</sub> nanofibers on day 1 (A, C) and on day 4 (B, D) after seeding. The cells were stained with Live/Dead Viability/Cytotoxicity assay kit; living cells are stained in green and dead cells are stained in red. Olympus IX 71 microscope, IX71 digital camera, scale bar = 200  $\mu$ m.

## 4. Conclusions

Nanofibrous scaffolds is one of the most promising materials for tissue engineering. At the experimental level, they have been used for construction or regeneration of almost all tissues in the human organism. Nanofibers are also applicable for the drug and gene delivery, gene silencing, biosensing, electrical stimulation of cells, and wound dressings. Nanofibers can be fabricated from a wide range of materials, mainly from natural and synthetic polymers, but also from ceramic and carbon materials, including SiO<sub>2</sub> and diamond. The main method recently used for fabrication of nanofibers is electrospinning. In order to enhance the attractiveness of nanofibers for the cell adhesion and growth, they can be loaded with various growth, angiogenic, and differentiation factors and/or functionalized with oligopeptidic ligands for the cell adhesion receptors. Other important modifications which improved the cell behavior on nanofibrous scaffolds were the activation of nanofibers by plasma treatment or coating of nanofibers with fibrin, as revealed by our earlier studies. For hard tissue engineering, the nanofibers can be reinforced with ceramic, metal-, or carbon- based nanoparticles, or by biomineralization. In our earlier studies, addition of hydroxyapatite to synthetic polymeric nanofibers increased the growth and osteogenic differentiation of human osteoblast-like cells. However, the addition of diamond nanoparticles to these nanofibers had controversial effects, which depended on the preparation method and physicochemical properties of the nanoparticles. Diamond nanoparticles prepared by PACVD method had

stimulatory effects on the cell adhesion and growth, while the effects of diamond nanoparticles prepared by detonation synthesis were rather negative. A novel material promising for tissue engineering is the diamond-coated SiO<sub>2</sub> nanofiber, recently developed and tested by our group.

## Acknowledgements

Our studies included in this review were supported by the Agency for Healthcare Research, Ministry of Health of the Czech Republic (grant no. 15-33018A), the Grant Agency of the Czech Republic (grant no. 14-04790S), and the Technology Grant Agency of the of the Czech Republic (grant no. TA04010065).

## Author details

Lucie Bacakova<sup>1\*</sup>, Marketa Bacakova<sup>1</sup>, Julia Pajorova<sup>1</sup>, Radmila Kudlackova<sup>1</sup>, Lubica Stankova<sup>1</sup>, Elena Filova<sup>1</sup>, Jana Musilkova<sup>1</sup>, Stepan Potocky<sup>2</sup> and Alexander Kromka<sup>2</sup>

\*Address all correspondence to: lucie.bacakova@fgu.cas.cz

1 Institute of Physiology, Czech Academy of Sciences, Videnska, Prague, Czech Republic

2 Institute of Physics, Czech Academy of Sciences, Cukrovarnicka, Prague, Czech Republic

## References

- [1] Sampath M, Lakra R, Korrapati P, Sengottuvelan B. Curcumin loaded poly (lactic-co-glycolic) acid nanofiber for the treatment of carcinoma. *Colloids and Surfaces B: Biointerfaces* 2014; 117: 128–134. doi:10.1016/j.colsurfb.2014.02.020.
- [2] Xue J, He M, Niu Y, Liu H, Crawford A, Coates P, Chen D, Shi R, Zhang L. Preparation and in vivo efficient anti-infection property of GTR/GBR implant made by metronidazole loaded electrospun polycaprolactone nanofiber membrane. *International Journal of Pharmaceutics* 2014; 475(1–2): 566–577. doi:10.1016/j.ijpharm.2014.09.026.
- [3] Meier C, Weil T, Kirchhoff F, Münch J. Peptide nanofibrils as enhancers of retroviral gene transfer. *Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology* 2014; 6(5): 438–451. doi:10.1002/wnan.1275.
- [4] Monteiro N, Ribeiro D, Martins A, Faria S, Fonseca NA, Moreira JN, Reis RL, Neves NM. Instructive Nanofibrous Scaffold Comprising Runt-Related Transcription Factor

- 2 Gene Delivery for Bone Tissue Engineering. *ACS Nano* 2014; 8(8): 8082–8094. doi:10.1021/nm5021049.
- [5] Goonoo N, Jeetah R, Bhaw-Luximon A, Jhurry D. Polydioxanone-based bio-materials for tissue engineering and drug/gene delivery applications. *European Journal of Pharmaceutics and Biopharmaceutics* 2015; 97: 371–391. doi:10.1016/j.ejpb.2015.05.024.
- [6] Diao HJ, Low WC, Lu QR, Chew SY. Topographical effects on fiber-mediated microRNA delivery to control oligodendroglial precursor cells development. *Biomaterials* 2015; 70: 105–114. doi:10.1016/j.biomaterials.2015.08.029.
- [7] Khadka DB, Haynie DT. Protein- and peptide-based electrospun nanofibers in medical biomaterials. *Nanomedicine: Nanotechnology, Biology and Medicine* 2012; 8(8): 1242–1262. doi:10.1016/j.nano.2012.02.013.
- [8] Dallas P, Georgakilas V. Interfacial polymerization of conductive polymers: Generation of polymeric nanostructures in a 2-D space. *Advances in Colloid and Interface Science* 2015; 224: 46–61. doi:10.1016/j.cis.2015.07.008.
- [9] Arslan A, Şimşek M, Aldemir SD, Kazaroğlu NM, Gümüşderelioğlu M. Honey-based PET or PET/chitosan fibrous wound dressings: effect of honey on electrospinning process. *Journal of Biomaterials Science, Polymer Edition* 2014; 25(10): 999–1012. doi:10.1080/09205063.2014.918455.
- [10] Unnithan AR, Gnanasekaran G, Sathishkumar Y, Lee YS, Kim CS. Electrospun antibacterial polyurethane–cellulose acetate–zein composite mats for wound dressing. *Carbohydrate Polymers* 2014; 102: 884–892. doi:10.1016/j.carbpol.2013.10.070.
- [11] Zhong, Teo WE, Zhu X, Beuerman R, Ramakrishna S, Yung LYL. Formation of collagen–glycosaminoglycan blended nanofibrous scaffolds and their biological properties. *Biomacromolecules* 2005; 6(6): 2998–3004. doi:10.1021/bm050318p.
- [12] Cao H, Chen MM, Liu Y, Liu YY, Huang YQ, Wang JH, Chen JD, Zhang QQ. Fish collagen-based scaffold containing PLGA microspheres for controlled growth factor delivery in skin tissue engineering. *Colloids and Surfaces B: Biointerfaces* 2015; 136: 1098–1106. doi:10.1016/j.colsurfb.2015.10.022.
- [13] Sell SA, McClure MJ, Garg K, Wolfe PS, Bowlin GL. Electrospinning of collagen/biopolymers for regenerative medicine and cardiovascular tissue engineering. *Advanced Drug Delivery Reviews* 2009; 61(12): 1007–1019. doi:10.1016/j.addr.2009.07.012.
- [14] Zeng L, Teng W, Jiang L, Cappello J, Wu X. Ordering recombinant silk-elastin-like nanofibers on the microscale. *Applied Physics Letters* 2014; 104(3): 033702. doi:10.1063/1.4863077.
- [15] Min BM, Lee G, Kim SH, Nam YS, Lee TS, Park WH. Electrospinning of silk fibroin nanofibers and its effect on the adhesion and spreading of normal human keratinocytes and fibroblasts in vitro. *Biomaterials* 2004; 25(7–8): 1289–1297. doi:10.1016/j.biomaterials.2003.08.045.

- [16] Shao W, He J, Sang F, Ding B, Chen L, Cui S, Li K, Han Q, Tan W. Coaxial electrospun aligned tussah silk fibroin nanostructured fiber scaffolds embedded with hydroxyapatite–tussah silk fibroin nanoparticles for bone tissue engineering. *Materials Science and Engineering: C* 2016; 58: 342–351. doi:10.1016/j.msec.2015.08.046.
- [17] Wang W, Itoh S, Konno K, Kikkawa T, Ichinose S, Sakai K, Ohkuma T, Watabe K. Effects of Schwann cell alignment along the oriented electrospun chitosan nanofibers on nerve regeneration. *Journal of Biomedical Materials Research Part A* 2009; 91A(4): 994–1005. doi:10.1002/jbm.a.32329.
- [18] Shi Q, Li Y, Sun J, Zhang H, Chen L, Chen B, Yang H, Wang Z. The osteogenesis of bacterial cellulose scaffold loaded with bone morphogenetic protein-2. *Biomaterials* 2012; 33(28): 6644–6649. doi:10.1016/j.biomaterials.2012.05.071.
- [19] Yao S, Wang X, Liu X, Wang R, Deng C, Cui F. Effects of ambient relative humidity and solvent properties on the electrospinning of pure hyaluronic acid nanofibers. *Journal of Nanoscience and Nanotechnology* 2013; 13(7): 4752–4758. doi:10.1166/jnn.2013.7197.
- [20] Deng Y, Zhang X, Zhao Y, Liang S, Xu A, Gao X, Deng F, Fang J, Wei S. Peptide-decorated polyvinyl alcohol/hyaluronan nanofibers for human induced pluripotent stem cell culture. *Carbohydrate Polymers* 2014; 101: 36–39. doi:10.1016/j.carbpol.2013.09.030.
- [21] Leszczak V, Place LW, Franz N, Popat KC, Kipper MJ. Nanostructured biomaterials from electrospun demineralized bone matrix: a survey of processing and crosslinking strategies. *ACS Applied Materials & Interfaces* 2014; 6(12): 9328–9337. doi:10.1021/am501700e.
- [22] Ainslie KM, Bachelder EM, Borkar S, Zahr AS, Sen A, Badding JV, Pishko MV. Cell Adhesion on nanofibrous polytetrafluoroethylene (nPTFE). *Langmuir* 2007; 23(2): 747–754. doi:10.1021/la060948s.
- [23] Hu Z jun, Li Z lun, Hu L yu, He W, Liu R ming, Qin Y sen, Wang S ming. The in vivo performance of small-caliber nanofibrous polyurethane vascular grafts. *BMC Cardiovascular Disorders* 2012; 12(1): 115. doi:10.1186/1471-2261-12-115.
- [24] Dou Y, Wu C, Chang J. Preparation, mechanical property and cytocompatibility of poly(l-lactic acid)/calcium silicate nanocomposites with controllable distribution of calcium silicate nanowires. *Acta Biomaterialia* 2012; 8(11): 4139–4150. doi:10.1016/j.actbio.2012.07.009.
- [25] Cai Q, Shi Y, Shan D, Jia W, Duan S, Deng X, Yang X. Osteogenic differentiation of MC3T3-E1 cells on poly(l-lactide)/Fe<sub>3</sub>O<sub>4</sub> nanofibers with static magnetic field exposure. *Materials Science and Engineering: C* 2015; 55: 166–173. doi:10.1016/j.msec.2015.05.002.
- [26] Xing ZC, Chae WP, Huh MW, Park LS, Park SY, Kwak G, Yoon KB, Kang IK. In vitro anti-bacterial and cytotoxic properties of silver-containing poly(l-lactide-co-glycolide)

- nanofibrous scaffolds. *Journal of Nanoscience and Nanotechnology* 2011; 11(1): 61–65. doi:10.1166/jnn.2011.3551.
- [27] Zhang S, Prabhakaran MP, Qin X, Ramakrishna S. Poly-3-hydroxybutyrate-co-3-hydroxyvalerate containing scaffolds and their integration with osteoblasts as a model for bone tissue engineering. *Journal of Biomaterials Applications* 2015; 29(10): 1394–1406. doi:10.1177/0885328214568467.
- [28] Song W, Markel DC, Wang S, Shi T, Mao G, Ren W. Electrospun polyvinyl alcohol–collagen–hydroxyapatite nanofibers: a biomimetic extracellular matrix for osteoblastic cells. *Nanotechnology* 2012; 23(11): 115101. doi:10.1088/0957-4484/23/11/115101.
- [29] Manjumeena R, Elakkiya T, Duraibabu D, Feroze Ahamed A, Kalaichelvan P, Venkatesan R. “Green” biocompatible organic–inorganic hybrid electrospun nanofibers for potential biomedical applications. *Journal of Biomaterials Applications* 2015; 29(7): 1039–1055. doi:10.1177/0885328214550011.
- [30] Vines JB, Lim DJ, Anderson JM, Jun HW. Hydroxyapatite nanoparticle reinforced peptide amphiphile nanomatrix enhances the osteogenic differentiation of mesenchymal stem cells by compositional ratios. *Acta Biomaterialia* 2012; 8(11): 4053–4063. doi:10.1016/j.actbio.2012.07.024.
- [31] Sankaran KK, Vasanthan KS, Krishnan UM, Sethuraman S. Development and evaluation of axially aligned nanofibres for blood vessel tissue engineering: small-diameter aligned nanofibrous vascular graft. *Journal of Tissue Engineering and Regenerative Medicine* 2014; 8(8): 640–651. doi:10.1002/term.1566.
- [32] Wang Y, Hu J, Jiao J, Liu Z, Zhou Z, Zhao C, Chang LJ, Chen YE, Ma PX, Yang B. Engineering vascular tissue with functional smooth muscle cells derived from human iPS cells and nanofibrous scaffolds. *Biomaterials* 2014; 35(32): 8960–8969. doi:10.1016/j.biomaterials.2014.07.011.
- [33] Hussain A, Collins G, Yip D, Cho CH. Functional 3-D cardiac co-culture model using bioactive chitosan nanofiber scaffolds. *Biotechnology and Bioengineering* 2013; 110(2): 637–647. doi:10.1002/bit.24727.
- [34] Khan M, Xu Y, Hua S, Johnson J, Belevych A, Janssen PML, Gyorke S, Guan J, Angelos MG. Evaluation of changes in morphology and function of human induced pluripotent stem cell derived cardiomyocytes (HiPSC-CMs) cultured on an aligned-nanofiber cardiac patch. *PLOS ONE* 2015; 10(5): e0126338. doi:10.1371/journal.pone.0126338.
- [35] Jahnvi S, Kumary TV, Bhuvaneshwar GS, Natarajan TS, Verma RS. Engineering of a polymer layered bio-hybrid heart valve scaffold. *Materials Science and Engineering: C* 2015; 51: 263–273. doi:10.1016/j.msec.2015.03.009.
- [36] Punnoose AM. Electrospun Type 1 collagen matrices using a novel benign solvent for cardiac tissue engineering. *Journal of Cellular Physiology* 2016; 231(3): 744. doi:10.1002/jcp.25115.

- [37] Jana S, Leung M, Chang J, Zhang M. Effect of nano- and micro-scale topological features on alignment of muscle cells and commitment of myogenic differentiation. *Biofabrication* 2014; 6(3): 035012. doi:10.1088/1758-5082/6/3/035012.
- [38] Ostrovidov S, Shi X, Zhang L, Liang X, Kim SB, Fujie T, Ramalingam M, Chen M, Nakajima K, Al-Hazmi F, Bae H, Memic A, Khademhosseini A. Myotube formation on gelatin nanofibers—multi-walled carbon nanotubes hybrid scaffolds. *Biomaterials* 2014; 35(24): 6268–6277. doi:10.1016/j.biomaterials.2014.04.021.
- [39] Bradshaw M, Ho D, Fear MW, Gelain F, Wood FM, Iyer KS. Designer self-assembling hydrogel scaffolds can impact skin cell proliferation and migration. *Scientific Reports* 2014; 4: 6903. doi:10.1038/srep06903.
- [40] Wang Z, Qian Y, Li L, Pan L, Njunge LW, Dong L, Yang L. Evaluation of emulsion electrospun polycaprolactone/hyaluronan/epidermal growth factor nanofibrous scaffolds for wound healing. *Journal of Biomaterials Applications* 2016; 30(6): 686–698. doi:10.1177/0885328215586907.
- [41] Zhou T, Wang N, Xue Y, Ding T, Liu X, Mo X, Sun J. Development of biomimetic tilapia collagen nanofibers for skin regeneration through inducing keratinocytes differentiation and collagen synthesis of dermal fibroblasts. *ACS Applied Materials & Interfaces* 2015; 7(5): 3253–3262. doi:10.1021/am507990m.
- [42] Sahoo S, Ang LT, Cho-Hong Goh J, Toh SL. Bioactive nanofibers for fibroblastic differentiation of mesenchymal precursor cells for ligament/tendon tissue engineering applications. *Differentiation* 2010; 79(2): 102–110. doi:10.1016/j.diff.2009.11.001.
- [43] Naghashzargar E, Farè S, Catto V, Bertoldi S, Semnani D, Karbasi S, Tanzi MC. Nano/micro hybrid scaffold of PCL or P3HB nanofibers combined with silk fibroin for tendon and ligament tissue engineering. *Journal of Applied Biomaterials & Functional Materials* 2015; 13(2): 0–0. doi:10.5301/jabfm.5000216.
- [44] Boomer L, Liu Y, Mahler N, Johnson J, Zak K, Nelson T, Lannutti J, Besner GE. Scaffolding for challenging environments: materials selection for tissue engineered intestine: scaffolding for challenging environments. *Journal of Biomedical Materials Research Part A* 2014; 102(11): 3795–3802. doi:10.1002/jbm.a.35047.
- [45] Kobayashi M, Lei NY, Wang Q, Wu BM, Dunn JCY. Orthogonally oriented scaffolds with aligned fibers for engineering intestinal smooth muscle. *Biomaterials* 2015; 61: 75–84. doi:10.1016/j.biomaterials.2015.05.023.
- [46] Jang YS, Jang CH, Cho YB, Kim M, Kim GH. Tracheal regeneration using polycaprolactone/collagen-nanofiber coated with umbilical cord serum after partial resection. *International Journal of Pediatric Otorhinolaryngology* 2014; 78(12): 2237–2243. doi:10.1016/j.ijporl.2014.10.022.
- [47] Mahoney C, Conklin D, Waterman J, Sankar J, Bhattarai N. Electrospun nanofibers of poly( $\epsilon$ -caprolactone)/depolymerized chitosan for respiratory tissue engineering

- applications. *Journal of Biomaterials Science, Polymer Edition* 2016; 27(7): 611–625. doi: 10.1080/09205063.2016.1144454.
- [48] Shakhssalim N, Rasouli J, Moghadasali R, Aghdas FS, Naji M, Soleimani M. Bladder smooth muscle cells interaction and proliferation on PCL/PLLA electrospun nanofibrous scaffold. *The International Journal of Artificial Organs* 2013; 36(2): 113–120. doi: 10.5301/ijao.5000175.
- [49] Wei G, Li C, Fu Q, Xu Y, Li H. Preparation of PCL/silk fibroin/collagen electrospun fiber for urethral reconstruction. *International Urology and Nephrology* 2015; 47(1): 95–99. doi: 10.1007/s11255-014-0854-3.
- [50] Mareková D, Lesný P, Jendelová P, Michálek J, Kostecká P, Přádný M, Martinová L, Pantoflíček T, Ryska M, Syková E. Hepatocyte growth on polycaprolactone and 2-hydroxyethylmethacrylate nanofiber sheets enhanced by bone marrow-derived mesenchymal stromal cells. *Hepato-Gastroenterology* 2013; 60(125): 1156–1163. doi: 10.5754/hge11948.
- [51] Bishi DK, Mathapati S, Venugopal JR, Guhathakurta S, Cherian KM, Verma RS, Ramakrishna S. A patient-inspired ex vivo liver tissue engineering approach with autologous mesenchymal stem cells and hepatogenic serum. *Advanced Healthcare Materials* 2016; n/a–n/a. doi:10.1002/adhm.201500897.
- [52] Chow LW, Wang L jia, Kaufman DB, Stupp SI. Self-assembling nanostructures to deliver angiogenic factors to pancreatic islets. *Biomaterials* 2010; 31(24): 6154–6161. doi: 10.1016/j.biomaterials.2010.04.002.
- [53] Uzunalli G, Tumas Y, Delibasi T, Yasa O, Mercan S, Guler MO, Tekinay AB. Improving pancreatic islet in vitro functionality and transplantation efficiency by using heparin mimetic peptide nanofiber gels. *Acta Biomaterialia* 2015; 22: 8–18. doi:10.1016/j.actbio.2015.04.032.
- [54] Fon D, Zhou K, Ercole F, Fehr F, Marchesan S, Minter MR, Crack PJ, Finkelstein DI, Forsythe JS. Nanofibrous scaffolds releasing a small molecule BDNF-mimetic for the re-direction of endogenous neuroblast migration in the brain. *Biomaterials* 2014; 35(9): 2692–2712. doi:10.1016/j.biomaterials.2013.12.016.
- [55] Sudwilai T, Ng JJ, Boonkrai C, Israsena N, Chuangchote S, Supaphol P. Polypyrrole-coated electrospun poly(lactic acid) fibrous scaffold: effects of coating on electrical conductivity and neural cell growth. *Journal of Biomaterials Science, Polymer Edition* 2014; 25(12): 1240–1252. doi:10.1080/09205063.2014.926578.
- [56] Rafalowska J, Sulejczak D, Chrapusta SJ, Gadamski R, Taraszewska A, Nakielski P, Kowalczyk T, Dziewulska D. Original article Non-woven nanofiber mats—a new perspective for experimental studies of the central nervous system? *Folia Neuropathologica* 2014; 4: 407–416. doi:10.5114/fn.2014.47841.
- [57] Wang J, Zheng J, Zheng Q, Wu Y, Wu B, Huang S, Fang W, Guo X. FGL-functionalized self-assembling nanofiber hydrogel as a scaffold for spinal cord-derived neural

- stem cells. *Materials Science and Engineering: C* 2015; 46: 140–147. doi:10.1016/j.msec.2014.10.019.
- [58] Yan L, Zhao B, Liu X, Li X, Zeng C, Shi H, Xu X, Lin T, Dai L, Liu Y. Aligned nanofibers from polypyrrole/graphene as electrodes for regeneration of optic nerve via electrical stimulation. *ACS Applied Materials & Interfaces* 2016; 8(11): 6834–6840. doi:10.1021/acsami.5b12843.
- [59] Popelka Š, Studenová H, Abelová L, Ardan T, Studený P, Straňák Z, Klíma J, Dvořánková B, Kotek J, Hodan J, Rypáček F. A frame-supported ultrathin electrospun polymer membrane for transplantation of retinal pigment epithelial cells. *Biomedical Materials* 2015; 10(4): 045022. doi:10.1088/1748-6041/10/4/045022.
- [60] Xia H, Chen Q, Fang Y, Liu D, Zhong D, Wu H, Xia Y, Yan Y, Tang W, Sun X. Directed neurite growth of rat dorsal root ganglion neurons and increased colocalization with Schwann cells on aligned poly(methyl methacrylate) electrospun nanofibers. *Brain Research* 2014; 1565: 18–27. doi:10.1016/j.brainres.2014.04.002.
- [61] Brady MA, Renzing A, Douglas TEL, Liu Q, Wille S, Parizek M, Bacakova L, Kromka A, Jarosova M, Godier G, Warnke PH. Development of composite poly(lactide-co-glycolide)-nanodiamond scaffolds for bone cell growth. *Journal of Nanoscience and Nanotechnology* 2015; 15(2): 1060–1069. doi:10.1166/jnn.2015.9745.
- [62] Duan S, Yang X, Mei F, Tang Y, Li X, Shi Y, Mao J, Zhang H, Cai Q. Enhanced osteogenic differentiation of mesenchymal stem cells on poly(L-lactide) nanofibrous scaffolds containing carbon nanomaterials: enhanced osteocompatibility of nanofibrous scaffolds. *Journal of Biomedical Materials Research Part A* 2015; 103(4): 1424–1435. doi:10.1002/jbm.a.35283.
- [63] Zhang X, Meng S, Huang Y, Xu M, He Y, Lin H, Han J, Chai Y, Wei Y, Deng X. Electrospun gelatin/ $\beta$ -TCP composite nanofibers enhance osteogenic differentiation of BMSCs and *in vivo* bone formation by activating  $\text{Ca}^{2+}$ -sensing receptor signaling. *Stem Cells International* 2015; 2015: 1–13. doi:10.1155/2015/507154.
- [64] Kung FC, Lin CC, Lai WFT. Osteogenesis of human adipose-derived stem cells on hydroxyapatite-mineralized poly(lactic acid) nanofiber sheets. *Materials Science and Engineering: C* 2014; 45: 578–588. doi:10.1016/j.msec.2014.10.005.
- [65] Mertaniemi H, Escobedo-Lucea C, Sanz-Garcia A, Gandía C, Mäkitie A, Partanen J, Ikkala O, Yliperttula M. Human stem cell decorated nanocellulose threads for biomedical applications. *Biomaterials* 2016; 82: 208–220. doi:10.1016/j.biomaterials.2015.12.020.
- [66] Erisken C. Viscoelastic and biomechanical properties of osteochondral tissue constructs generated from graded polycaprolactone and beta-tricalcium phosphate composites. *Journal of Biomechanical Engineering* 2010; 132(9): 091013. doi:10.1115/1.4001884.
- [67] Münchow EA, Pankajakshan D, Albuquerque MTP, Kamocki K, Piva E, Gregory RL, Bottino MC. Synthesis and characterization of CaO-loaded electrospun matrices for

- bone tissue engineering. *Clinical Oral Investigations* 2015. doi:10.1007/s00784-015-1671-5.
- [68] Parizek M, Douglas TE, Novotna K, Kromka A, Brady MA, Renzing A, Voss E, Jarosova M, Palatinus L, Tesarek P, Ryparova P, Lisa V, dos Santos AM, Bacakova L. Nanofibrous poly(lactide-co-glycolide) membranes loaded with diamond nanoparticles as promising substrates for bone tissue engineering. *International Journal of Nanomedicine* 2012; 1931. doi:10.2147/IJN.S26665.
- [69] Joshi MK, Pant HR, Tiwari AP, Maharjan B, Liao N, kim HJ, Park CH, Kim CS. Three-dimensional cellulose sponge: fabrication, characterization, biomimetic mineralization, and in vitro cell infiltration. *Carbohydrate Polymers* 2016; 136: 154–162. doi:10.1016/j.carbpol.2015.09.018.
- [70] Yang M, Zhou G, Castano-Izquierdo H, Zhu Y, Mao C. Biomineralization of natural collagenous nanofibrous membranes and their potential use in bone tissue engineering. *Journal of Biomedical Nanotechnology* 2015; 11(3): 447–456. doi:10.1166/jbn.2015.2038.
- [71] Çakmak S, Çakmak AS, Gümüşderelioğlu M. RGD-bearing peptide-amphiphile-hydroxyapatite nanocomposite bone scaffold: an in vitro study. *Biomedical Materials* 2013; 8(4): 045014. doi:10.1088/1748-6041/8/4/045014.
- [72] Pasuri J, Holopainen J, Kokkonen H, Persson M, Kauppinen K, Lehenkari P, Santala E, Ritala M, Tuukkanen J. Osteoclasts in the interface with electrospun hydroxyapatite. *Colloids and Surfaces B: Biointerfaces* 2015; 135: 774–783. doi:10.1016/j.colsurfb.2015.08.045.
- [73] Jia X, Tang T, Cheng D, Zhang C, Zhang R, Cai Q, Yang X. Micro-structural evolution and biomineralization behavior of carbon nanofiber/bioactive glass composites induced by precursor aging time. *Colloids and Surfaces B: Biointerfaces* 2015; 136: 585–593. doi:10.1016/j.colsurfb.2015.09.062.
- [74] Liu Y, Sagi S, Chandrasekar R, Zhang L, Hedin NE, Fong H. Preparation and characterization of electrospun SiO<sub>2</sub> nanofibers. *Journal of Nanoscience and Nanotechnology* 2008; 8(3): 1528–1536.
- [75] Ganesh VA, Dinachali SS, Raut HK, Walsh TM, Nair AS, Ramakrishna S. Electrospun SiO<sub>2</sub> nanofibers as a template to fabricate a robust and transparent superamphiphobic coating. *RSC Advances* 2013; 3(12): 3819. doi:10.1039/c3ra22968h.
- [76] Potocký š., Ižák T, Rezek B, Tesárek P, Kromka A. Transformation of polymer composite nanofibers to diamond fibers and films by microwave plasma-enhanced CVD process. *Applied Surface Science* 2014; 312: 188–191. doi:10.1016/j.apsusc.2014.05.119.
- [77] Ruffinatto S, Girard HA, Becher F, Arnault JC, Tromson D, Bergonzo P. Diamond porous membranes: a material toward analytical chemistry. *Diamond and Related Materials* 2015; 55: 123–130. doi:10.1016/j.diamond.2015.03.008.
- [78] Ralchenko VG, Sovyk DN, Bolshakov AP, Homich AA, Vlasov II, Kurdyukov DA, Golubev VG, Zakhidov AA. Diamond direct and inverse opal matrices produced by

- chemical vapor deposition. *Physics of the Solid State* 2011; 53(6): 1131–1134. doi:10.1134/S106378341106028X.
- [79] Gao F, Nebel CE. Diamond-based supercapacitors: realization and properties. *ACS Applied Materials & Interfaces* 2015. doi:10.1021/acsami.5b07027.
- [80] Kabiri M, Oraee-Yazdani S, Shafiee A, Hanaee-Ahvaz H, Dodel M, Vaseei M, Soleimani M. Neuroregenerative effects of olfactory ensheathing cells transplanted in a multi-layered conductive nanofibrous conduit in peripheral nerve repair in rats. *Journal of Biomedical Science* 2015; 22(1) . doi:10.1186/s12929-015-0144-0.
- [81] Fraczek-Szczypta A. Carbon nanomaterials for nerve tissue stimulation and regeneration. *Materials Science and Engineering: C* 2014; 34: 35–49. doi:10.1016/j.msec.2013.09.038.
- [82] Kharaziha M, Shin SR, Nikkhah M, Topkaya SN, Masoumi N, Annabi N, Dokmeci MR, Khademhosseini A. Tough and flexible CNT–polymeric hybrid scaffolds for engineering cardiac constructs. *Biomaterials* 2014; 35(26): 7346–7354. doi:10.1016/j.biomaterials.2014.05.014.
- [83] Dolati F, Yu Y, Zhang Y, Jesus AMD, Sander EA, Ozbolat IT. In vitro evaluation of carbon-nanotube-reinforced bioprintable vascular conduits. *Nanotechnology* 2014; 25(14): 145101. doi:10.1088/0957-4484/25/14/145101.
- [84] Norouzi M, Shabani I, Ahvaz HH, Soleimani M. PLGA/gelatin hybrid nanofibrous scaffolds encapsulating EGF for skin regeneration: PLGA/gelatin hybrid nanofibrous scaffolds. *Journal of Biomedical Materials Research Part A* 2015; 103(7): 2225–2235. doi:10.1002/jbm.a.35355.
- [85] Lai HJ, Kuan CH, Wu HC, Tsai JC, Chen TM, Hsieh DJ, Wang TW. Tailored design of electrospun composite nanofibers with staged release of multiple angiogenic growth factors for chronic wound healing. *Acta Biomaterialia* 2014; 10(10): 4156–4166. doi:10.1016/j.actbio.2014.05.001.
- [86] Sridhar S, Venugopal JR, Ramakrishna S. Improved regeneration potential of fibroblasts using ascorbic acid-blended nanofibrous scaffolds: regeneration potential of ascorbic acid-blended nanofibrous scaffolds. *Journal of Biomedical Materials Research Part A* 2015; 103(11): 3431–3440. doi:10.1002/jbm.a.35486.
- [87] Bacakova M, Musilkova J, Riedel T, Stranska D, Brynda E, Bacakova L, Zaloudkova M. The potential applications of fibrin-coated electrospun polylactide nanofibers in skin tissue engineering. *International Journal of Nanomedicine* 2016: 771. doi:10.2147/IJN.S99317.
- [88] Srinivasan DK, Gandhimathi C, Venugopal JR, Bhaarathy V, Ramakrishna S. Biocomposite nanofibrous strategies for the controlled release of biomolecules for skin tissue regeneration. *International Journal of Nanomedicine* 2014: 4709. doi:10.2147/IJN.S65335.

- [89] Li J, Fu R, Li L, Yang G, Ding S, Zhong Z, Zhou S. Co-delivery of dexamethasone and green tea polyphenols using electrospun ultrafine fibers for effective treatment of keloid. *Pharmaceutical Research* 2014; 31(7): 1632–1643. doi:10.1007/s11095-013-1266-2.
- [90] Kim JI, Pant HR, Sim HJ, Lee KM, Kim CS. Electrospun propolis/polyurethane composite nanofibers for biomedical applications. *Materials Science and Engineering: C* 2014; 44: 52–57. doi:10.1016/j.msec.2014.07.062.
- [91] Park SJ, Lee BK, Na MH, Kim DS. Melt-spun shaped fibers with enhanced surface effects: fiber fabrication, characterization and application to woven scaffolds. *Acta Biomaterialia* 2013; 9(8): 7719–7726. doi:10.1016/j.actbio.2013.05.001.
- [92] Flaibani M, Elvassore N. Gas anti-solvent precipitation assisted salt leaching for generation of micro- and nano-porous wall in bio-polymeric 3D scaffolds. *Materials Science and Engineering: C* 2012; 32(6): 1632–1639. doi:10.1016/j.msec.2012.04.054.
- [93] Pokorny P, Kostakova E, Sanetnik F, Mikes P, Chvojka J, Kalous T, Bilek M, Pejchar K, Valtera J, Lukas D. Effective AC needleless and collectorless electrospinning for yarn production. *Phys Chem Chem Phys* 2014; 16(48): 26816–26822. doi:10.1039/C4CP04346D.
- [94] Orgill D, Blanco C, editors. *Biomaterials for treating skin loss*. Boca Raton, FL: CRC Press; 2009.
- [95] Wojtowicz AM, Oliveira S, Carlson MW, Zawadzka A, Rousseau CF, Baksh D. The importance of both fibroblasts and keratinocytes in a bilayered living cellular construct used in wound healing: two cell type importance in wound healing. *Wound Repair and Regeneration* 2014; 22(2): 246–255. doi:10.1111/wrr.12154.
- [96] Ng KW, Tham W, Lim TC, Werner Hutmacher D. Assimilating cell sheets and hybrid scaffolds for dermal tissue engineering. *Journal of Biomedical Materials Research Part A* 2005; 75A(2): 425–438. doi:10.1002/jbm.a.30454.
- [97] van Dorp AGM, Verhoeven MCH, Koerten HK, van Blitterswijk CA, Ponc M. Bilayered biodegradable poly(ethylene glycol)/poly(butylene terephthalate) copolymer (Polyactive?) as substrate for human fibroblasts and keratinocytes. *Journal of Biomedical Materials Research* 1999; 47(3): 292–300. doi:10.1002/(SICI)1097-4636(19991205)47:3<292::AID-JBM2>3.0.CO;2-B.
- [98] Idrus RH, Rameli MA bin P, Low KC, Law JX, Chua KH, Latiff MBA, Saim AB. Full-thickness skin wound healing using autologous keratinocytes and dermal fibroblasts with fibrin: bilayered versus single-layered substitute. *Advances in Skin & Wound Care* 2014; 27(4): 171–180. doi:10.1097/01.ASW.0000445199.26874.9d.
- [99] Marston WA, Sabolinski ML, Parsons NB, Kirsner RS. Comparative effectiveness of a bilayered living cellular construct and a porcine collagen wound dressing in the treatment of venous leg ulcers: effectiveness of BLCC vs. SIS in VLU. *Wound Repair and Regeneration* 2014; 22(3): 334–340. doi:10.1111/wrr.12156.

- [100] Monteiro IP, Gabriel D, Timko BP, Hashimoto M, Karajanagi S, Tong R, Marques AP, Reis RL, Kohane DS. A two-component pre-seeded dermal–epidermal scaffold. *Acta Biomaterialia* 2014; 10(12): 4928–4938. doi:10.1016/j.actbio.2014.08.029.
- [101] Wendt H, Hillmer A, Reimers K, Kuhbier JW, Schäfer-Nolte F, Allmeling C, Kasper C, Vogt PM. Artificial skin—Culturing of different skin cell lines for generating an artificial skin substitute on cross-weaved spider silk fibres. *PLoS One* 2011; 6(7): e21833. doi:10.1371/journal.pone.0021833.
- [102] Bacakova M, Lopot F, Hadraba D, Varga M, Zaloudkova M, Stranska D, Suchy T, Bacakova L. Effects of fiber density and plasma modification of nanofibrous membranes on the adhesion and growth of HaCaT keratinocytes. *Journal of Biomaterials Applications* 2015; 29(6): 837–853. doi:10.1177/0885328214546647.
- [103] Filová E, Brynda E, Riedel T, Chlupáč J, Vandrovová M, Švindrych Z, Lisá V, Houska M, Pirk J, Bačáková L. Improved adhesion and differentiation of endothelial cells on surface-attached fibrin structures containing extracellular matrix proteins: endothelial cells on surface-attached fibrin. *Journal of Biomedical Materials Research Part A* 2014; 102(3): 698–712. doi:10.1002/jbm.a.34733.
- [104] Xie J, MacEwan MR, Willerth SM, Li X, Moran DW, Sakiyama-Elbert SE, Xia Y. Conductive core-sheath nanofibers and their potential application in neural tissue engineering. *Advanced Functional Materials* 2009; 19(14): 2312–2318. doi:10.1002/adfm.200801904.
- [105] Wang Y fei, Guo H feng, Ying D jun. Multilayer scaffold of electrospun PLA-PCL-collagen nanofibers as a dural substitute: PLA-PCL-collagen as a dural substitute. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2013; 101(8): 1359–1366. doi:10.1002/jbm.b.32953.
- [106] Nedjari S, Eap S, Hébraud A, Wittmer CR, Benkirane-Jessel N, Schlatter G. Electrospun honeycomb as nests for controlled osteoblast spatial organization: electrospun honeycomb as nests for controlled. *Macromolecular Bioscience* 2014; 14(11): 1580–1589. doi:10.1002/mabi.201400226.
- [107] Valarezo E, Tammara L, Malagón O, González S, Armijos C, Vittoria V. Fabrication and characterization of poly(lactic acid)/poly( $\epsilon$ -caprolactone) blend electrospun fibers loaded with amoxicillin for tunable delivering. *Journal of Nanoscience and Nanotechnology* 2015; 15(6): 4706–4712. doi:10.1166/jnn.2015.9726.
- [108] Novotna K, Havelka P, Sopuch T, Kolarova K, Vosmanska V, Lisa V, Svorcik V, Bacakova L. Cellulose-based materials as scaffolds for tissue engineering. *Cellulose* 2013; 20(5): 2263–2278. doi:10.1007/s10570-013-0006-4.
- [109] Bačáková L, Novotná K, Pařízek M. Polysaccharides as cell carriers for tissue engineering: the use of cellulose in vascular wall reconstruction. *Physiological Research/Academia Scientiarum Bohemoslovaca* 2014; 63(Suppl. 1): S29–S47.

- [110] Bacakova L, Novotna K, Sopuch T, Havelka P. Cell interaction with cellulose-based scaffolds for tissue engineering—a review. In: *Cellulose and Cellulose Derivatives: Synthesis, Modification, Nanostructure and Applications*. Ibrahim H. Mondal (ed.), Nova Science Publishers, Inc., New York, USA, 2015, chapter 13, pp. 341–376, ISBN: 978-1-63483-571-8.
- [111] Kwak MH, Kim JE, Go J, Koh EK, Song SH, Son HJ, Kim HS, Yun YH, Jung YJ, Hwang DY. Bacterial cellulose membrane produced by *Acetobacter* sp. A10 for burn wound dressing applications. *Carbohydrate Polymers* 2015; 122: 387–398. doi:10.1016/j.carbpol.2014.10.049.
- [112] Bottan S, Robotti F, Jayathissa P, Hegglin A, Bahamonde N, Heredia-Guerrero JA, Bayer IS, Scarpellini A, Merker H, Lindenblatt N, Poulikakos D, Ferrari A. Surface-structured bacterial cellulose with guided assembly-based biolithography (GAB). *ACS Nano* 2015; 9(1): 206–219. doi:10.1021/nn5036125.
- [113] Song SH, Kim JE, Lee YJ, Kwak MH, Sung GY, Kwon SH, Son HJ, Lee HS, Jung YJ, Hwang DY. Cellulose film regenerated from *Styela clava* tunics have biodegradability, toxicity and biocompatibility in the skin of SD rats. *Journal of Materials Science: Materials in Medicine* 2014; 25(6): 1519–1530. doi:10.1007/s10856-014-5182-8.
- [114] Hu Y, Catchmark JM. Integration of cellulases into bacterial cellulose: toward bioabsorbable cellulose composites. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* 2011; 97B(1): 114–123. doi:10.1002/jbm.b.31792.
- [115] Lee SY, Bang S, Kim S, Jo SY, Kim BC, Hwang Y, Noh I. Synthesis and in vitro characterizations of porous carboxymethyl cellulose-poly(ethylene oxide) hydrogel film. *Biomaterials Research* 2015; 19(1) . doi:10.1186/s40824-015-0033-3.
- [116] Atila D, Keskin D, Tezcaner A. Cellulose acetate based 3-dimensional electrospun scaffolds for skin tissue engineering applications. *Carbohydrate Polymers* 2015; 133: 251–261. doi:10.1016/j.carbpol.2015.06.109.
- [117] Kim M, Kim G. 3D multi-layered fibrous cellulose structure using an electrohydrodynamic process for tissue engineering. *Journal of Colloid and Interface Science* 2015; 457: 180–187. doi:10.1016/j.jcis.2015.07.007.
- [118] Vatankhah E, Prabhakaran MP, Jin G, Mobarakeh LG, Ramakrishna S. Development of nanofibrous cellulose acetate/gelatin skin substitutes for variety wound treatment applications. *Journal of Biomaterials Applications* 2014; 28(6): 909–921. doi:10.1177/0885328213486527.
- [119] Novotna K, Zajdlova M, Suchy T, Hadraba D, Lopot F, Zaloudkova M, *et al.* Polylactide nanofibers with hydroxyapatite as growth substrates for osteoblast-like cells. *Journal of Biomedical Materials Research Part A* 2014; 102(11): 3918–3930. doi:10.1002/jbm.a.35061.

- [120] Behler KD, Stravato A, Mochalin V, Korneva G, Yushin G, Gogotsi Y. Nanodiamond-polymer composite fibers and coatings. *ACS Nano* 2009; 3(2): 363–369. doi:10.1021/nn800445z.
- [121] Bacakova L, Kopova I, Stankova L, Liskova J, Vacik J, Lavrentiev V, Kromka A, Potocky S, Stranska D. Bone cells in cultures on nanocarbon-based materials for potential bone tissue engineering: a review: bone cells in cultures on nanocarbon-based materials. *Physica Status Solidi (a)* 2014; 211(12): 2688–2702. doi:10.1002/pssa.201431402.
- [122] Potocky S, Kromka A, Potmesil J, Remes Z, Polackova Z, Vanecek M. Growth of nanocrystalline diamond films deposited by microwave plasma CVD system at low substrate temperatures. *Physica Status Solidi (a)* 2006; 203(12): 3011–3015. doi:10.1002/pssa.200671110.