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Chapter 3

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

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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 funcionalized 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_2 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 elecrospinning [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_3O_4 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_2 [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_2 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 α and β, platelet-derived growth factor, fibroblast growth factor, tumor necrosis factor α, and interferons α and β. 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 synthetize 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, sweet 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,

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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**).

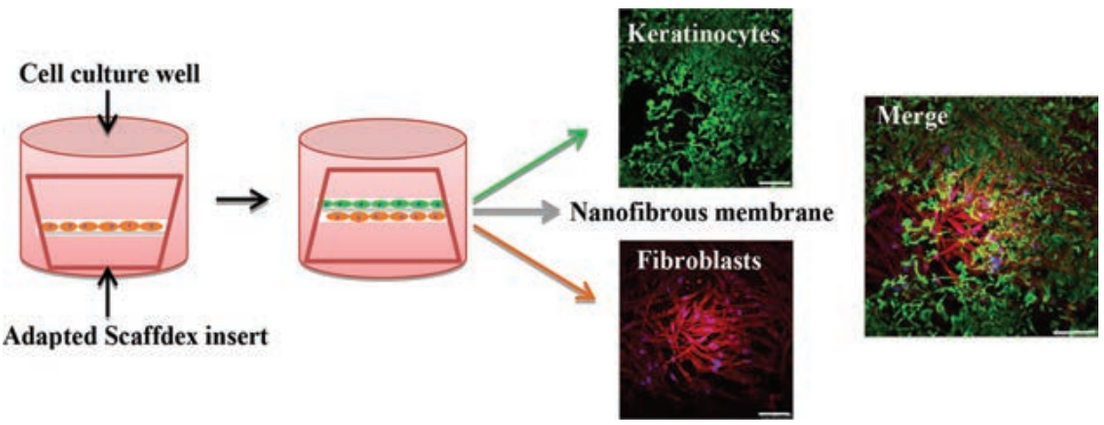

Figure 1. Development of a bilayered construct of HaCaT keratinocytes and neonatal human dermal fibroblasts on the opposite sides of a nanofibrous membrane (scale bar = 100 μm). The keratinocytes were stained by immunofluorescence against cytokeratin 5, an early marker of keratinocyte differentiation. The fibroblasts were stained with Texas Red C_2-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].

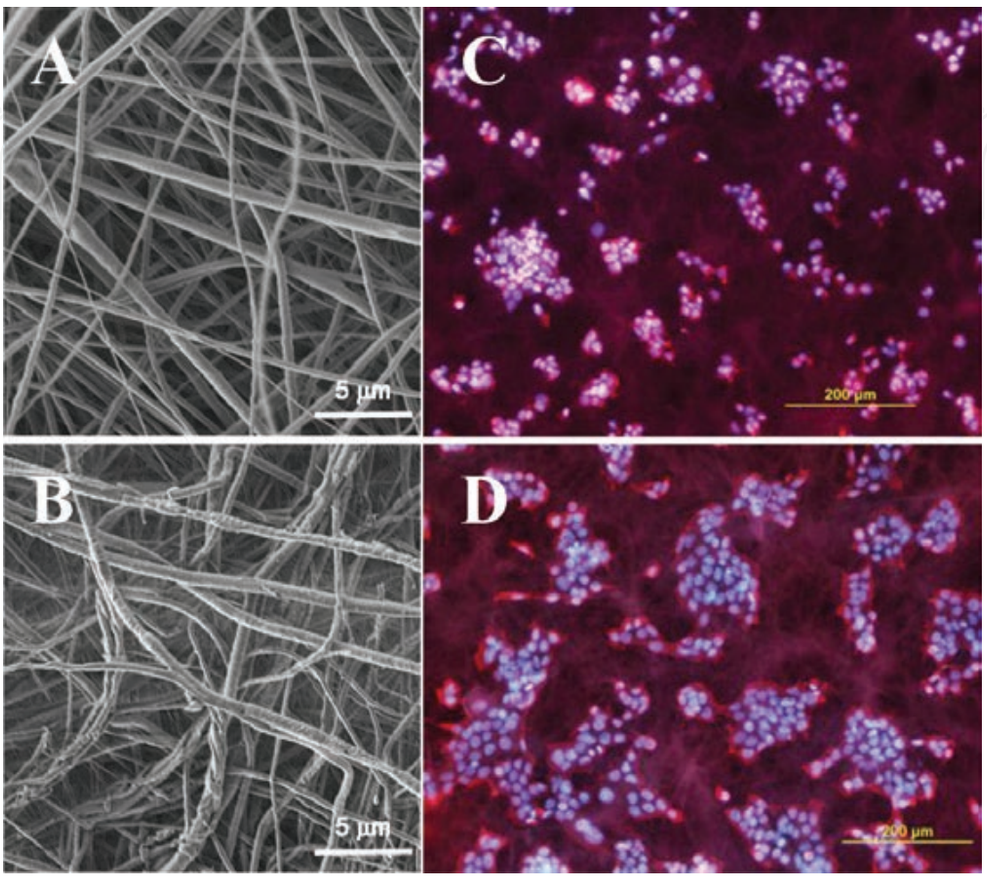

Figure 2. The morphology of unmodified nanofibrous polylactide scaffolds (A) and scaffolds treated by oxygen plasma (power 75 W, time 30 min, B), and the morphology of human HaCaT keratinocytes on these scaffolds (C and D). Note larger keratinocyte islands on plasma-treated scaffolds (D) than on untreated scaffolds (C). A, B: FE-SEM Tescan MIRA3 scanning electron microscope, objective magnification 10,000×, scale bar 5 μm. C, D: Cells stained with Texas Red C_2-Maleimide and Hoechst #33342. Olympus IX 51 microscope, obj. 10×, DP 70 digital camera, scale bar = 200 μm. 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].

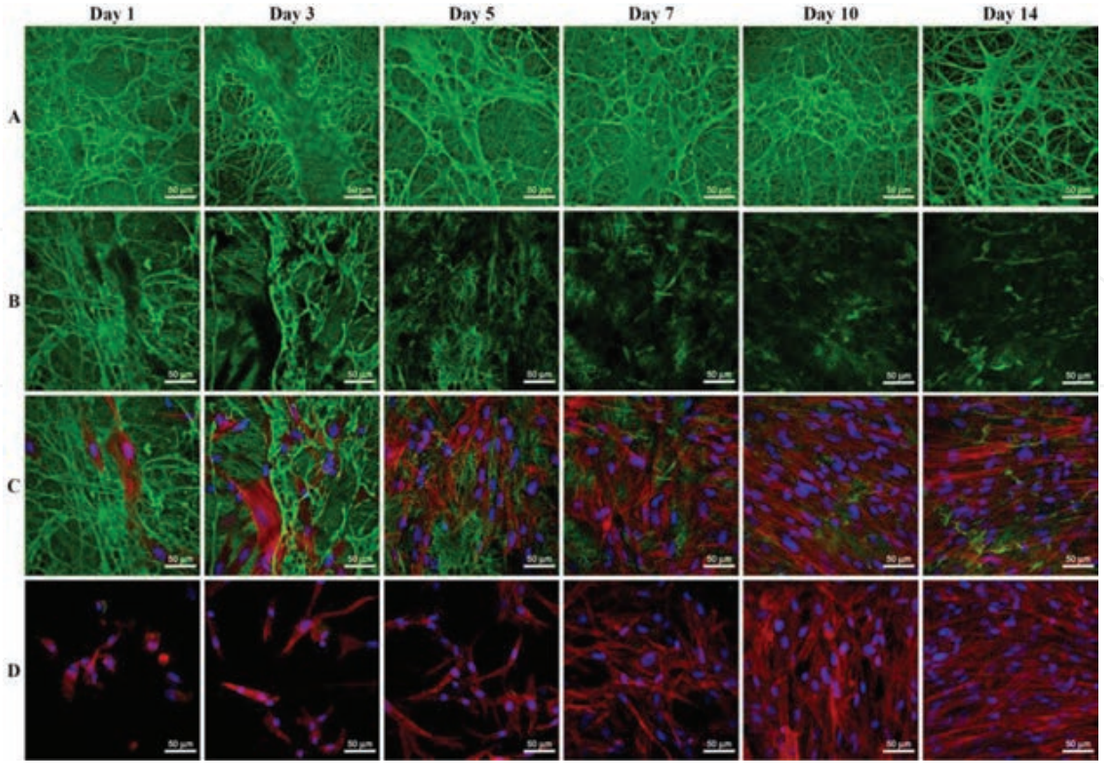

Figure 3. Morphology of fibrin coatings (green immunofluorescence) on nanofibrous PLA membranes in six time intervals incubated without cells at 37°C, 5% CO_2 (A), or incubated with human dermal fibroblasts (B—only fibrin, C—fibrin with cells). (D) Cells on non-modified PLA membranes. The cells were 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-ε-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^2), 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].

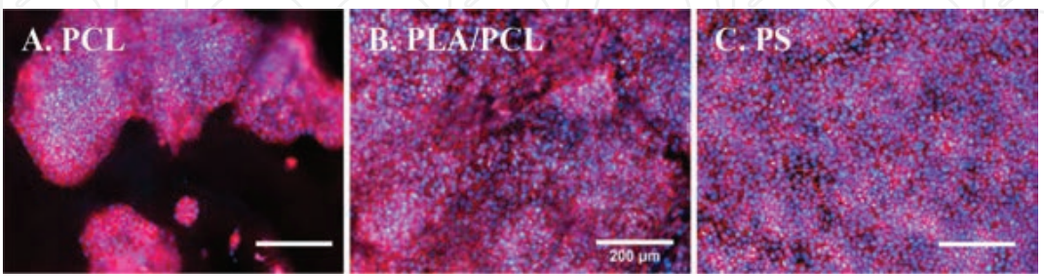

Figure 4. Human HaCaT keratinocytes on day 7 after seeding on nanofibrous membranes made (A) of poly-ε-caprolactone (PCL), (B) of a copolymer of PCL and polylactide (PLA/PCL) and (C) on standard cell culture polystyrene dishes (PS). Cells stained with phalloidin conjugated with TRITC (red fluorescence),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

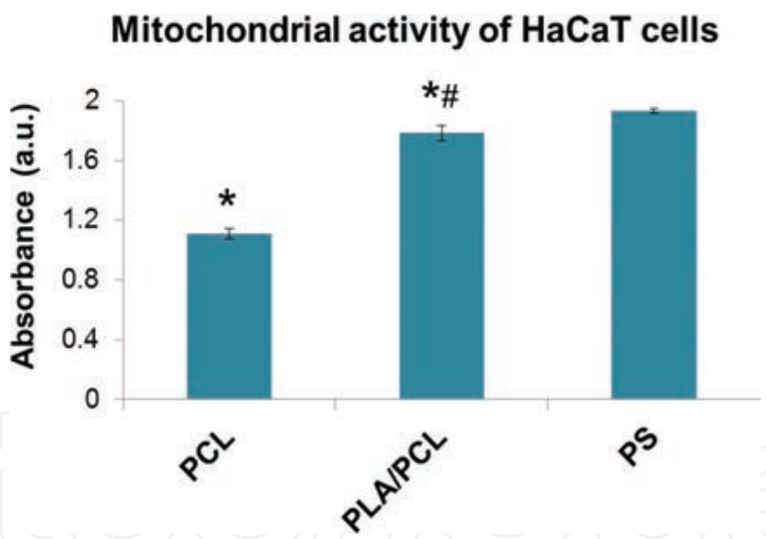

Figure 5. Activity of mitochondrial enzymes, measured by the WST-1 test in human HaCaT keratinocytes on day 7 after seeding on nanofibrous meshes made of poly-ε-caprolactone (PCL) and its copolymer with PLA (PLA/PCL), and on standard cell culsture polystyrene dishes (PS). Mean ± SEM (standard error of 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^2 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].

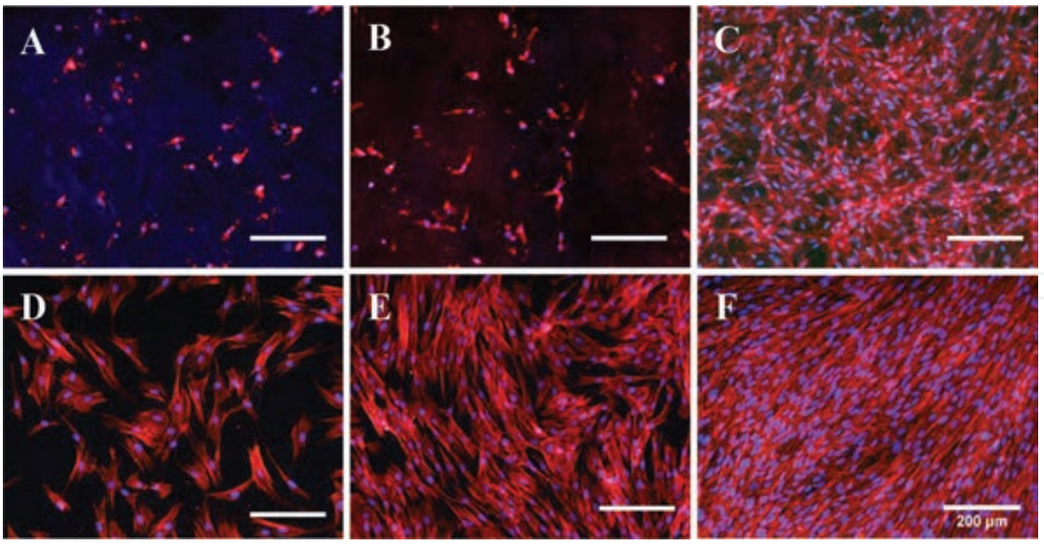

Figure 6. Human dermal fibroblasts on day 1 (A, D), day 3 (B, E), and day 7 (C, F) after seeding on nanofibrous membranes made of cellulose acetate (A–C) and on standard cell culture polystyrene dishes (D–F). Cells stained with phalloidin conjugated with TRITC (red fluorescence), and the cell nuclei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

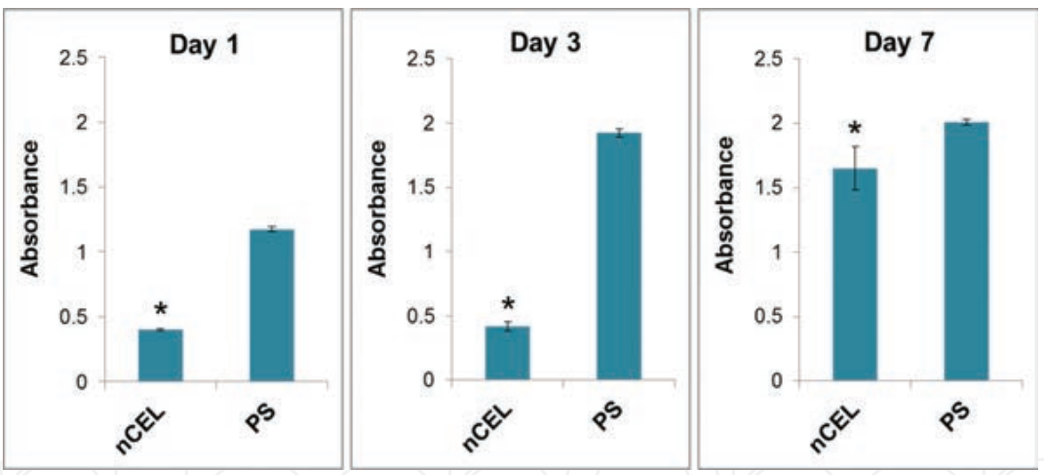

Figure 7. Activity of mitochondrial enzymes, measured by the WST-1 test in human dermal fibroblasts on days 1, 3, and 7 after seeding on nanofibrous membranes made of cellulose acetate (nCEL) and on standard cell culture polystyrene dishes (PS). Mean ± SEM (standard error of mean) from nine measurements 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_2 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**).

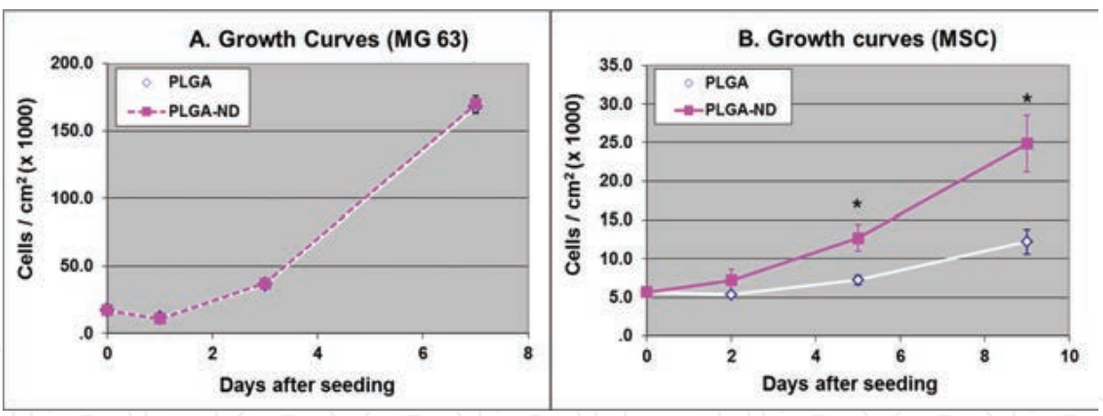

Figure 8. Growth curves of human osteoblast-like MG 63 cells (A) and human bone marrow mesenchymal stem cells (MSC, B) in cultures on pure poly(lactide-*co*-glycolide) (PLGA) scaffolds and scaffolds loaded with 23 wt.% of diamond nanoparticles (PLGA-ND). Mean ± SEM (standard error of mean) from 8 to 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 of 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 ± S.E.M. from 17 to 22 measurements for each experimental group and cell type. ANOVA, Student–Newman–KeulsStudent–Newman–Keuls method. Statistical significance: $^{A,\ B}p \leq 0.05$ 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_2 nanofibers prepared by electrospinning and then coated by a thin diamond film. SiO_2 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_2 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^2, 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_2 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_2 nanofibers. However, on day 4, the cell number on pure SiO_2 remained similar as on day 1, while on diamond-coated SiO_2 nanofibers, it increased, and the cells formed islands (**Figure 10**).

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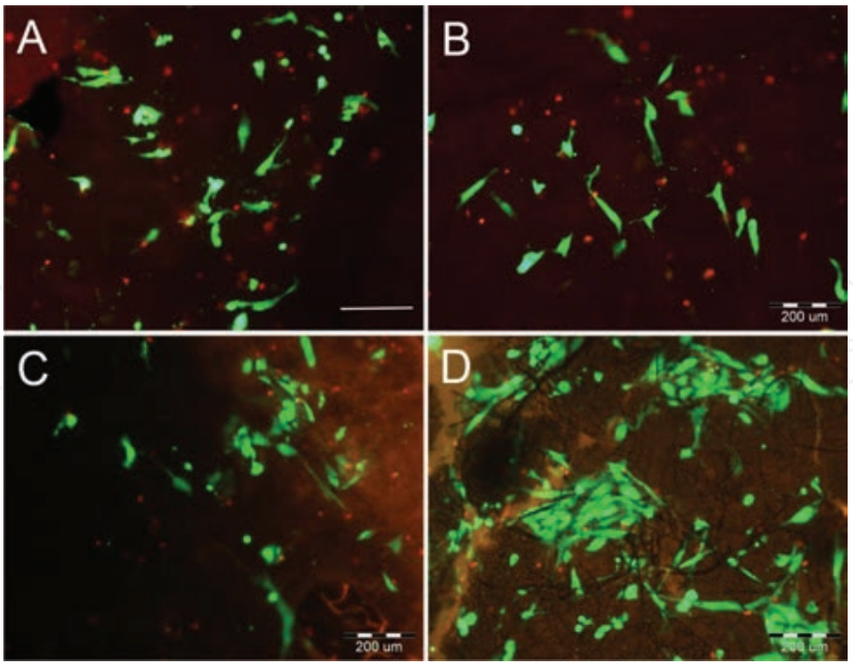

Figure 10. Human umbilical vein endothelial cells (HUVEC) cells grown on O-terminated SiO_2 nanofibers (A, B) and on O-terminated diamond-SiO_2 nanofibers on day 1 (A, C) and on day 4 (B, D) after seeding. The cells were stained with Live/Dead Viability/Cytotoxicity assay kit; living cells are stained in green and dead cells are stained in red. Olympus IX 71 microscope, IX71 digital camera, scale bar = 200 μm.

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_2 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_2 nanofiber, recently developed and tested by our group.

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