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Review

Electrospun Nanofibrous Membranes for Tissue Engineering and Cell Growth

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Abstract: In biotechnology, the field of cell cultivation is highly relevant. Cultivated cells can be used, for example, for the development of biopharmaceuticals and in tissue engineering. Commonly, mammalian cells are grown in bioreactors, T-flasks, well plates, etc., without a specific substrate. Nanofibrous mats, however, have been reported to promote cell growth, adhesion, and proliferation. Here, we give an overview of the different attempts at cultivating mammalian cells on electrospun nanofiber mats for biotechnological and biomedical purposes. Starting with a brief overview of the different electrospinning methods, resulting in random or defined fiber orientations in the nanofiber mats, we describe the typical materials used in cell growth applications in biotechnology and tissue engineering. The influence of using different surface morphologies and polymers or polymer blends on the possible application of such nanofiber mats for tissue engineering and other biotechnological applications is discussed. Polymer blends, in particular, can often be used to reach the required combination of mechanical and biological properties, making such nanofiber mats highly suitable for tissue engineering and other biotechnological or biomedical cell growth applications.

Keywords: cell growth; electrospinning; nanofibrous membrane; adherent cells; biomedicine

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1. Introduction

The cultivation of cells in T-flasks is a common method in biotechnology. For the classical cultivation of adherent cells in roller bottles or cell culture pellets, manufacturing costs are very high [1]. Microcarriers and hollow fibers are an alternative to the classical systems and can replace these bioreactor systems [2,3]. Such hollow fibers can be prepared from various polymers [4,5].

Today's single-use fixed-bed bioreactors provide consistent product quality from batch to batch, which is vital in pharmaceutical processes. With continuous bioprocesses, smaller production volumes and higher product concentrations are possible. Such ready-to-use bioreactors are packed with non-woven macroporous carriers or rolled membranes. The fabric often consists of polyethylene terephthalate (PET) microfibers, which are surface treated to make them hydrophilic and biocompatible for cell attachment [6–8].

There are, however, approaches to support cell growth more effectively, e.g., by using nanofiber mats as substrates for cell growth [9]. Such new nanostructured substrates can improve cell growth in tissue engineering and biotechnology by guiding cell growth and even improving stem cell differentiation. On the other hand, tissue engineering often necessitates 3D substrates, which cannot be reached by bioreactors or T-flasks, while nanofiber mats can be draped according to the requirements of an experiment.

Such nanofiber mats can be produced by electrospinning from various polymers and polymer blends, as well as polymers with nanoparticles included [10–12]. With electrospinning techniques being developed further, nanofiber mats have been increasingly used for biotechnological applications, including cell cultivation [13–16].

Besides the large specific surface of such nanofiber mats, specific electrospinning techniques or substrate preparations enable spinning oriented fibers, which is not only

supportive in terms of mechanical properties, but also for growing cells on these substrates in a defined orientation and in many cases with a higher proliferation rate [17–20].

Here, we give a brief overview of recent electrospinning techniques, followed by reviewing materials typically used for the preparation of electrospun nanofiber mats as scaffolds for mammalian cell growth. The focus is put on Chinese hamster ovary (CHO) cells, which are often used for experiments in such cell cultures, not only for the production of therapeutic proteins, but also as model cells for biotechnological studies [21–23].

2. Electrospinning

Generally, electrospinning is performed by introducing a polymer solution or melt into a strong electric field, either by pressing it through a needle, or by coating a wire or a rotating cylinder etc. with the fluid polymer. The latter, also called needleless technology, usually has a higher production performance and can often be scaled up from laboratory to industrial scale [24–26].

Using a static collector plate, the produced nanofiber mats usually contain incidentally oriented nanofibers [27,28]. These kinds of nanofiber mats are often used in biotechnology and biomedicine. As previously mentioned, aligned nanofiber mats can be advantageous for cell growth. They can be prepared by using a rapidly rotating collector along with blades [17,18,29]. For special cases, as with magnetic nanofibers, a magnetic field can be used to support fiber alignment [19,30], while introducing conductive, and ideally also grounded, areas in the substrate can also be used to prepare oriented nanofiber mats [31]. Another method for modifying the electric field during electrospinning is given by adding dielectric coatings on parts of the substrate. Nguyen et al. report on a cylindrical collector rotating at high speed that is shielded by two dielectric films [32]. This diverts the airflow due to the cylinder rotation and also amplifies the electronic field in a thin gap between them, in this way providing alignment and position control of the fibers. Moreover, nanofiber layers partially grown on conductive structures and partly consolidated can lead to an interesting mixture of aligned and chaotic layers, with different mechanical properties [33].

Electrospinning of nanofiber mats can be carried out from different polymers and polymer blends [34–36]. Some polymers can be electrospun from an aqueous solution, which makes the process very simple and environmentally friendly. However, a crosslinking step is then required if the nanofiber mats are to be used in humid environments [37–40]. Most of the water-resistant polymers need to be spun from toxic or corrosive solvents, while only some of them can also be electrospun from low-toxic solvents such as dimethyl sulfoxide (DMSO) [41]. The spinnability from DMSO is one of the reasons that polyacrylonitrile (PAN) is often used in electrospinning [16–18,42–44]. The possibility of using PAN as a precursor for carbon nanofibers is another reason why this material is often electrospun [45–47]. The broad range of spinnable and co-spinnable polymers and other materials makes nanofiber mats attractive for applications in many fields, not only in biotechnology and biomedicine [13,15,16,48–50]. Here, however, we concentrate on materials applicable for tissue engineering and other cell growth applications.

3. Materials for Tissue Engineering and Cell Growth Experiments

Generally, the most important prerequisite of a material usable for tissue engineering, or more generally as a scaffold for cell growth, is its biocompatibility. Interestingly, this does not necessarily mean that all substances used during electrospinning and a possible post-treatment step, e.g., crosslinking, have to be nontoxic themselves, as directly visible by the often highly toxic crosslinking chemicals which nevertheless can be used to prepare nontoxic nanofiber scaffolds [40]. Besides biocompatible in the sense of “bioinert” materials, there have also been attempts to achieve a positive interaction between substrate and cells [51]. On the other hand, it is necessary to distinguish between biodegradable and permanent materials, which can be used for different purposes.

In the next sections, we will give an overview of some often used and highly interesting materials used for the electrospinning of scaffolds for cell growth, such as poly(lactic acid) (PLA), polyurethane, poly(acrylonitrile) (PAN), poly(ε-caprolactone) (PCL), collagen and gelatin, chitosan and chitin, and others. It should be mentioned that due to the large bandwidth of possible polymers and polymer blends applicable as electrospun scaffolds, our choice of a few of them is necessarily subjective. However, we hope to show some interesting blends of well-known materials and give some inspiration for researchers starting with this topic.

4. Gelatin and Collagen

Collagen belongs to the native extracellular matrix (ECM) of the human body. The ECM is responsible for providing the mechanical structure of tissues, as well as for wound healing and controlling other cellular processes [52]. Thus, collagen belongs to the highly interesting materials for tissue engineering. Gelatin, which is derived from collagen by partial hydrolysis, has partly similar and partly different physical properties, varying depending on the origin and processing of the material [53,54].

As usual for many biopolymers, collagen and gelatin are water-soluble and thus need a crosslinking step [40]. Alternatively, the water-resistance and mechanical properties can be enhanced by blending them with water-stable polymers. This is why many studies found in the literature report on blends of collagen or gelatin with other polymers.

For example, Chen et al. prepared polyurethane/collagen core-shell nanofibers by coaxial electrospinning that were crosslinked by glutaraldehyde (GA) vapor [55]. Thermoplastic polyurethanes (TPUs) are known to have not only good mechanical properties, but also good biocompatibility, resulting in their frequent use in implants, catheters, etc. [56]. Special medical-grade TPU can additionally avoid in vivo degradation [57]. The coaxial electrospinning technique used here allows spinning two different polymers through the inner and the outer part of the needle, driven by two independent syringe pumps and fed from separate reservoirs [55]. In comparison with pure collagen, the core-shell fibers showed significantly improved mechanical properties, combined with the highest viability of pig iliac endothelial cells (PIECs) cultured on the different electrospun nanofiber mats, even higher than on pure collagen fibers. All nanofiber mats showed a better cell viability than reference tests on coverslips. The authors mentioned that the fiber diameters, mat porosity, and mechanical properties of the nanofiber mats significantly influenced cell growth and migration, which resulted in compound nanofibers—especially produced by coaxial electrospinning of collagen around TPU—having the best growth conditions. They also mentioned that the coaxially collagen-coated TPU fibers were much more attached cells, while simply coating a pure TPU nanofiber mat macroscopically with collagen did not have the same effect of 3D ingrowth into the scaffold, showing the importance of combining material and structure. Similar results were found by the same group with TPU/collagen blended nanofibers [58].

A polymer blend from collagen and poly((D,L-lactic)-*co*-glycolide) (PLGA) was investigated by Yang et al. [59]. PLGA belongs to the synthetic biodegradable polymers, which are often studied for tissue engineering applications, but usually show insufficient cell adhesion and proliferation if used purely [60]. Blending PLGA with collagen, however, led to electrospun nanofiber mats which did not need to be crosslinked to avoid swelling in culture medium and on which human dermal fibroblasts (HDFs) and mice fibroblasts containing green fluorescent protein (GFP) showed a significantly higher viability than on pure PLGA nanofiber mats [59]. This was attributed to the chemical composition of the matrix material, since the fiber diameters were kept constant in all nanofiber mats. The authors explained that collagen and its integrin-binding domains facilitated the cell attachment for anchorage-dependent cell types as the fibroblasts used in this study. A comparison with tissue culture plates additionally showed much higher cell numbers inside the nanofibrous mats due to the available void volume.

Quite a different approach was chosen by Akhshabi et al., who combined collagen with chondroitin sulfate, a negatively sulfated glycosaminoglycan [61]. The electrospun collagen/chondroitin nanofiber mat was cross-linked with EDC/NHS (1-ethyl-3-(3-dimethylaminopropyl)-1-carbodiimide hydrochloride/*N*-hydroxy succinimide), before cytocompatibility was investigated using corneal epithelial cells. Crosslinking was found to significantly increase the biostability of the nanofiber mats, while chondroitin was reported to significantly promote cell proliferation, as compared to pure collagen. This was explained by the role of chondroitin sulfate, which induces skin tissue regeneration [62], osteoblast adhesion [63], and fibroblast proliferation [64].

Due to the chemical similarity of gelatin and collagen, gelatin can be expected to show similar effects in increasing cell adhesion and proliferation. Baiguera et al. used genipin cross-linked gelatin nanofiber mats as the base to include decellularized rat brain extracellular matrix as an active agent, before mesenchymal stromal cells (MSCs) from rat bone marrow were seeded onto these scaffolds [65]. They found the MSCs grew not only on top of the substrate, but also in the inner volume of the decellularized samples. Seeding these cells on pure gelatin nanofiber mats and those with an additional decellularized rat brain extracellular matrix showed growth on both sides of the nanofiber mats in multilayered cultures, especially for the second tissue. Incorporation of a decellularized rat brain extracellular matrix into gelatin nanofiber mats, however, was found to trigger differentiation of MSCs towards neural/glial precursor cells.

An interesting approach was chosen by Li et al., who blended gelatin with the conductive polymer polyaniline (PAni) to prepare conductive electrospun scaffolds [66]. With conductive scaffolds, cell attachment, proliferation, migration, and even differentiation can be stimulated [67]. PAni, on the other hand, is known to be biocompatible [68] and thus a good candidate to make a nanofiber mat conductive. Li et al. seeded rat cardiac myoblast cells on these electrospun nanofiber mats and found they supported cell attachment and proliferation with different amounts of PAni, as compared to glass and other substrates, but also in comparison with pure gelatin nanofiber mats [66]. They found the cell morphology depended on the substrate structure, with cells growing on fibers with larger diameters showing microfilament-rich pseudopodia attached to single fibers (Figure 1a–c), while cells on thinner fibers were more spread out and exhibited a smooth muscle-like morphology (Figure 1d), similar to cells grown on glass substrates (Figure 1e) [66]. This was attributed to the higher roughness of the fibrous substrates, which provided a larger surface area on which cells could attach, as compared to smooth glass substrates and tissue culture-treated polystyrene, which is commonly regarded as the gold-standard.

Like collagen, gelatin can also be blended with water-resistant or slowly degrading polymers to reduce or avoid crosslinking. Gautam et al. report about PCL/gelatin blended nanofiber mats used for tissue engineering [69]. They seeded mouse fibroblast cells on nanofiber mats with different blend ratios and found significantly increased cell viability and cell proliferation for the blends as compared to pure PCL scaffolds. This was attributed to the presence of gelatin in these nanofiber mats, which is generally known to result in good cell adhesion and proliferation. Combinations of gelatin with PCL are thus often found in the literature [70–72].

There are, however, many other gelatin blends reported to be advantageous. Comparing PLGA and PLGA/gelatin blended nanofiber mats, Meng et al. found increased cell viability on PLGA/gelatin blends compared to pure PLGA, and in addition they also found better MC3T3-E1 cell proliferation and guidance for aligned nanofibers than for randomly oriented ones [73]. They mentioned that avoiding crosslinking induced easier cell attachment to the scaffolds, as crosslinking decreased bioactivity and hydrophilicity of the nanofiber mats. Ba Linh et al. investigated polyvinyl alcohol (PVA)/gelatin nanofiber mats after physical crosslinking by methanol and reported osteoblasts to attach firmly on these scaffolds, facilitated by gelatin and the crosslinking process [74]. Moreover, the hydrophilic nature of PVA and gelatin, as well as the interconnected porous structure of the nanofiber mats, were found to be advantageous for cell proliferation and multilayer growth. A special

polyurethane "Tecophilic" was chosen by Vatankhah et al. as a blend partner for gelatin to produce tubular scaffolds for blood vessels, which showed good mechanical properties, higher cell viability and proliferation than pure polyurethane, and good compatibility with blood [75]. Here again, a deep cellular infiltration into the nanofibrous scaffold was found, which would be impossible on the common flat surfaces, allowing for a larger number of attached cells per area. In addition, triple-blends including gelatin were tested for tissue engineering applications [76–78].

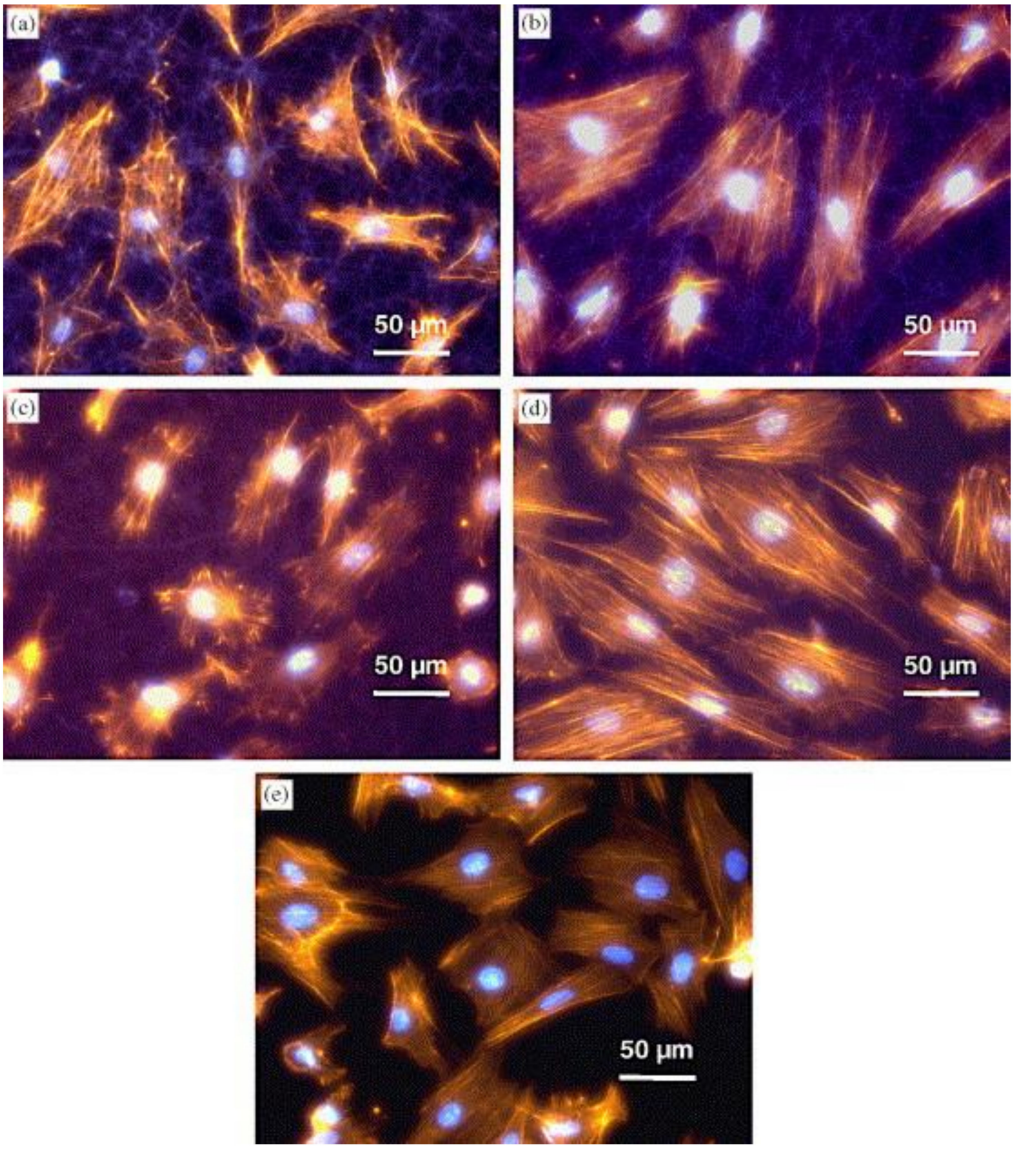

Figure 1. Morphology of myoblast cells 20 h after seeding on: (**a**) gelatin nanofiber mat; (**b**) 15:85 PAni-gelatin blend nanofibers; (**c**) 30:70 PAni-gelatin blend nanofibers; (**d**) 45:55 PAni-gelatin blend nanofibers; and (**e**) glass matrices. Staining for nuclei-bisbenzimide and actin cytoskeleton-phalloidin. Reprinted from [66], with permission from Elsevier.

5. Chitosan and Chitin

Chitin belongs to the highly abundant natural polymers and is mainly found in marine crustaceans, shrimps, and crabs. It is insoluble in common solvents. Chitosan, on the other hand, is an important derivative of chitin, obtained by partial deacetylation of chitin under alkaline conditions or by enzymatic hydrolysis and is soluble in acidic aqueous media [79].

In spite of the problems in dissolving chitin, it is regularly reported in the literature as a possible substrate material for tissue engineering [80], since it shows similar properties to glycosaminoglycan, which is part of the ECM [81]. Noh et al. thus prepared electrospun chitin nanofiber mats [81]. To dissolve the chitin powder, it was first irradiated by Co^{60} γ-irradiation and then dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) for 20 days. Electrospinning resulted in ultrafine nanofibers with an average diameter 163 nm. These nanofiber mats degraded in vitro faster than chitin microfibers, promoted cell attachment and spreading of normal human keratinocytes and fibroblasts better than the chitin microfibers, and after coating with type I collagen also promoted cellular response in all cells under investigation. The authors interpreted these findings as a higher functional activity of collagen-coated chitin nanofibers in terms of cell attachment and spreading for normal human keratinocytes as well as fibroblasts. In a similar manner, Min et al. prepared a chitin solution by γ-irradiation and dissolving the resulting chitin in HFIP [82]. In addition, they investigated deacetylation for transforming the original chitin matrix into a chitosan matrix.

Most groups, however, combine chitin or its derivatives with other polymers. For example, Shalumon et al. blended the water-soluble carboxymethyl chitin with PVA to prepare an aqueous electrospinning solution [83]. The resulting nanofiber mats were crosslinked by glutaraldehyde vapor before human mesenchymal stem cells (hMSCs) were seeded on them. The cells were found to attach and spread in the washed nanofiber mats, which supported cell adhesion and proliferation (Figure 2).

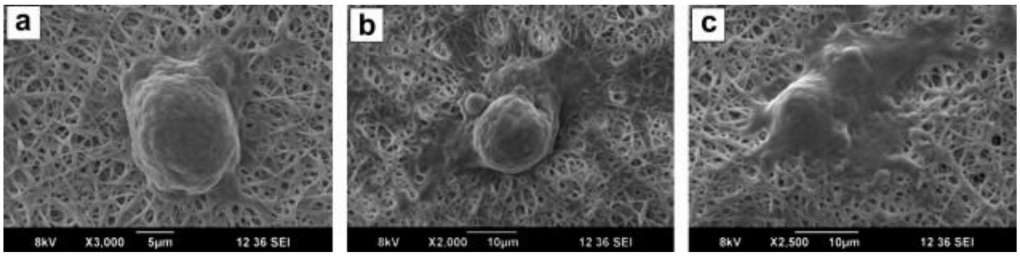

Figure 2. SEM images of hMSCs attached on the surfaces of CMC/PVA scaffolds after (**a**) 12 h; (**b**) 24 h; and (**c**) 48 h of incubation. Reprinted from [83], with permission from Elsevier.

Pangon et al. prepared chitosan/PVA nanofibers with chitin whiskers in different concentrations from succinic acid/water as solvent [84]. The resulting nanofiber mats were crosslinked by GA vapor, followed by mineralizing hydroxyapatite onto them by applying ten-fold concentrated simulated body fluid and seeding MC3T3-E1 osteoblast cells. They found an increased cell viability and proliferation due to the addition of the chitin whiskers, as compared to pure chitosan/PVA nanofiber mats. This effect was attributed to the clearly modified surface of the nanofiber mats, which showed a rough, uneven structure due to the hybridization with hydroxyapatite, and resulting in significantly improved cell viability and proliferation.

Electrospun nanocomposites of chitin nanofibrils and PCL were prepared by Ji et al. [85]. The chitin nanofibrils were dispersed in 2,2,2-trifluoroethanol (TFE) in which PCL could also be dissolved, allowing for spinning nanofibers with different chitin:PCL ratios, which showed strongly decreasing average diameters with increasing chitin content. For high chitin nanofibril contents, the water contact angle was significantly reduced. When seeding human dermal fibroblasts (hDF) on these nanofiber mats, the nanofiber mats with high chitin content, in particular, showed cell penetration and migration inside the scaffold; oppositely to pure PCL nanofiber mats, where the cells stayed on the surface. This finding is similar to those reported in other papers, where 3D structures and increased surface areas generally supported cell growth due to the improved possibility of cell infiltration and migration into the scaffold. The authors mentioned that for this effect, the pores in the electrospun nanofiber mats do not have to be larger than the cell diameters, but cells were found to perform amoeboid movements to migrate even through smaller pores by pushing

the neighboring fibers away. Instead, hydrophilicity and biochemical signals were found to be more important to promote cell ingrowth than perfectly fitting pore sizes.

Min et al. used an electrospinning method based on two separate syringes to prepare PLGA nanofibers with chitin electrosprayed nanoparticles [86]. They seeded normal human oral keratinocytes and normal human epidermal keratinocytes on these nanofiber composites and found the PLGA/chitin nano-composite fibers to be well suitable for tissue engineering scaffolds, due to their biomimetic 3D structure, similar to the collagen/glycosaminoglycan composite structure in the extracellular matrix.

Another blend was investigated by Park et al., who used electrospinning to prepare chitin/silk fibroin (SF) blends from HFIP as solvent [87]. Normal human epidermal keratinocytes (NHEK) and fibroblasts (NHEF) were seeded onto these nanofiber mats after crystallization of the SF fraction in water vapor. While for attachment of NHEK cells, the reference polystyrene substrate performed best, cell spreading of NHEF cells was superior on chitin/SF nanofiber mats, as visible in Figure 3. This finding was explained by the high biocompatibility and other supportive properties of chitin and SF.

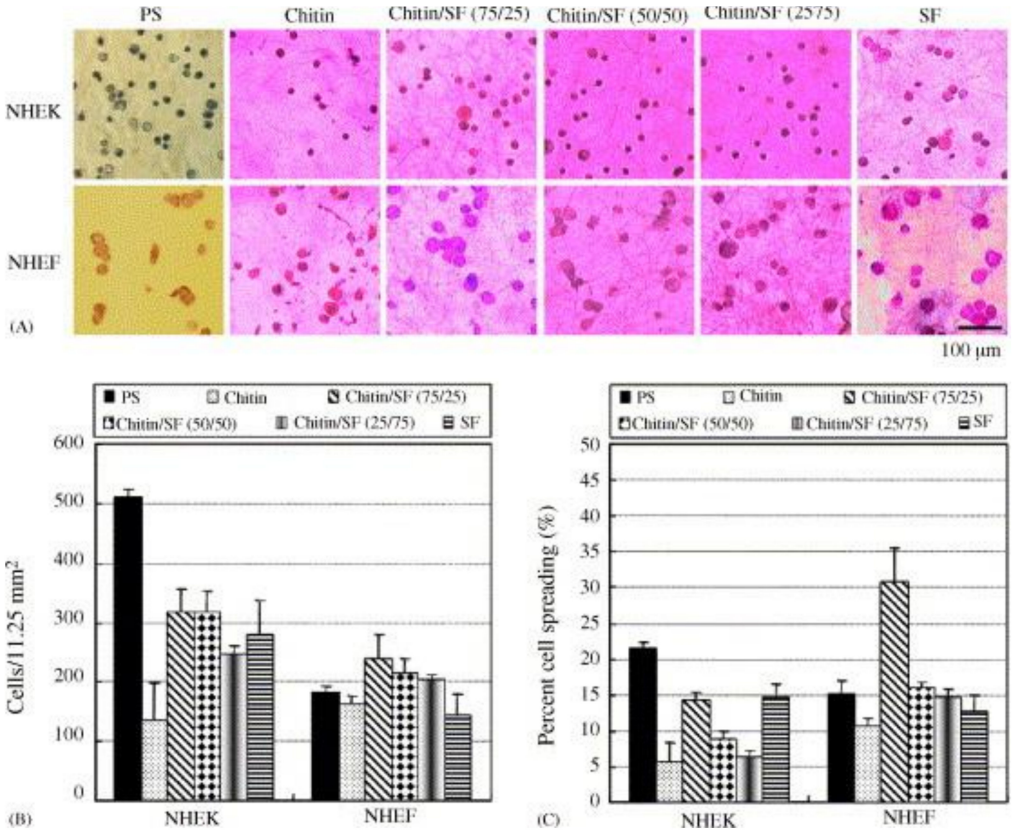

Figure 3. (**A**) Photographs and (**B**) numbers of NHEK and NHEK adhered on the substrates; (**C**) percentage of cell spreading (n = 4). Reprinted from [87], with permission from Elsevier.

As mentioned before, chitosan combines many of the positive physical and chemical properties of chitin with water-solubility, making it more easily electrospinnable. This is why diverse groups investigated electrospun chitosan or chitosan blend fibers. A recent review of electrospinning chitosan-based solutions for tissue engineering was given by Qasim et al. [88]. Some exemplary blend partners for chitosan are poly(ethylene oxide) PEO [39,89], collagen [90], PVA [91,92], PCL [93], and hydroxyapatite [94].

After this brief overview of materials correlated to the extracellular matrix, the next sections will show progress in using electrospun man-made polymers as scaffolds in tissue engineering.

6. PLA

PLA is a biodegradable polyester which is produced from renewable resources and used in many applications; from biomedicine to 3D printing. PLA is rarely electrospun solely [95], but mostly in combination with diverse blend partners. For example, Xu et al. used an alginate/PLA blend for emulsion electrospinning [96]. After making the sodium alginate water-insoluble by ion exchange of Na^+ in a $CaCl_2$ solution, these nanofiber mats showed significantly increased mechanical properties, as compared to pure PLA, as well as significantly enhanced hydrophilicity. The latter point was reflected by significantly increased cell proliferation and cell differentiation on alginate/PLA nanofiber mats, which was also supported by structures in the nanofiber mats similar to the ECM, including a rougher fiber surface than in pure PLA fibers. Similar results were obtained by Ye et al., working with periodontal ligament cells and bone marrow stromal cells [97].

Carbonated calcium deficient hydroxyapatite (CDHA) was blended with PLA by Zhou et al. [98]. They used chloroform and dimethylformamide (DMF) as solvent for the electrospinning process. The PLA/CDHA nanofiber mats degraded faster than pure PLA fibers and allowed for more uniform apatite coating, besides buffering the pH decrease that usually occurs during PLA degradation. Furthermore, they showed improved bioactivity and biocompatibility, as compared to pure PLA nanofiber mats. The improved cell attachment and proliferation was attributed to the CDHA forming a similar crystalline structure, as in deproteinated bone apatite.

To combine the mechanical properties of PLA with cell recognition properties of some biopolymers, Gugutkov et al. blended PLA with fibrinogen [99]. Seeding human umbilical endothelial cells on the received nanofiber mats, they found good adhesion and reduced cell movement in randomly oriented nanofiber, but elongated cell shape and significantly increased cell mobility on aligned nanofibers. They verified this finding by testing the artificial wound coverage, which was found to be superior for the aligned samples, while directional migration was almost not visible on the random nanofiber mats. On the other hand, randomly spun nanofiber mats were suggested for endothelization of implants, as opposed to directed nanofibers, which could be used for guided neovascularization. Here, fiber orientation was clearly the reason for the different growth of the cells, since the residual parameters were kept constant.

Another approach was chosen by Zhou et al., who used maleic anhydride grafted PLA as a matrix with incorporated cellulose nanocrystals (CNCs) [100]. They found decreased nanofiber diameters with increasing CNC content, improved thermal and mechanical stability, as well as higher stability during in vitro degradation. Additionally, by seeding human adult adipose derived mesenchymal stem cells, the nanofiber mats were found to be nontoxic and to support cell proliferation.

A multi-blend of PLA, human-like collagen (HLC), chitosan, and PEO in different ratios and spun through two needles, was investigated by Zhu et al. [101]. They found that the blended fibers had a smaller diameter than pure PLA and a larger one than HLC/chitosan/PEO alone. Depending on the amount of HLC, chitosan, and PEO, they found varying hemocompatibility and better biocompatibility than for pure PLA, which was suggested to result from the interaction between chitosan and blood cells.

An approach without biopolymers was reported by Vaz et al., who spun PLA/PCL bilayer nanofiber tubes with an inner PCL layer between outer PLA layers and used 3T3 mouse fibroblasts, as well as human venous myofibroblasts, to test cell attachment, proliferation, and migration [102]. This scaffold was shown to allow for attachment, spreading, and growth of mouse fibroblasts and human myofibroblasts. For future research, they suggested controlling the pore size, porosity, and fiber diameters, as well as testing the structural integrity during degradation, since these parameters were assumed to mostly influence the cell attachment and growth.

Another polymer blend was reported by Abudula et al., who combined PLA with poly(butylene succinate) (PBS) with a reinforcement by cellulose nano-fibrils [103]. They found increased protein adsorption for the blended nanofiber mats and accelerated biodegra-

dation under proteinase K, as compared to pure PLA, as well as good wetting; all together resulting in good cell attachment and proliferation.

Blending a PLA-PEG (poly(ethylene glycol) block copolymer with PLGA, Luu et al. used electrospun scaffolds as the base for a polymer/plasmid DNA composite [104]. Such scaffolds could be used for therapeutic applications in gene delivery. They found a sustained release of intact DNA over three weeks, with a maximum burst after approx. 2 h. The bioactivity of the DNA was tested by transfection of pre-osteoblastic cells. In addition, the mechanical properties of these nano-composites were similar to those of skin and cartilage.

Coaxial electrospun PLA/PVA nanofibers were investigated by Alharbi et al., who compared both combinations of core and shell with pure PLA and pure PVA fibers [105]. In comparison with pure PVA, the core-shell nanofibers showed significantly reduced water contact angles and an increase in tensile strength and strain at failure. PLA/PVA core/shell nanofibers, in particular, showed good attachment and proliferation of human embryonic kidney (HEK) cells, as visible in Figure 4, and due to the improved hydrophilicity and good water stability of these nanofiber mats.

Besides PLA, there are other man-made polymers that are typically used for tissue engineering.

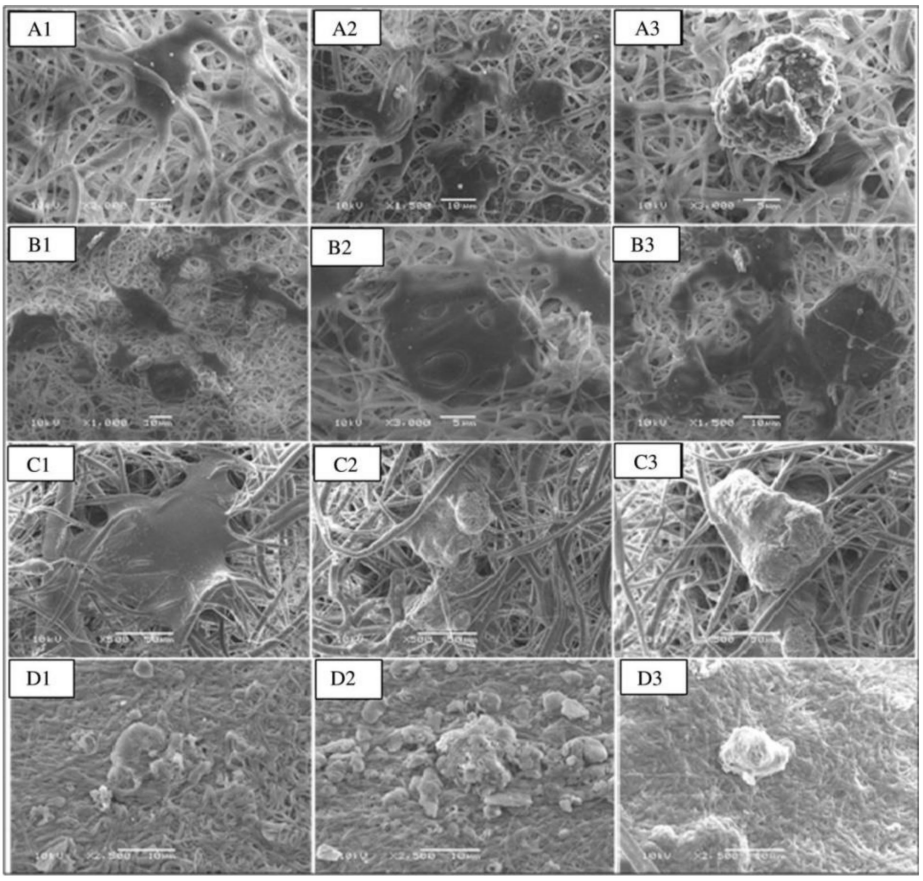

Figure 4. Scanning electron microscopy (SEM) images of HEK cells on a (**A1**–**A3**) PLA/PVA core/shell; (**B1**–**B3**) PVA/PLA core/shell; (**C1**–**C3**) pure PLA; (**D1**–**D3**) pure PVA nanofiber mats. Reprinted from [105], with permission from Elsevier. Each rows shows different locations or magnifications for the same material.

7. PCL

PCL was reported in the previous sections as an often used blend partner for different polymers. The material is sometimes used to prepare pure electrospun nanofiber mats for tissue engineering [106–108]; however, in many cases blends are produced to combine the advantages of two or more materials.

Combining PCL with PLGA, Hiep and Lee used electrospinning to prepare nanofiber mats [109]. They found an increasing biocompatibility with increasing amount of PLGA in the blend, and it even showed a higher proliferation than on common tissue culture plates. This was attributed to the PLGA support in the PCL nanofiber mats, which apparently increased biocompatibility, cell attachment, and proliferation. In addition, the blended nanofiber mats showed higher mechanical strength than pure PCL.

To reach a higher biodegradation of a PCL nanofiber mat, Kim et al. blended it with poly(*N*-vinyl-2-pyrrolidone) (PVP), which could be washed out in cell culture medium, resulting in nanoporous fibers [110]. This nanoporosity, not only supported biodegradation, but also the attachment and spreading of adipose derived stem cells (ADSCs). The finding that the number of elongated cells decreased with increasing PVP content was explained by changes in the cytoskeletal organization influencing the cell response and the chemical composition, as well as morphology, of the substrate affecting the cytoskeletal organization.

For bone tissue engineering, Heydari et al. used PCL nanofiber mats with embedded octacalcium phosphate (OCP) particles [111]. On the one hand, the average fiber diameter was significantly reduced by adding OCP, which was attributed to the increased solution conductivity during electrospinning, as a result of the additional OCP particles. This was correlated with a slight increase in the ultimate tensile stress, strain, and Young's modulus. Moreover, PCL/OCP was found to form a hydroxyapatite layer on its surface when soaked in simulated body fluid (SBF). Seeding human osteoblast cells on the scaffold showed a positive influence of OCP on their growth due to both these factors.

On the other hand, Rad et al. combined PCL with zein and gum arabic to prepare skin tissue engineering scaffolds [112]. They found an increase in fibroblast proliferation, as compared to tissue culture polystyrene, due to the improved hydrophilic properties and functional groups of zein and gum arabicum, but also a slightly reduced viability, as compared to the reference sample. In addition, antibacterial properties against *E. coli* were found, due to the antibacterial properties of the gum arabic. These antibacterial properties could be further increased by adding *Calendula officinalis* extract in different ways to the nanofiber mats [113].

Luginina et al. produced electrospun PCL/poly(glycerol-sebacate) (PGS) nanofiber mats with diverse PCL/PGS ratios and additional bioactive glass particles [114]. PGS increased the average fiber diameter as compared to pure PCL nanofiber mats, while bioglass increased this value's standard deviation, but not the mean value. Release of bioglass particles was found for all composite nanofiber mats, making them usable for therapeutic ion release. When seeding a stromal cell line onto these substrates, however, PGS was found to reduce cell viability (samples S1–S4 in Figure 5) as compared to pure PCL, with or without bioglass (samples S5–S7 in Figure 5), which was attributed to PGS degradation from the nanofiber surface, thus reducing the cell adhesion capability.

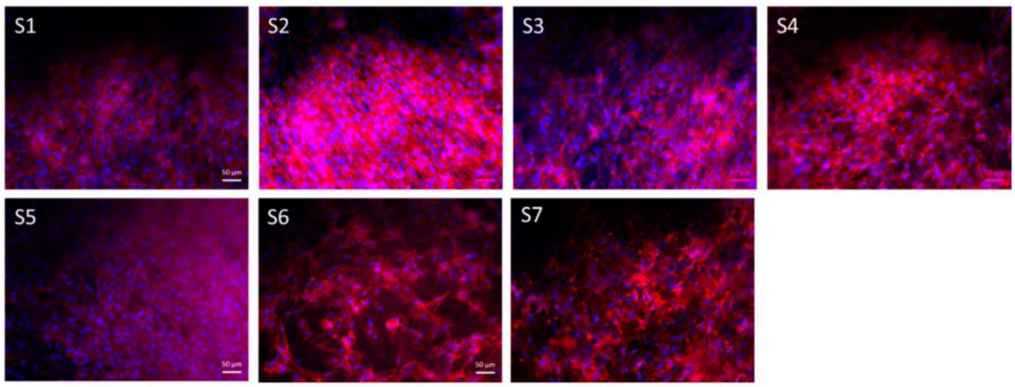

Figure 5. Fluorescent images of ST-2 cells on as-spun nanofiber mats of different PCL/PGS ratios S1–S7 after 7 days of incubation. Reprinted from [114], originally published under a CC-BY license.

8. Polyurethane

Polyurethane (PU) belongs to the materials that were only briefly mentioned before as a possible partner for one of the other polymers; however, PU is biocompatible, non-toxic, and has good elastic and mechanical properties, which makes it suitable for soft tissue engineering, e.g., for the soft tissues of the cardiovascular system [115].

Gabriel et al. prepared PU nanofiber mats by electrospinning and tested attachment, proliferation, and viability of VERO fibroblast cells (African green monkey kidney fibroblasts) in vitro [116]. They found no cytotoxic effect after 24 h but did after longer times. Nevertheless, the live/dead assay showed mainly living (stained green) cells and only a few dead (stained red) cells after 24–72 h (Figure 6). Generally, the nanofiber mats were found to show an ideal porosity, with suitable pore size and pore interconnectivity, to support cell penetration into the scaffolds, as well as the exchange of nutrients and metabolic products.

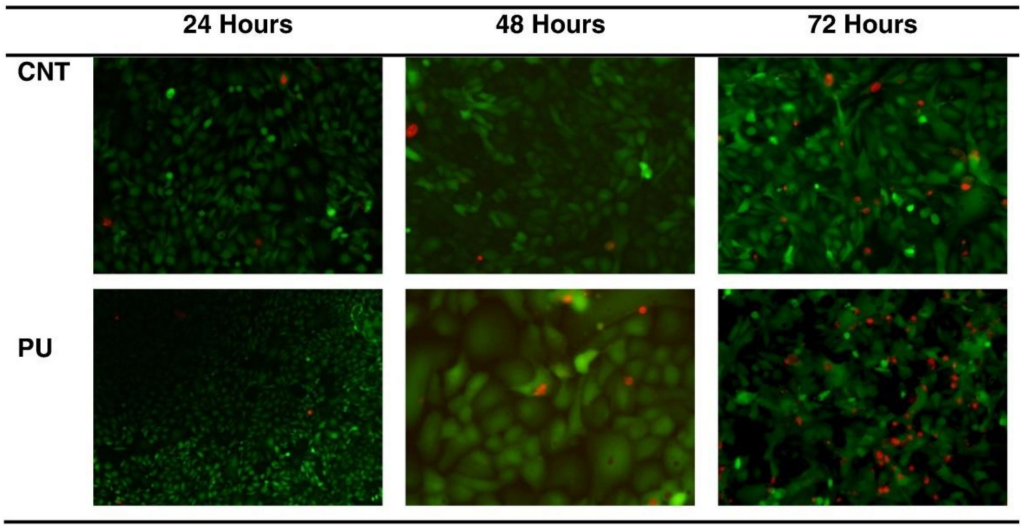

Figure 6. Live/dead assays showing fibroblasts incorporated in control and PU membranes, after 24, 48, and 72 h. Reprinted from [116], with permission from Elsevier.

One of the possible blending partners for PU is hydroxyapatite (HA), as already mentioned in combination with chitosan and PLA. Tetteh et al. investigated PU nanofiber mats with HA particles in different ratios, electrospun from various combinations of DMF and tetrahydrofuran (THF), and for use in bone tissue engineering and found that beads vanished when THF was part of the solvent, while fibers became more uniform and thicker [117]. The inclusion of HA particles significantly increased the tensile properties of the nanofiber mats. Tests with osteoblastic mouse cells and human embryonic mesenchymal

progenitor cells showed no difference of viability on all tested PU scaffolds, while nano-HA was shown to increase the cell proliferation rate. This finding was attributed to a stronger interaction between the polymer and ceramic phase for a large HA surface area, which allowed for improved protein attachment. Significantly lower cell viability was additionally found on nanofiber mats with significantly larger average fiber diameters; underlining the importance of the scaffold morphology for cell growth and viability. Similar results were found in other studies [118].

Similarly addressing bone tissue engineering, Jaganathan et al. added corn and neem oil to polyurethane scaffolds and found slightly reduced fiber diameters, as compared to pure PU nanofiber mats, increased tensile strength, and hydrophobic or hydrophilic behavior for PU/corn oil or PU/corn oil/neem oil, respectively [119]. The hydrophilicity of PU/corn oil/neem oil was found to be in an ideal range of contact angles between 40° and 70°, which was mentioned as a possible reason for the increase of the adhesion and proliferation of the fibroblast cells seeded on these substrates. Furthermore, blood compatibility assessments showed anticoagulant properties, as opposed to pure PU nanofiber mats, and non-toxicity against red blood cells and human fibrost cells.

Combining PU with PCL and graphene oxide (GO), the resulting nanofiber mats showed an increasing fiber diameter with increasing GO concentration [120]. Seeding human skin fibroblast cells on them, Sadeghianmaryan et al. found good biocompatibility and showed that GO could significantly increase the hydrophilicity and biocompatibility, as compared to pure PU/PCL nanofiber mats, in this way leading to stretched cell growth on the more "cell-friendly" surface.

Additionally aiming at skin tissue engineering, Movahedi et al. investigated PU/starch core-shell nanofibers, partly with additional hyaluronic acid in the shell [121]. They showed that the average fiber diameters decreased with adding starch to the PU solution and decreased further with additional hyaluronic acid. On the other hand, the porosity was significantly increased by adding starch and further by also adding hyaluronic acid. Seeding mouse fibroblasts on these nanofiber mats, the cell morphology and viability were found much better for the blended fibers, which was attributed to these scaffolds better mimicking the extracellular matrix and having a higher specific surface area for attachment and stretching of the cells. Further in vivo studies also showed good wound dressing properties for the PU/starch-hyaluronic acid nanofiber mats.

Another blend for bone tissue engineering was suggested by Jiang et al., who added wintergreen and TiO_2 to PU [122]. Both PU/wintergreen and PU/wintergreen/TiO_2 nanofiber mats showed smaller fiber diameters than pure PU nanofiber mats, in this way again offering a larger specific surface area. While PU/wintergreen had a smaller wettability than pristine PU, this value was increased for PU/wintergreen/TiO_2. Moreover, the anticoagulant properties were improved by both blends, and fibroblasts were shown to attach and proliferate well on the composite nanofiber mats. Both showed increased calcium content in a bone apatite formation study as compared to pure PU nanofiber mats. In another study, the group investigated blending PU with rosemary oil or copper sulfate and found suitable wettability and roughness for bone tissue engineering, as well as improved blood and cytocompatible properties [123].

Besides these often reported applications for skin or bone tissue engineering, PU blends are also used for aortic valve or cardiovascular tissue engineering [78,124,125] and other applications.

9. Poly(acrylonitrile)

Poly(acrylonitrile) (PAN) belongs to the polymers that can be electrospun from dimethyl sulfoxide (DMSO), is a low-toxic solvent, and should thus be highly attractive for biomedical and biotechnological applications [9,12,41]. Nevertheless, it is less often used for tissue engineering than other polymers.

Ramezani et al. blended PAN with Fe(III)-metal-organic framework (MOF), with different amounts of Fe-MOF, and investigated cell viability on this electrospun nano-

composite [126]. They found that for lower percentages of Fe-MOF, the electrospun nanofiber mats showed high porosity, with a suitable average fiber diameter, as well as chemical stability. The cell attachment, proliferation, and spreading were better than in pure PAN nanofiber mats or scaffolds with higher amounts of Fe-MOF, as shown for human umbilical vein endothelial cells (HUVEC) in Figure 7. For low amounts of Fe-MOF, cytotoxic effects could be excluded; in vivo implantation showed no inflammatory response for the lower amounts of Fe-MOF. The optimum concentration of Fe-MOF was explained by finding a balance between scaffold degradation and increasing cytotoxicity upon high concentrations of Fe-MOF; i.e., negative impact of this material, and modulation of the pH value at the biointerface towards optimum values for cell activity, i.e., the positive influence of the Fe-MOF.

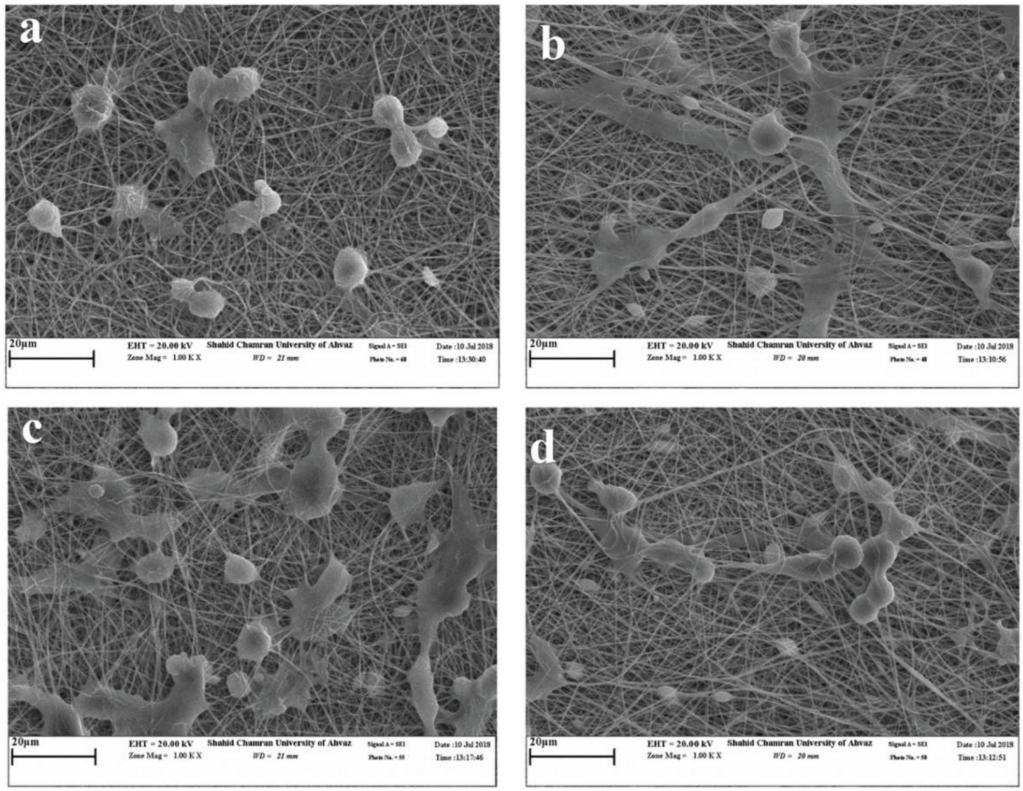

Figure 7. Scanning electron micrographs showing the morphology of plated HUVEC on various scaffolds 24 h after seeding. (**a**) Plated HUVEC on PAN scaffold, (**b**) Plated HUVEC on PAN/5%Fe-MOF, (**c**) Plated HUVEC on PAN/10%Fe-MOF scaffold, and (**d**) Plated HUVEC on PAN/20%Fe-MOF. Reprinted from [126], with permission from Elsevier.

Combining PAN with ZnO as a negative reference and with a maltodextrin/protein blend, as well as with casein/gelatin, Wehlage et al. showed significantly increased cell growth and viability on electrospun PAN/chitosan/gelatin nanofiber mats, as compared to pure PAN; which was attributed to the positive influence of the chemical modification on cell attachment [127]. The same group showed in a previous study the suitability of PAN/gelatin nanofiber mats for the growth of CHO (Chinese hamster ovary) cells [9].

Wu et al. studied PAN combined with the aforementioned hydroxyapatite as a possible scaffold for bone tissue engineering [128]. They prepared a highly three-dimensional scaffold by electrospinning and biomineralization. Seeding bone marrow mesenchymal stem cells onto the scaffolds, they found significantly higher cell growth, osteogenic differentiation, and mineralization than on two-dimensional scaffolds of the same material composition; underlining the importance of a 3D structure.

Similarly working with PAN and hydroxyapatite, Rajzer et al. carbonized the PAN nanofibers after electrospinning [129]. They determined the bioactivity of these scaffolds by assessing crystalline apatite formation on the nanofiber mat upon immersion in simulated body fluid. In comparison with carbonized nanofiber mats without hydroxyapatite, a significant increase of mineralization activity was measured.

Wu et al. added MoS_2 to PAN nanofibers by electrospinning and doping and found good biocompatibility, as well as increased growth of bone marrow mesenchymal stem cells, high cellular activity, increased contact between cells, and improved cell proliferation [130]. These positive properties of PAN/MoS_2 nanofiber mats were attributed to the good biocompatibility and the structure mimicking the extracellular matrix, as well as the support of the alkaline phosphatase expression by MoS_2.

Besides these examples, only a few reports about pure PAN or PAN blends being applied for tissue engineering can be found in the literature.

10. Conclusions

In biotechnology, cultivation is classically carried out in large bioreactors or experimentally in T-bottles. By integrating other materials and shapes, e.g., electrospun nanofiber mats, new possibilities are opened for faster or more directed cell growth and higher cell differentiation, etc. This is why many research groups investigate the possibility of preparing such nanostructured substrates for tissue engineering and other biomedical and biotechnological applications, in which flexible substrate shapes have to be combined with high stem cell differentiation or the good adhesion and proliferation of mammalian cells.

Here, we give an overview of the diverse polymers typically used to prepare electrospun nanofiber mats as scaffolds for tissue engineering. We show that in many cases, polymer blends are applied in order to reach the desired combination of mechanical and biological properties. Moreover, adding hydroxyapatite or other non-polymeric nanoparticles, the cell adhesion, proliferation, and even differentiation of stem cells can be tailored towards a desired application, e.g., for bone or soft tissue engineering. Generally, cells grow best in an environment that is most similar to their original environment in the human or animal body. This defines clearly the optimum chemical and morphological environment which should be mimicked as well as possible with such tailored substrates.

This review can be used as a basis for future research, giving a concise overview of the possible polymers, with their typical blend partners for specific applications.

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