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Polymeric Nanofibers in Tissue Engineering

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Polymeric nanofibers can be produced using methods such as electrospinning, phase separation, and self-assembly, and the fiber composition, diameter, alignment, degradation, and mechanical properties can be tailored to the intended application. Nanofibers possess unique advantages for tissue engineering. The small diameter closely matches that of extracellular matrix fibers, and the relatively large surface area is beneficial for cell attachment and bioactive factor loading. This review will update the reader on the aspects of nanofiber fabrication and characterization important to tissue engineering, including control of porous structure, cell infiltration, and fiber degradation. Bioactive factor loading will be discussed with specific relevance to tissue engineering. Finally, applications of polymeric nanofibers in the fields of bone, cartilage, ligament and tendon, cardiovascular, and neural tissue engineering will be reviewed.

Introduction

Tissue engineering approaches typically involve three key elements: scaffolds, cells, and biochemical and/or mechanical stimuli. Scaffolds generally serve as the foundation for many strategies to promote tissue formation. Although a wide range of scaffold materials are available, polymeric scaffolds are commonly employed to support tissue growth and to serve as carriers for bioactive factor delivery. Since polymeric nanofibers are well suited for such applications, they are gaining popularity in tissue engineering and have been used in attempts to regenerate a variety of tissues. The popularity of nanofibers is demonstrated by the number of reviews focusing on their production,[1–3] application,[1,2,4] and interaction with cells.[5] This review discusses information not previously reviewed with regard to the fabrication of polymeric nanofibers in the context of its effect on the physical properties of nanofibrous scaffolds, relevant to tissue engineering, including fiber degradation and control of pore structure and cell infiltration. Finally, bioactive factor loading and applications of polymeric nanofibers in the fields of bone, cartilage, ligament and tendon, cardiovascular, and neural tissue engineering will be presented to demonstrate the utility of nanofibers in tissue engineering.

The unique properties of polymeric nanofibers make them a valuable tool to tissue engineers. In this field, the term "nanofiber" is typically used to describe fibers with diameters ranging from 1 to 1000 nm.[4] The small diameter of nanofibers closely matches the size scale of extracellular matrix (ECM) fibers, allowing them to be used as biomimetic scaffolds,[6–8] and the high surface area-to-volume ratio is ideal for cell attachment[9] and drug loading.[3,10] Compared to macroscale surfaces, nanofibers have shown higher rates of protein adsorption, a key mediator in cell attachment to a biomaterial surface. For example, poly(L-lactic acid) (PLLA) fibers with diameters ranging from 50 to 500 nm were shown to have four times higher rates of protein adsorption than porous PLLA constructs with macroscale features. Additionally, the nanofibrous constructs were found to selectively enhance the adsorption of specific proteins, such as fibronectin and vitronectin,[11] which is significant as fibronectin is one protein known to mediate cell adhesion and to bind many growth factors.[12]

Further, polymeric nanofibers have been shown to display unique mechanical properties. Specifically, the tensile modulus,[13–16] tensile strength,[15] and shear modulus[17] have been shown to increase as fiber diameter decreases. Although this occurrence is not fully understood, one explanation is that the decrease in fiber diameter leads to an increase in macromolecular chain alignment within the fibers,[17–19] with nanofibers of a smaller diameter having a higher degree of crystallinity.[20] This might especially be true of electrospun fibers, where flow-induced crystallization is thought to occur during spinning.[21] These unique mechanical properties are useful for modulating cell behavior as well as providing adequate tension and strength to resist the forces from the cell cytoskeleton.[15]

Production Methods

When producing polymeric nanofibers for tissue engineering, three production methods are commonly used: electrospinning, phase separation, and self-assembly. Each technique has inherent advantages and disadvantages. Although these production methods have been reviewed by other authors,[1,2] they will be discussed briefly here to

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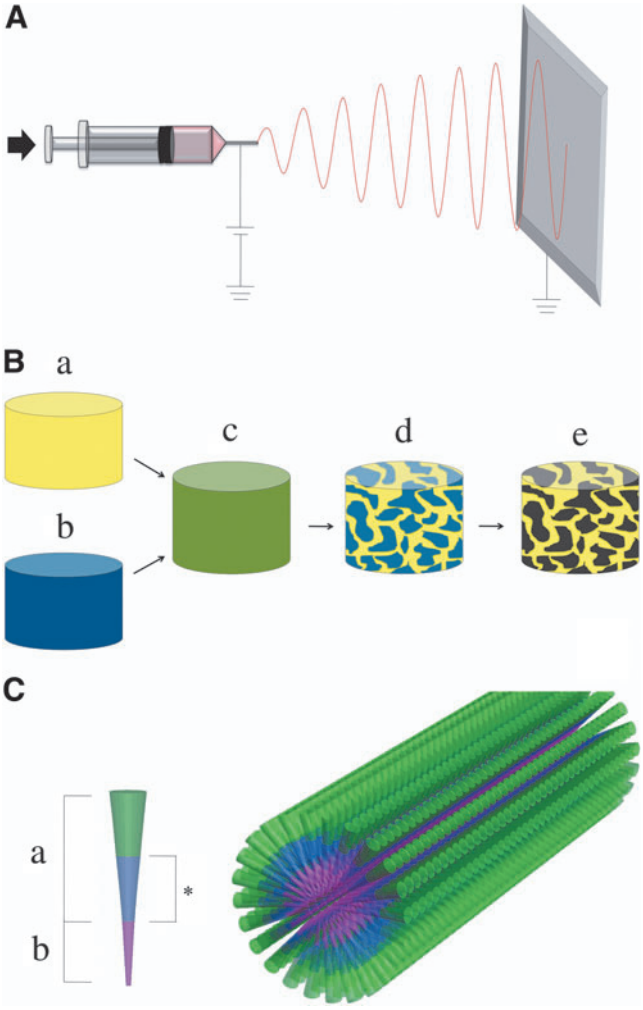

FIG. 1. Production methods. This figure presents schematics summarizing methods used to fabricate polymeric nanofibers in tissue engineering. **(A)** Electrospinning requires only a few relatively inexpensive pieces of equipment. A syringe pump is used to extrude the polymer from the syringe. A power supply is used to apply an electric charge to the needle and ground the collecting plate. **(B)** Phase separation does not require specialized equipment but requires several steps to produce nanofibers. The steps are summarized in the figure. The polymer **(a)** and solvent **(b)** are combined in solution **(c)**. The solution is then rapidly cooled, which induces phase separation **(d)** of the polymer and solvent. Finally, the solvent is then removed **(e)**, leaving a network of nanofibers. **(C)** Peptide amphiphiles are a common building block for self-assembled nanofibers. This schematic shows the structure of peptide amphiphiles, which consist of a hydrophilic peptide sequence of four or more amino acids **(a)** attached by an amide bond to a hydrophobic aliphatic tail **(b)**. The peptides will assemble into cylindrical structures with the hydrophobic tails clustered in the core. This structure leaves the hydrophilic regions positioned on the exterior of the fiber. The four amino acids closest to the core (*) have been shown to be responsible for the formation of beta-sheet hydrogen bonds oriented down the *z*-axis of the fiber, and the disruption of these bonds will lead to the formation of a spherical nanostructure.

highlight the influence each technique has on key scaffold properties.

Electrospinning

Electrospinning is a time- and cost-efficient technique to produce polymer fibers and is the most commonly used method to produce fiber meshes in tissue engineering.[1,3] The technique is capable of producing long, continuous fibers ranging from 3 nm to 10 μm in diameter.[3,22] The process is relatively simple and relies on the electrostatic repulsion of a polymer solution to form polymer fibers. As shown in Figure 1A, the polymer solution is extruded, typically from a syringe and needle aimed at a collecting plate. Surface tension holds the polymer to the needle tip, but with the application of an electric field, a repulsive charge builds within the polymer. Once this repulsive force overcomes the attractive force of surface tension, a jet of polymer solution forms, directed toward the grounded collecting plate. The solvent evaporates from the polymer jet before it reaches the plate, creating a fibrous polymer mesh on the plate. There are several variables in this procedure that can be adjusted to control the fiber diameter. The main variables include the concentration of polymer dissolved in solution, polymer solution flow rate, magnitude of applied voltage, and distance from the needle to the collecting plate.[1–3] By collecting fibers on a rotating mandrel, rather than a flat plate, the fiber orientation can be directed.[23–27] Other variations include coaxial spinning,[28,29] which can create hollow tube nanofibers or fibers with an inner core composed of one material and an outer layer composed of a second material[30] that can be used to alter the chemical[31] or mechanical[31,32] properties of the fibers. Advantages of this technique include the efficiency and simplicity of the procedure, the inexpensive setup, and the ability to control many factors, such as the fiber diameter, orientation, and composition; disadvantages include the use of organic solvents and the limited control of pore structures.[1–3]

A variety of natural and synthetic polymers have been electrospun into nanofibers. Typically, synthetic polymers are considerably easier than natural polymers to electrospin,[33] which is reflected by the larger number of synthetic polymers that have been electrospun into nanofibers. Natural polymers are often blended with synthetic polymers or salts to increase the solution viscosity and consistency in electrospinning.[34] For example, to electrospin alginate/chitosan composite fibers, poly(ethylene oxide) (PEO) has been added to increase the chain entanglements and decrease the conductivity of the charged polysaccharide solution. PEO can be leached from the fibers with water after electrospinning.[35] Other natural polymers include gelatin[33] and collagen, which was spun with diameters as small as 100 nm and displayed structural properties similar to native collagen.[6] Synthetic polymers include poly(ε-caprolactone) (PCL),[36,37] poly(L-lactic acid) (PLLA),[24] polyurethane,[26] copolymers of poly(ethylene glycol) (PEG) and PCL,[38] poly(L-lactic acid-*co*-ε-caprolactone) (P(LLA-CL)),[23,39] and poly (D,L-lactic–*co*–glycolic acid) (PLGA).[40,41] Additionally, composite fibers can be created, such as chitosan-poly(vinyl alcohol) nanofibers[42] and silk, PEO, and hydroxyapatite nanoparticle composites.[43] Fibers containing a blend of collagen and elastin were electrospun with diameters ranging from 220 to 600 nm.[34] Similarly, polymer/nanotube composites can be

created; for example, poly(methyl methacrylate)/nanotube composite nanofibers were made in which the nanotubes assemble along the axis of the fiber, enhancing the mechanical properties of the fibers.[44] Further, nanofibers made of different P(LLA-CL) compositions, ranging from 70/30 to 30/70 PLLA to PCL, exhibited tunable fiber mechanical properties.[9] These few examples illustrate the tremendous versatility of electrospinning.

Phase separation

Phase separation is a method that has long been used to create porous polymer membranes and scaffolds by inducing the separation of a polymer solution into a polymer-poor phase and a polymer-rich phase.[45,46] More recently, the method has been used to produce polymeric nanofibrous constructs from aliphatic polyesters. The matrices were shown to have up to 98.5% porosity and fiber diameters ranging from 50 to 500 nm. To produce the matrices (Fig. 1B), a polymer, such as PLLA, is dissolved in a solvent, such as tetrahydrofuran, and rapidly cooled to induce phase separation. Afterward, the solvent is exchanged with water, and the construct is freeze-dried. Nanofibers can be achieved by selecting the appropriate gelling temperature. Higher gelling temperatures were shown to lead to microfiber formation, but with lower gelling temperatures the diameter was reduced to nanofiber dimensions.[47] However, adjustments of the gelling temperature within the temperature range capable of creating nanofibers was shown to not significantly affect fiber diameter.[7] Likewise, it has been shown that fiber diameter is not influenced by polymer concentration.[7] Increasing the polymer concentration has been found to increase the tensile modulus and tensile strength of the constructs, allowing the mechanical properties to be tailored to the specific application without affecting fiber diameter.[7] Variations of this technique can be used to create nanofibrous constructs with controlled and carefully designed macroporous architectures.[48] Originally, the technique was demonstrated with PLLA or PLGA, but more recently, the technique has been performed with polyhydroxyalkanoate,[49] chitosan,[47] gelatin,[50] and gelatin/apatite composites.[51] The advantages of this method are that it does not require specialized equipment, and there is little variation between batches. Additionally, constructs can be produced in a mold to achieve a specific geometry. However, this process can only be performed with a limited number of polymers and would be difficult to scale-up to a commercial setting.[1,7]

Self-assembly

Self-assembly is a bottom-up approach to nanofiber fabrication that relies on weak noncovalent interactions to build nanofibers from small molecules, proteins, peptides, and nucleic acids.[8,52,53] Several approaches have been demonstrated,[8] but all rely on intermolecular forces to assemble small units into fibers with diameters of approximately 10 nm, arranged into networks with a very high water content (>99.5%).[8,54] The building blocks can be naturally occurring or designed for the intended application.[2,8,54,55] Further, this approach could be used to assemble the nanofibers *in vivo* to create an injectable scaffold for tissue repair[56]; however, competition with natural amphiphiles present *in vivo* could complicate this application. While this approach creates nanofibers of the smallest scale (5–8 nm), the fabrication process is a challenging technique, limited to a few polymers, and can only create short fibers with lengths of one to several μm.[1,57]

Peptide-amphiphiles (PAs) are a common building block for self-assembled nanofibers (Fig. 1C) and have been used for over a decade.[58] PAs consist of a hydrophobic aliphatic tail attached by an amide bond to a hydrophilic peptide sequence of four or more amino acids.[54,59] Initiation of PA assembly can be controlled by adjusting the ion content of the PA solution, which results in a gel-like structure. When the electrostatic repulsion between molecules is neutralized, the peptides will spontaneously assemble into cylindrical, micelle-like structures with the hydrophobic tails clustered in the core. This structure leaves the hydrophilic regions positioned on the exterior of the fiber and available to interact with cells. The four amino acids closest to the core have been shown to be responsible for the formation of beta-sheet hydrogen bonds oriented down the *z*-axis of the fiber, and the disruption of these bonds will lead to the formation of a spherical nanostructure.[59] The mechanical properties of the gels can be controlled by the structure and concentration of PAs; however, the ability to control mechanical properties by modifying the interactions between fibers has also been demonstrated.[60]

Pore structure and cellular infiltration

Control of pore structure is an important aspect of scaffold fabrication, as it directly affects cell infiltration. Of the three production methods discussed, phase separation allows for the greatest control of pore structure.[48] The limited control of pore structure is a significant disadvantage of the electrospinning technique. The pore size of electrospun scaffolds is dependent on the fiber diameter, with smaller diameter fibers leading to smaller average pore sizes, which in turn leads to decreased cellular infiltration. In some cases infiltration can be limited to a very thin layer of cells on top of the nanofibrous scaffold. This occurrence limits the potential benefits of the nanofibers for certain tissue engineering applications. The cell–nanofiber interaction is reduced to the outer regions of the scaffold, which, while beneficial, reduces the advantages of three-dimensional tissue culture. The importance of pore structure can be seen in comparisons of microfibrous and nanofibrous constructs. In some cases, the larger pore size of microfibrous scaffolds has been shown to promote higher levels of stem cell differentiation, in addition to improved cell infiltration.[61]

The importance of pore structures has led to the development of strategies aimed at increasing the pore size of the electrospun constructs, while maintaining nanoscale features. These include salt leaching techniques, where salt crystals are mixed with the fibers during fabrication and leached after spinning.[62] Similarly, researchers have induced the formation of ice crystals on the collecting plate, which leads to larger pores in the construct after melting the ice crystals.[63] A dual electrospinning setup has been created with the additional stream of polymer serving to create a sacrificial fiber that is eluted after spinning, increasing the void space in the construct.[64] Although these strategies have successfully increased scaffold pore size, the mechanical strength of the constructs was reduced.[62] Recently, it was

shown that by using a spherical collecting dish with metallic pegs dispersed throughout, an uncompressed, cotton ball-like mesh of nanofibers can be electrospun. These meshes have much larger pores and have shown improved cell infiltration compared to nanofiber meshes spun onto flat collecting plates.[65] Other approaches include dispersing nanofibers in a microfiber framework. The microfibers serve to increase the pore size in the construct, whereas the nanofibers are dispersed to allow cell contact with nanofibers.[36] In one example, nanofibers were electrospun on top of microfibers created by a fiber bonding process. Here the mesenchymal stem cells (MSCs) were able to interact with the nanofibers, which contributed to a change in morphology compared to MSCs cultured on microfibers alone.[66] Similarly, collagen type I nanofibers have been electrospun onto starch based microfibers, which were present to provide macroscale support to the scaffold.[67] In another example, PCL nanofibers, 600 nm in diameter, were coelectrospun with fibers 5 μm in diameter and seeded with MSCs cultured in osteogenic media. Here the nanofibers did increase cell spreading but still limited the cell infiltration into the scaffold.[36] Alternatively, electrospun microfibers with nanoporous features have been created and shown to lead to increased spreading of human MSCs (hMSCs), compared to smooth fibers, while maintaining the same pore size.[68] Other methods include the use of mechanical force to aid cells in infiltrating small pores. For example, flow perfusion bioreactor culture was shown to increase cell infiltration in multilayer nanofiber/microfiber scaffolds; however, infiltration through the nanofiber layers was still limited.[36] Finally, alignment of nanofibers has been shown to increase cell infiltration both *in vivo* and *in vitro*, and addition of grafted collagen,[69] heparin,[70] and cationized gelatin[71] to the surface polymer nanofibers was shown to increase cell infiltration *in vivo* and *in vitro*. Thus, while efforts have been aimed at increasing cell infiltration in nanofibrous constructs, infiltration remains a challenge and is a major drawback of nanofibrous constructs. These factors must be considered when such constructs are being designed for cell cultures.

Bioactive Factor Loading

In addition to serving as scaffolds to support cell infiltration and tissue formation, nanofibrous constructs can be designed to serve as bioactive factor delivery vehicles to induce a desired cellular or tissue response. Toward this end, nanofibrous constructs can be loaded with bioactive factors via several methods (Fig. 2). Regardless of the chosen method, a significant burst release is often observed, which can be undesirable in tissue engineering, but may be desirable in cases requiring rapid delivery. A burst release will provide an initial high delivery rate and a much lower release rate over an extended period, limiting the overall time course of drug release at effective levels.[29] In the case of polymeric nanofibers, the degree of burst release varies depending on the method of loading and the steps taken to control the delivery. For example, altering the polymer composition or coating the surface of the fibers with a polymer[72] can be performed to reduce the degree of initial burst and extend the time course of release.

Bioactive factors can be directly loaded into nanofibers produced via electrospinning. Blending the bioactive factor into the polymer solution during electrospinning has been

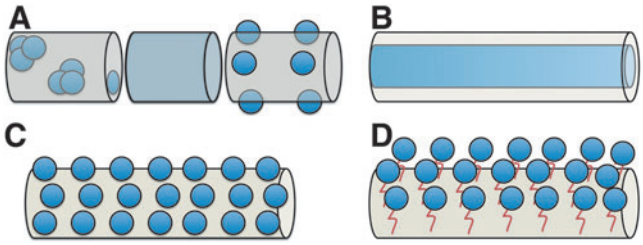

FIG. 2. Bioactive factor loading. Nanofibrous constructs can be loaded with drugs, proteins, and nucleic acids via several mechanisms. Blending during electrospinning **(A)** can lead to the factors being distributed in aggregates, homogeneously dispersed or oriented on the surface of the fibers. Coaxial electrospinning **(B)** localizes the factors to the center of the fibers. Adsorption **(C)** or immobilization **(D)** can be used to load the factors onto the nanofiber surfaces.

used to load proteins,[15,40,43,73] small molecules,[15,74] and nucleic acids[75–77] for release. Depending on the relative properties of the polymer and bioactive factors, this method can lead to the factors being homogenously dispersed in the fibers,[15] randomly dispersed in aggregates throughout the fibers,[15,73] or localized to the surface of the fibers.[10,75] Additionally, this technique has been used to create a controlled gradient of factors throughout the thickness of the construct to spatially control the differentiation of cells.[78] This approach is relatively straightforward, yet several disadvantages are inherent to this process. For example, a significant burst release is reported when using this approach alone.[29,72] Consequently, approaches have been taken to alter the release profiles. Varying the polymer composition of the fibers has been shown to alter the release profile, and in some cases significantly extend the release. For example, release from poly(ethylene-*co*-vinyl acetate) (PEVA) microfibers or a 50/50 blend of PEVA and PLA was shown to exhibit a smoother and longer release than from PLA microfibers.[79] Additionally, coating the surface of the fibers with a polymer has been shown to delay release and reduce the degree of burst release.[72] Further, while some factors could be easily blended with polymers in organic or aqueous solvents,[43] others such as those that are highly charged and large are difficult to homogenously dissolve in organic solvents typically used in electrospinning,[10] and some simply cannot be electrospun using this method.[40] Additionally, phase separation between proteins and the polymer solution can occur,[15,73,80] reducing the encapsulation efficiency[73,80] and leading to weak, brittle fibers.[15] In some instances a surfactant can be added to prevent phase separation.[80] Additionally, the exposure of bioactive factors to organic solvents should be limited to prevent a reduction in activity after release.[10,80] Thus, while the simplicity of this approach is advantageous, there are several key challenges that must be overcome for its practical implementation in sustained bioactive factor delivery for tissue engineering.

Coaxial electrospinning is one method that can be used to overcome some of these challenges. Here, two solutions are simultaneously pumped through concentric needles or cylinders and electrospun to create a fiber with a core and sheath of varied composition.[29] Typically, the two components have different solubility in aqueous and organic solvents, which prevents the mixing of the two phases during

the electrospinning process. A hydrophilic core is beneficial to load factors and preserve their bioactivity as they are protected from organic solvents used to dissolve the outer polymer, whereas a hydrophobic sheath promotes fiber formation.[28,29] In this case, the factors will be restricted to the central core of the fibers, creating a reservoir for factor release. Factors contained in the inner core can be released through pores in the outer sheath or over time as the sheath degrades. The core-sheath strategy has similar encapsulation efficiency as blending but reduces the burst release of the compounds and extends the time course of release.[29] Coaxial production has been shown to extend the release of basic fibroblast growth factor (bFGF) from PLGA nanofibers from 1 to 2 weeks.[40] Similarly, a fluorescently labeled bovine serum albumin (BSA) protein loaded into a core of PEG with a PCL sheath was shown to be released continuously for more than 5 months. Further, the release rate was dependent on the fiber diameter, with smaller fibers leading to faster release rates, and the system was shown to limit the burst release with an efficiency that was dependent on BSA loading.[29]

After nanofiber fabrication, proteins can be immobilized or adsorbed onto the surface of fibers. Techniques of surface modification, such as plasma or wet chemical treatment, can be used to treat the fibers before factor immobilization. Alternatively, factors can be immobilized using techniques such as surface graft polymerization or chemical immobilization. A unique method of surface modification involves the treatment of the hydrophobic surface of PCL nanofibers with hydrophobins, which self-assemble on the fiber surface due to their hydrophobic regions, but leave hydrophilic regions of the protein exposed. Antibodies were then immobilized to the surface through protein–protein interactions. Some advantages of this technique are that it does not require chemical agents for cross-linking and will not compromise the mechanical integrity of the fibers.[81] Further details of immobilization and adsorption techniques have been reviewed by other authors[10] and are similar to those used with other biomaterials regardless of the structure, so the advantages and disadvantages of methods will be only briefly described here. Immobilization leads to low levels of release from nanofibers[80] until degradation of the polymer occurs. In contrast, adsorbed proteins can be released by competitive adsorption of proteins with a higher affinity for the polymer surface. While the simplicity of adsorbing proteins onto the surface of fibers can be favorable, immobilization has several advantages. The two methods were compared in a study evaluating the immobilization and adsorption of bFGF and epidermal growth factor (EGF) on PLLA nanofibers. Simply adsorbing the growth factors to the surface of the fibers was found to have a low efficiency with bFGF, and while the EGF successfully adsorbed to the surface, it was found to have little effect on human embryonic stem cells (hESCs), indicating a loss of bioactivity. However, immobilization with a heparin linkage had high efficiency with both bFGF and EGF, and both growth factors appeared to maintain bioactivity.[82] Further, immobilization of growth factors can lead to a different signaling effect compared to a soluble form of the same factor, as the immobilization can reduce the endocytosis of growth factor receptors.[12] Human EGF chemically conjugated to the surface of nanofibers electrospun from copolymer PCL-PEG/PCL was found to be an effective tool in the treatment for diabetic ulcers. *In vitro* results found the immobilized EGF led to upregulation of keratinocytic genes in human primary keratinocytes, and *in vivo* results demonstrated improved wound closure in diabetic mice.[38] Finally, immobilization or adsorption could be used in combination with another approach, such as coaxial loading, to create a biphasic release of factors, which has been demonstrated with BSA protein and PCL-PEG nanofibers.[80]

Self-assembled peptide nanofiber hydrogels can also be used as carriers, with release profiles dependent on the structure of the nanofiber hydrogel and factor to be released. Specifically, it has been shown that the release kinetics of molecules from self-assembled peptide hydrogels can be controlled by the hydrogel peptide concentrations. The apparent diffusivities of various dyes in such hydrogels were measured, and it was determined that the diffusivities of the dyes decreased with increasing hydrogel peptide concentrations.[83] Additional studies used single-molecule fluorescence correlation spectroscopy to determine the diffusion coefficient and release kinetics of proteins in acetyl-$(Arg\text{-}Ala\text{-}Asp\text{-}Ala)_4$-$CONH_2$[Ac-$(RADA)_4$-$CONH_2$] peptide hydrogels. Here, it was found that in addition to nanofiber density, the release depended on the size of the protein to be released. Additionally, the system was found to have an initial burst release over the first hour, which was likely due to proteins being released from the surface of the gel and through the larger pores in the construct. Finally, the secondary and tertiary structures of the proteins were found to remain intact at the conclusion of the processing, potentially maintaining bioactivity.[84] These studies demonstrated the feasibility of using self-assembled nanofiber hydrogel networks as a reservoir for drug release and revealed key parameters governing the release.

Characterization of Nanofibers

A variety of methods are used to characterize nanofibers and nanofibrous constructs after production and drug loading. Most of these methods of characterization are not unique to nanofibers; however, in many cases special consideration must be taken due to the small size of the fibers.

Morphology

Fiber diameter, alignment, and geometry are commonly determined with scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM).[40,52] SEM is probably the most commonly used due to availability and ease of use, but the electron beam can damage fibers with diameters <200 nm, reducing the accuracy. Additionally, nonconductive samples must be coated with a thin layer of a conductive metal such as gold, which can lead to questionable accuracy for very thin fibers. For these reasons, TEM or AFM are better suited for characterizing the morphology of especially small fibers.[52]

The pore size of fibrous materials is a crucial parameter in tissue engineering as it directly affects the ability of cells to infiltrate the material. Pore characterization includes determination of the porosity of the construct, as well as the pore size and distribution. SEM can be used to characterize surface pore structure but is unable to evaluate the interior of the construct. More in depth pore characterization is commonly performed using mercury porosimetry.[2,9,14,26,36,52] The technique can be complicated by nanofibrous scaffolds with small pores[36] or very thin samples.[52]

Drug and protein distribution

The distribution of drugs and proteins loaded in polymer fibers can be determined by loading fluorescently tagged proteins, such FITC-conjugated BSA, in the constructs and observing with fluorescent microscopy[15,40] or with attenuated total reflectance-Fourier transform infrared spectroscopy (FTIR).[40,43]

Molecular structure

X-ray diffraction is commonly used to determine the crystal structure of polymer fibers.[15,20,42,52] Often after chemical modification or the attachment of functional groups, the molecular structure is determined using FTIR[42,43,85,86] or nuclear magnetic resonance analysis.[52]

Mechanical characterization

Mechanical properties are a key parameter in tissue scaffold design. The mechanical strength of a construct is essential for *in vivo* applications, where the construct often must withstand repeated mechanical loading. Mechanical characterization of the bulk nanofibrous construct can be performed using traditional methods such as tensile testing,[57] and is commonly performed. In addition to the bulk scaffold properties, the mechanical properties of individual fibers can affect cell and tissue growth. As such, the mechanical properties of nanofibers themselves are evaluated. Specialized methods of testing individual fibers have been developed. Other authors have provided a more in depth review of mechanical characterization of individual nanofibers,[87] but the methods will be briefly described here to demonstrate the inherent difficulty.

Mechanical testing of individual nanofibers is complicated by their small size.[57,87] Obtaining and handling individual fibers are just a few of the difficulties. Further, specialized equipment is needed, such as a force transducer with high sensitivity and accuracy and an actuator with resolution large enough to precisely apply force.[87] Due to these complications, mechanical testing of individual fibers is rarely performed. Despite these challenges, methods for tensile testing, bending, and nanoindentation of individual fibers have been reported. Several systems have been used to perform tensile testing of single fibers, most of which are custom-made. One such method uses a piezoresistive AFM cantilever to grip one end of a single fiber, which is connected on the opposite end to a movable, optical microscope stage. The stage is used to apply tensile force to the fiber, and a microscope and camera are used to observe the fiber during testing.[87,88] A few commercial nanotensile testers have also been produced. One such system uses a cardboard frame to grip the nanofiber. The fiber is produced directly on the frame, which is then cut on both sides to allow uninhibited stretching of the fiber.[87] Three-point bending tests can be used to determine the tensile modulus and fracture strength of individual fibers.[87] Polymeric nanofibers are typically laid across a groove or hole in the testing surface and deflected with an AFM cantilever tip. Often the fibers are produced directly on the testing surface. Using beam bending theory and measuring the applied force and fiber deflection, the tensile modulus of the fiber can be estimated.[14,87] Nanoindentation can be used to determine the elastic modulus.[87] In these tests, an AFM probe is commonly used to indent the nanofiber, but many factors must be controlled, such as the humidity, the underlying surface, and the fiber surface roughness.[13]

Due to the technical difficulties of mechanical testing of individual nanofibers, models that could accurately predict the mechanical properties of fibers based on the fiber diameter, structure, material composition, and processing techniques would have much utility in the field of tissue engineering. Such models are underdeveloped but would allow more knowledge to be gained from how the mechanical properties of individual fibers influence fiber–cell interactions. This knowledge would allow for more intelligent design of nanofibrous scaffolds in tissue engineering.

Degradation Characteristics

Degradable biomaterials are often used in tissue engineering to support the tissue during regeneration and are designed to degrade when the support is no longer necessary. In this case, the optimal degradation rate would match the rate of tissue growth. For this reason, the degradation characteristics of polymeric nanofibers are an important factor to understand for successful implementation in tissue engineering. The rate of polymer degradation depends on several parameters, including the material composition and structure.

Few studies have evaluated the influence of nanofiber structure on scaffold degradation rate. However, it has been seen that rapid degradation of nanofibrous constructs can adversely affect the ability of the scaffolds to support tissue growth, as it was shown that cell viability on poly(glycolic acid) (PGA) scaffolds was significantly reduced after 5 days due to degradation of the nanofibrous construct.[89] Further, rapidly degrading materials are generally not suitable for long-term cell culture but, depending on the specific application, may be well suited for rapid delivery of drugs.

Most studies of nanofiber degradation have emphasized the influence of material selection; however, the nanofiber structure is thought to play a significant role in degradation characteristics. In one study, PGA, poly(D,L-lactic acid), PLLA, PLGA, and PCL nanofibrous constructs were immersed in aqueous medium for 6 weeks. This study found that all constructs, except those composed of PLLA and PCL, were significantly degraded over the course of the study.[2] The structure of polymeric nanofibers is thought to affect their degradation in several ways. The large surface area-to-volume ratio of nanofibers makes them especially vulnerable to hydrolytic degradation, which could lead to an increased rate of degradation compared to microfibers.[2,90,91] Alternatively, an increase in crystallinity and chain orientation in small diameter fibers could lead to a reduction in the rate of degradation, as crystallinity in polymers is known to reduce the rate of degradation.[89] Finally, reduction in fiber diameter and increase in surface area increases the rate of diffusion of degradation byproducts from the fibers, which could decrease the rate of autocatalytic degradation.[89–92] It is likely that all three of these factors play a role in polymeric nanofiber degradation and should be taken into consideration when designing nanofibrous constructs for tissue engineering. Further, the material composition of polymeric nanofibers should be tailored to the intended application to support tissue growth or the release of bioactive factors over the proper time course. The following sections highlight examples of nanofibrous polymeric scaffolds developed for the

engineering of specific tissues within the context of the unique requirements for each application.

Specific Applications of Nanofibrous Polymeric Scaffolds in Tissue Engineering

Polymeric nanofibers have been applied to many areas of tissue engineering as both cell scaffolds and carriers for bioactive factors. Although they have been used in many other fields, such as skin[93,94] or kidney[95] tissue engineering, this section will focus on a sample of recent applications in the fields of bone, cartilage, tendon and ligament, neural, and cardiovascular tissue engineering, which are summarized in Tables 1–5.

Bone tissue engineering

Collagen composes about 90% of the organic bone matrix, and 95% of this collagen is in the form of collagen type I fibrils. In bone, the native fibers are approximately 50 nm in diameter[96] and can be organized in aligned or irregular patterns, which lead to the distinguishing characteristics of lamellar and woven bone.[97] In bone engineering, polymeric nanofibers can be uniquely designed to approximate the size scale and fibrous nature of bone ECM.

The ability of polymeric nanofibers to serve as scaffolds for bone engineering both *in vitro* and *in vivo* has been demonstrated by several studies. Electrospun PCL scaffolds with fibers ranging from 20 nm to 5 μm in diameter have been shown to support mineralization and differentiation of bone marrow-derived rat MSCs *in vitro*,[98] and PCL scaffolds with average fiber diameter of approximately 370 nm were shown to support the adhesion and proliferation of MSCs and contribute to higher levels of alkaline phosphatase activity, mineralization, and osteocalcin and osteopontin production compared to two-dimensional control surfaces.[99] The effectiveness of polymeric nanofibers *in vivo* was evaluated using nanofibrous PCL scaffolds seeded with MSCs and implanted in rat omenta for 4 weeks. Cells were shown to differentiate and infiltrate the scaffolds, and ECM production, including collagen type I, and mineralization were evident throughout the scaffold.[100] Further, self-assembled PAs designed to promote cell binding and mineralization have been formed by including the amino acid sequence Arg-Gly-Asp (RGD) and phosphoserine. The fibers were able to reversibly crosslink and were shown to promote mineralization with the alignment of hydroxyapatite crystals in a manner that replicates their orientation in natural bone.[54]

Table 1. Applications of Polymeric Nanofibers in Bone Tissue Engineering Discussed in This Review

Diameter	*Fiber composition*	*Application*	*Key results*	*Reference*
20 nm–5 μm	Electrospun PCL	*In vitro* culture of rat MSCs	Nanofiber scaffolds supported mineralized tissue formation.	98
372 ± 179 nm	Electrospun PCL	*In vitro* culture of rat MSCs	Compared to controls, cells grown on nanofibers displayed increased adhesion, proliferation, ALP activity, and osteocalcin and osteopontin production.	99
Alternating layers of 600 nm and 5 μm	Electrospun PCL	*In vitro* culture of rat MSCs	Nanofibers enhanced cell spreading but limited cell infiltration.	36
100 nm–5 μm	Electrospun PCL	*In vivo* implantation in rat omenta for 4 weeks with rat MSCs	Mineral deposits, cells, and ECM were found throughout scaffold, which had a rigid bone-like appearance.	100
180 ± 31 nm	Electrospun chitosan-based hydroxyapatite composite doped with collagen	*In vitro* culture of human fetal osteoblasts	Compared to controls, cells grown on construct displayed increased proliferation, ALP activity, and mineral deposits.	101
520 ± 55 nm	Electrospun composite fibers containing silk, PEO, hydroxyapatite, and BMP-2	*In vitro* culture of human MSCs	BMP-2 and hydroxyapatite greatly enhanced bone formation.	43
400 nm	Electrospun starch/PCL nanofibers on top of fiber-bonded microfibers	*In vitro* culture of SaOs-2 cells and rat MSCs	Compared to microfiber controls, nanofibers led to stretched morphology in cells, higher viability, and increased ALP activity.	66
7.6 ± 1 nm	Self-assembled PA with RGD amino acid sequence and phosphoserine	*In vitro* mineralization (acellular)	PAs promoted mineralization and alignment of hydroxyapatite crystals, whose orientation replicated natural bone.	54

PCL, poly(ε-caprolactone); MSCs, mesenchymal stem cells; ALP, alkaline phosphatase; ECM, extracellular matrix; PEO, poly(ethylene oxide); PA, peptide-amphiphiles.

Several approaches have been investigated in bone engineering with scaffolds composed of a combination of nano- and microfibers. The microfibers serve to increase the pore size in the construct, whereas the nanofibers are dispersed to allow cell contact with nanofibers.[36] In one example, nanofibers were electrospun on top of microfibers created by a fiber bonding process. The presence of nanofibers was shown to contribute to MSCs having a stretched morphology compared to microfibers alone, which resulted in a more rounded morphology.[66] In another example, PCL nanofibers, 600 nm in diameter, were electrospun with fibers 5 μm in diameter and seeded with MSCs cultured in osteogenic media. Here the nanofibers were found to limit the cell infiltration into the scaffold, and while the presence of nanofibers did not enhance cell attachment, it did enhance cell spreading. The degree of cell spreading could affect the cell proliferation and differentiation.[36]

Composite fibers containing osteoinductive factors have also been created. Fibers consisting of hydroxyapatite, collagen, and chitosan have been electrospun to create scaffolds with a mean fiber diameter of 180 nm and were shown to enhance osteoblast activity *in vitro*.[101] Similarly, composite nanofibers were electrospun containing silk, PEO, hydroxyapatite nanoparticles, and BMP-2. The silk/PEO fibers were shown to support the osteogenic differentiation of hMSCs, and the presence of BMP-2 and hydroxyapatite nanoparticles was shown to greatly enhance bone formation *in vitro* as determined by calcium content and the transcription of bone-specific markers.[43]

Cartilage tissue engineering

Articular cartilage lines the surfaces of articulating joints to provide lubrication and protect the underlying bone. The tissue consists of chondrocytes, collagen (primarily type II), proteoglycans, and water. Much of the collagen is in the form of fibrils, which form a mesh structure, that provide the tensile properties of the tissue and trap other molecules in the network.[102] This fibrous structure of articular cartilage makes

Table 2. Applications of Polymeric Nanofibers in Cartilage Tissue Engineering Discussed in This Review

Diameter	*Fiber composition*	*Application*	*Key results*	*Reference*
700 nm	Electrospun PCL	*In vitro* culture of bovine chondrocytes	Higher levels of chondrogenic gene expression on nanofibers compared to tissue culture polystyrene.	103
500–900 nm	Electrospun PLLA	*In vitro* culture of bovine chondrocytes	Compared to microfibers, chondrocytes on nanofibers maintained rounded cell morphology and displayed higher proliferation rate.	105
Not reported	Self-assembled peptide KLD-12 hydrogel	*In vitro* culture of bovine chondrocytes	Chondrocytes maintained rounded morphology and produced cartilage-like ECM with enhanced mechanical properties.	106
400–1400 nm 300 nm–20 μm	Electrospun PCL and starch-compound PCL	*In vitro* culture of bovine chondrocytes	Nanofibers supported cartilage ECM production. Cells colonized both scaffolds with some migration into the interior.	107
700 nm	Electrospun PCL	*In vitro* culture of human MSCs	Nanofibers supported the multilineage differentiation of MSCs.	108
492 ± 120 nm 2796 ± 845 nm	Electrospun PCL	*In vitro* culture of human MSCs	Cells oriented with fibers. Could be used to create oriented tissue such as zonal organization of cartilage.	109
500–900 nm	Electrospun PCL	*In vitro* culture of human MSCs	Chondrogenesis of MSCs seeded on nanofiber scaffolds was comparable to pellet culture.	37
500–900 nm	Electrospun PCL	*In vivo* implantation of human MSCs in swine model	Implantation led to the formation of hyaline-like cartilage with smooth surface. Scaffolds were easily fixed to surrounding tissue.	110
0.29 ± 0.08 μm 1 ± 0.04 μm 5 ± 1.5 μm 9 ± 2.0 μm	Electrospun PLLA	*In vitro* culture of human MSCs	Chondrogenic gene expression was highest in micron-sized fibers.	61

PLLA, poly(L-lactic acid).

nanofibrous scaffolds an ideal option for engineering articular cartilage. For this reason, many researchers have investigated the influence of nanofibers in cartilage tissue engineering.

As the primary cell in cartilage tissue, chondrocytes are a common cell source in cartilage engineering; however, because these cells are present in low numbers in native cartilage, cell expansion is typically required. This poses a challenge, as maintaining the chondrocytic phenotype is difficult when culturing chondrocytes *in vitro*.[103] When expanded in monolayer culture, chondrocytes will dedifferentiate and halt the production of key molecules such as cartilage proteoglycans and collagen type II.[104] Several methods have been used to prevent the dedifferentiation and to promote the redifferentiation of chondrocytes, including the use of nanofibrous scaffolds. PCL nanofibrous scaffolds with an average diameter of 700 nm were evaluated for their ability to support chondrocyte expansion. Fetal bovine chondrocytes cultured in chondrogenic growth media on nanofibrous scaffolds proliferated and expressed higher levels of cartilage-associated genes compared to controls cultured on tissue culture polystyrene.[103] Additional studies compared the influence of fiber diameter on chondrocyte morphology. Primary bovine chondrocytes were seeded on PLLA electrospun scaffolds. Microfiber scaffolds contained fibers 15 μm in diameter, whereas the fiber diameters ranged from 500 to 900 nm in the nanofiber scaffolds. Both scaffolds supported cell proliferation; however, a higher proliferation rate was seen in the nanofibrous scaffolds. Further, the cell morphology varied between the scaffold types. Cells grown on microfibers appeared well spread, whereas cells on nanofibrous scaffolds had higher rates of proliferation and maintained a rounded morphology, which is characteristic of the chondrocyte phenotype.[105] Similarly, self-assembled peptide hydrogels have also been shown to maintain the chondrocytic phenotype and promote chondrogenic ECM deposition. Bovine chondrocytes suspended in self-assembled peptide hydrogels were shown to maintain a rounded morphology and produce cartilage-like ECM with enhanced mechanical properties.[106] Additionally, the ability of chondrocytes to infiltrate nanofiber scaffolds has been demonstrated *in vitro* with bovine articular chondrocytes seeded on PCL meshes with fiber diameters ranging from 400 to 1400 nm,[107] further supporting the potential of nanofibrous constructs as scaffolds for the support of chondrocytes *in vitro* or *in vivo*.

Nanofibrous scaffolds have also been used to support the chondrogenesis of progenitor cells. Electrospun PCL scaffolds with fiber diameters of approximately 700 nm were shown to support multilineage differentiation of bone marrow-derived hMSCs.[108] The level of chondrogenesis on the nanofiber scaffolds was shown to be equivalent and in some cases higher than the gold standard pellet cultures. However, nanofiber scaffolds have improved mechanical properties, making them an option for *in vivo* transplantation.[37] Implantation of hMSCs on nanofibrous PCL scaffolds in a swine model led to the formation of hyaline-like cartilage with a smooth cartilage surface. Additionally, the nanofibrous scaffolds were found to be easily fixed to the surrounding tissue with sutures and did not require a periosteal covering, which reduced the morbidity associated with the procedure.[109] While researchers have found higher levels of chondrogenesis with nanofiber scaffolds compared to microfiber scaffolds,[110] some groups have found higher levels of chondrogenic gene expression in progenitor cells grown on microfiber scaffolds compared to nanofiber scaffolds.[61] This occurrence could be due to the larger pore sizes in microfiber constructs.

Tendon and ligament tissue engineering

The mechanical loading of tendons and ligaments is restricted to one direction. For this reason, there is a high degree of ECM fiber alignment that leads to highly anisotropic mechanical properties. The tensile properties of the tissues can be 200 to 500 times higher in the direction of fiber

Table 3. Applications of Polymeric Nanofibers in Ligament and Tendon Tissue Engineering Discussed in This Review

Diameter	*Fiber composition*	*Application*	*Key results*	*Reference*
438 ± 156 nm 519 ± 127 nm	Electrospun, aligned, and randomly oriented PCL	*In vitro* culture of meniscal fibrocartilage cells and human MSCs	Aligned fibers can be used to mimic the properties of musculoskeletal tissue.	111
430 ± 170 nm 450 ± 110 nm	Electrospun, aligned, and randomly oriented PLLA	*In vitro* culture of human tendon stem cells	Alignment led to the upregulation of tendon-specific genes. Nanofibers led to spindle-shaped cells.	27
657 ± 183 nm	Electrospun, aligned PU	*In vitro* culture of human ligament fibroblasts	Aligned fibers led to increased collagen synthesis and spindle morphology in fibroblasts. There was no difference in cell proliferation with fiber alignment. Fibroblasts were more sensitive to strain in the longitudinal direction.	26
300–900 nm	Electrospun PLGA nanofibers on top of microfibers	*In vitro* culture of porcine MSCs	Nanofibers led to improved cell seeding and proliferation and higher levels of tendon/ligament-specific gene expression.	41

PU, polyurethane; PLGA, poly (D,L-lactic–*co*–glycolic acid).

alignment than in the normal direction.[111] Consequently, cells, commonly MSCs and fibroblasts,[27] are often cultured on aligned fibers to engineer a similarly anisotropic structure.

Aligned nanofibers have been shown to be a promising scaffold for the engineering of ligaments and tendons, as the structure mimics the anisotropy of the native tissue.[26] Human tendon progenitor cells seeded onto aligned PLLA nanofibers oriented themselves along the direction of the fibers and expressed higher levels of tendon specific genes than cells seeded on randomly oriented fibers.[27]

Braided fabrics are commonly used as scaffolds for ligament and tendon engineering; however, these constructs have poor mass transfer, cell seeding, cell infiltration, and mechanical strength. Knitted microfibers have been more effective for this application, yet cell seeding is complicated. As an alternative, knitted microfibers were used to provide mechanical strength, and nanofibers were added to increase the surface area for cell attachment.[41] PLGA nanofibers were spun on top of a PLGA microfiber scaffold and shown to promote cell seeding, proliferation, and function for use as a scaffold for the engineering of ligaments and tendons.[41]

Neural tissue engineering

In the field of neural tissue engineering, a significant effort is placed on developing effective neural guidance conduits aimed at bridging gaps in damaged peripheral or central neurons. These conduits are implanted into the tissue with the role of directing axonal sprouting, preventing the growth of fibrous tissue into the defect, and promoting the diffusion of neurotrophic factors. Nanofibers are well suited for this application as their structure not only mimics the fibrous components of the neural ECM, but also can be used to direct axon sprouting and to deliver neurotrophic factors to the site of injury.[112]

Several properties of nanofiber scaffolds have been shown to affect cell proliferation and differentiation. The arrangement of the nanofibers has been shown to influence the growth patterns of neural stem cells, with neural stem cell

Table 4. Applications of Polymeric Nanofibers in Neural Tissue Engineering Discussed in This Review

Diameter	*Fiber composition*	*Application*	*Key results*	*Reference*
300 nm 250 nm	Electrospun, aligned, and randomly oriented PLLA	*In vitro* culture of neural stem cells	Cell differentiation was higher on nanofibers than microfibers, independent of alignment. Cells aligned with aligned fibers, independent of fiber size.	24
800 ± 96 nm	Electrospun, aligned poly(acrylonitrile-*co*-methacrylate)	*In vitro* culture of Schwann cells	Fiber alignment promoted the alignment of fibronectin networks sourced from both serum and Schwann cells. Topographically organized fibronectin networks may contribute to Schwann cell migration and neurite outgrowth.	113
Not reported	Electrospun PLLA with adsorbed or heparin immobilized bFGF or EGF	*In vitro* culture of rat neural stem cells	Immobilization of bFGF or EGF promoted axon growth. Adsorption of bFGF and EGF was not effective in increasing axon growth.	82
238 ± 45 nm 749 ± 153 nm 1452 ± 312 nm	Electrospun polyethersulfone	*In vitro* culture of hESC derived neural cells	Fiber diameter influenced cell differentiation and proliferation. Decreased diameter led to increased proliferation and cell spreading and a lower degree of cell aggregation.	114
112–189 nm	Electrospun polyaniline, PCL, and gelatin	*In vitro* culture of neural stem cells	Incorporation of polyaniline allowed for electrical stimulation of cells and enhanced cell spreading and neurite outgrowth.	85
5–8 nm	Self-assembled IKVAV peptide hydrogel	*In vitro* culture of murine neural progenitor cells	Culture induced the rapid differentiation of cells into neurons and discouraged the development of astrocytes.	115

EGF, epidermal growth factor; bFGF, basic fibroblast growth factor; hESC, human embryonic stem cell.

elongation and neurite outgrowth in the direction of the aligned fibers,[24] possibly due to the alignment of fibronectin networks.[113] hESCs cultured on aligned PLLA nanofibers displayed enhanced axon growth compared to those cultured on randomly oriented fibers.[82] Further, the diameter of fibers has been shown to influence neural stem cell behavior, as smaller diameter fibers were shown to increase the rates of proliferation and differentiation.[24,114] The conductivity of nanofibers can also be controlled, and conductive nanofibrous scaffolds with average fiber diameters ranging from 112 to 189 nm were electrospun from polyaniline, PCL, and gelatin. Electrical stimulation was shown to lead to increased neural stem cell proliferation and neurite outgrowth for cells cultured on such constructs.[85]

Other work has shown that self-assembled PAs with an epitope known to promote neurite sprouting and growth can be used to encapsulate neural progenitor cells. The cells encapsulated in the nanofiber network were found to more rapidly differentiate into neurons, compared to controls.[115] Further, growth factors have been used in combination with nanofibers. Immobilizing EGF and bFGF onto the surface of PLLA fibers was shown to significantly enhance the axon growth.[82]

Cardiovascular tissue engineering

In cardiac tissue the ECM causes cardiomyocytes to form into fiber-like cell bundles. These fibrous bundles elongate and align allowing mechanical coupling of adjacent fibrils.[116] A polymeric structure that causes cardiomyocytes to align would mimic this specific feature of the natural tissue architecture. As such, electrospun fibers of P(LLA-CL) with average diameter of 550 nm have been shown to support the attachment and proliferation of human coronary artery

Table 5. Applications of Polymeric Nanofibers in Cardiovascular Tissue Engineering Discussed in This Review

Diameter	*Fiber composition*	*Application*	*Key results*	*Reference*
400–800 nm 550 ± 120 nm	Electrospun P(LLA-CL)	*In vitro* culture of human smooth muscle and endothelial cells	Scaffold is capable of supporting cell attachment and proliferation. Cells maintained phenotypic shape. Mechanical properties of fibers are comparable to human coronary artery. Smooth muscle cells attached and migrated along the axis of aligned fibers and expressed spindle-like contractile phenotype. Adhesion and proliferation were improved compared to polymer films.	23
200 nm–1 μm 200–500 nm	Electrospun PCL, plasma–treated, and covalently grafted with gelatin	*In vitro* culture of human coronary endothelial cells	Gelatin enhanced cell spreading and proliferation. Cells aligned with fibers.	25
400–500 nm 1.0–1.2 μm	Electrospun polymethylglutarimide with controlled positioning density	*In vitro* culture of rat cardiac cells	Cardiac cells elongated and grew along nanofibers to form an excitable cardiac tissue. Best ordering and alignment was found with fibers spaced ≤30 μm.	117
500–1500 nm	Electrospun PCL with hydrophobin coating to immobilize anti-CD31	*In vitro* culture of human umbilical vein endothelial cells	Anti-CD31 enhanced the binding of human umbilical vein endothelial cells.	81
100–200 nm	Electrospun, collagen blended P(LLA-CL)	*In vitro* culture of human coronary artery endothelial cells	Collagen grafting promoted cell spreading and viability and preserved the endothelial phenotype.	86
1000 ± 125 nm	Electrospun core/shell fibers with poly(glycerol sebacate)/gelatin	*In vitro* culture of rabbit cardiomyocytes and MSCs	Gelatin promoted cell adhesion and proliferation. Poly(glycerol sebacate) provided mechanical support. The scaffold supported MSC differentiation into cardiomyocytes.	31
Not reported	Self-assembled RAD16-II peptide hydrogel	Injected into mouse left ventricle	Scaffolds recruited endogenous endothelial and smooth muscle cells. Exogenously injected cells survived in the nanofiber matrix.	56

P(LLA-CL), poly(L-lactic acid-*co*-ε-caprolactone).

smooth muscle cells (SMC) and endothelial cells.[23,39] Constructs with aligned P(LLA-CL) fibers were found to have a higher rate of SMC adhesion and proliferation compared to controls. Cytoskeletal proteins were observed to arrange parallel to the direction of the fibers, and the cells migrated along the axis of the fibers and developed a contractile phenotype, which is desired.[23] Further, the positioning density of aligned nanofibers has been varied, and the best results were achieved with a density of 30–50 nanofibers/mm. In this case, cardiac cells took an elongated shape and formed excitable cardiac tissue.[117]

To further mimic the native tissue, several approaches have focused on altering the surface properties of nanofibers. A hydrophobin coating was used to immobilize anti-CD31 to the surface of PCL nanofibers. The processing was shown to enhance the binding of human umbilical vein endothelial cells, which shows promise as a technique for the vascularization of small diameter vascular grafts.[81] In mimicking the basal lamina, gelatin was covalently grafted onto surface-modified, electrospun PCL constructs. The nanofibrous constructs consisted of either aligned or random fiber orientations. The gelatin grafting was shown to enhance endothelial cell spreading and proliferation and cells cultured on aligned fibers were found to align in the direction of the fibers.[25] Other approaches have included collagen directly into the electrospinning process, creating collagen-blended P(LLA-CL) fibers, which were shown to promote endothelial cell spreading, attachment, and viability.[86] Similarly, core/shell nanofibers have been created with gelatin in the shell to promote cell adhesion and proliferation, whereas poly (glycerol sebacate) was used as the core to mimic the mechanical properties of heart muscle. These fibers were shown to support the cardiogenic differentiation of MSCs, indicating the potential of the nanofibers in the repair of myocardium.[31]

Self-assembling peptides have also been shown to be a promising tool for cardiovascular tissue engineers. Peptides were injected into the myocardium and were found to be able to self assemble into a nanofiber network *in vivo* and, after doing so, enhanced the recruitment of vascular cells.[56]

Conclusion

Polymeric nanofibers show great potential as an effective tool for tissue engineering, serving as a scaffold to support tissue growth or as a platform for the delivery of biochemical or mechanical stimuli. While the applicability of nanofibers in tissue engineering is quickly advancing, much remains to be accomplished. In the area of nanofiber production, methods to electrospin nanofibrous scaffolds with pore size and structure adequate for cell infiltration are under development. Other production methods, such as phase separation and self-assembly are not ideally suited for large scale production, and advances in fabrication technology would facilitate their implementation in a commercial setting. While methods to mechanically characterize individual nanofibers exist, the difficulty of implementing the procedures limits their application. As such, more efficient methods of working with individual nanofibers could lead to improved nanofiber characterization. Moreover, the field would benefit from more advanced mathematical models describing the behavior and mechanical properties of individual fibers. With regard to bioactive factor delivery, many techniques have been employed to reduce the burst release observed with nanofibrous scaffolds. As mechanisms of fiber degradation and interaction with bioactive factors are further understood, greater control of factor delivery should result. Additional understanding of such principles could lead to improved modeling of bioactive factor release from nanofibers, which is currently lacking. Finally, the ability to use chemical signals to modulate cell behavior is currently more developed than the ability to use mechanical signals from scaffold architecture to achieve the same result. However, the chemical and mechanical signals will often have a synergistic relationship and distinguishing between the effects of each is a challenge. Successfully expanding the current understanding of how nanofibrous structures modulate cell behavior would enable more rigorous design of nanofibrous scaffold architecture for tissue engineering applications. Improvement in the current level of technology in these numerous areas should have substantial impact on the field of tissue engineering.

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