Review

Polyblend nanofibers for biomedical applications: perspectives and challenges

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Advances in disease treatment and tissue regeneration are buoyed by new, multifaceted materials that emulate and coercively interact with the local microenvironment. Polyblend nanofibers represent an emerging class of biomimetic nanostructures that can act as proxies of the native tissue, while providing topographical and biochemical cues that promote healing. These fibers are prepared with mixtures of synthetically and naturally derived polymers that can behave cooperatively to demonstrate unique combinations of mechanical, biochemical and structural properties. This flexibility has led to the application of polyblend nanofibers in a wide assortment of tissue engineering and drug delivery systems. In this review, we will examine design criteria and properties of polymer-blend nanofibers and their use in tissue engineering and local therapeutic delivery applications.

Introduction

Controlled synthesis of nano-sized materials has offered the biomedical community the promise of a new class of regenerative and therapeutic devices with unique optical, magnetic, electrical and biochemical properties. These materials are particularly suited to medical application because they operate on the same size scale – tens to hundreds of nanometers – as biological molecules and structures. Nanomaterials shaped as particles, wires, fibers or with complex features have shown enhanced cellular interactions, including selective endocytosis, adhesion and orientation [1,2]. To this end, nanomaterials are uniquely capable of mimicking and reorganizing the biological microenvironment to supplement damaged or diseased tissue and to stimulate the regeneration of neotissue.

Polyblend nanofibers, long spaghetti-like masses of thin polymeric mixtures, represent a new class of nanomaterials that have been successfully prepared and used in regenerative medicine (tissue engineering scaffolds, wound dressings and vascular grafts) as well as in controlled, local delivery and release of small molecule drugs and biological agents (e.g. proteins and nucleic acids) [3–5]. These nanofibers are prepared from varying combinations of polymer components to manipulate the mechanical and biochemical properties of fibers. The high surface area to mass ratio of these structures gives nanofibrous mats (three-dimensional structures of deposited nonwoven nanofibers) high pore volumes with variable pore sizes. This provides nanofiber scaffolds with strong mechanical properties while maintaining a very low density.

These fibrous mats are commonly made with pore and fiber size distributions that match the structure of the extracellular matrix (ECM) of the body, the fibrous protein and glycosaminoglycan (GAG) network that surrounds and supports cellular activity. As temporary supplements to actual tissue matter, polyblend nanofibers can be engineered with the topographical and structural ordering of the native tissue in addition to having a degradation profile that will break down the material into biocompatible fragments over its lifespan as the native tissue gradually grows. In this review, we will assess recent advancements in nanofiber blend preparation, look at the biomedical areas these fibers can play a role and expose new areas of development.

Polyblend nanofibers

For biomedical applications, nanofibers have been prepared with synthetic or naturally derived polymers by one of three established techniques, including phase separation, self-assembly and electrospinning. All of these methods have produced three-dimensional nanofibrous scaffolds suitable for use in tissue engineering applications, and each has been touted for their individual advantages. The phase separation requires only simple equipment [6]; self-assembly can form very fine fiber diameters [7]; but electrospinning is the most widely adopted technique for the preparation of polymeric nanofibers owing to its versatility and flexibility and is the focus of this review. This scalable method produces nanofibers ranging from 3 nm to 6 μm in diameter and can process a wide range of raw polymer materials into nanofibers, including blends of multiple synthetic and natural polymers [8,9]. Fibers electrospun onto a flat surface produce a thin fibrous mat, but three-dimensional structures (e.g. tubes for vascular grafts) can also be produced modifications to the electrospinning setup. A detailed summary of electrospinning implementation is provided in Box 1.

Traditionally, electrospun nanofibers have been prepared from single polymer sources, but more recently, mixtures of polymers have been used to form so-called polyblend nanofibers. Nanofibers prepared from premixed or multiple polymer solutions share several advantages

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Box 1. Electrospinning nanofibers

The core electrospinning process involves the application of a high electric potential (several kV) to a pendant droplet of a starting polymer solution or melt to form a thin fiber, as shown in Figure I. Specifically, a voltage is applied to the spinneret of a polymer source aimed at a conductive collector that serves as a counterelectrode. The resulting external electrical field forms tangential stress on the polymer solution causing a deformation of the droplet into a conically shaped 'Taylor cone'. When the surface tension of the polymer solution is exceeded by the the electrical field, the conductive liquid is ejected from the Taylor cone towards the collector. Before reaching the collector, the solvent is quickly evaporated, and the stream undergoes significant bending and stretching. The resulting nanofibers form either a nonwoven or aligned fibrous mat depending upon the type of the collector used. Nonwoven mats are characterized by large, interconnected pores, which can be used for drug and gene delivery or to mimic the native ECM. Alternatively, aligned fibers can promote outstretched cell conformations and directionality. Polymer preparation and electrospinning parameters can affect final nanofiber properties. Some of the major working parameters include:

Applied voltage: an increase in the electric field strength typically leads to thinner nanofibers because of the increase in Coulombic repulsive forces in the fluid jet, causing stretching of the viscoelastic solution. Higher voltages can also lead to bead formation and lower polymer crystallinity.

Spinneret-to-collector distance: at shorter distances the solvent has less time to evaporate, which can lead to fibers merging on the collector. Longer distances can cause more polymer stretching (thinner fibers) or a decreased electrostatic field strength (thicker fibers).

Polymer solution feedrate: the feedrate is the speed at which the polymer is forced through the spinneret. Feedrates typically correspond to a given voltage to maintain a stable Taylor cone, where the feedrate is equal to the rate at which a solution is removed by electrical forces. When the feedrate is too high, nanofibers can form having large beads.

Polymer properties: polymer molecular weight (MW) affects viscosity and conductivity of polymer solutions. Typically, use of lower MW polymers leads to bead formation, whereas higher MW polymers yield larger diameter fibers.

Solution viscosity: increased viscosity owing to high polymer MW or concentration can result in larger fiber diameters. Beading is less likely to form with high viscosity solutions, whereas bead size and regularity increases with higher viscosity solutions.

Solution conductivity: highly conductive solutions can increase the stretching of the solution under electric fields leading to smoother, thinner fibers. Good electrospinning mixtures are prepared with highly conductive solvents or solutions loaded with salts or polyelectrolytes.

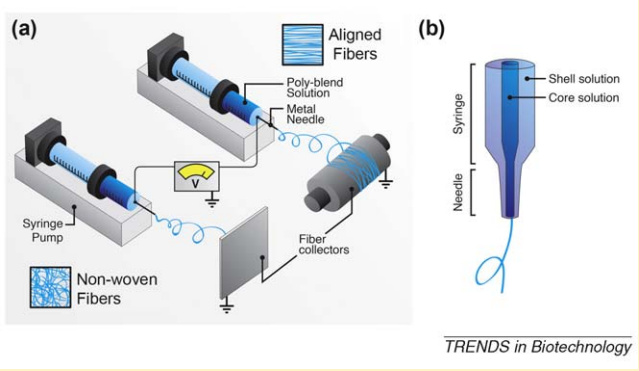

Figure I. Electrospinning apparatus. **(a)** Polymer fibers captured on a flat surface produce nonwoven mats, whereas fibers can be aligned using a rotating spindle target. **(b)** Coaxial nanofibers with core-shell structures are formed with a spinneret made from two coaxial capillaries carrying the different polymer solutions.

over single-component systems. First, polyblends combine the characteristic properties of several unique polymers instead of exhibiting the singular attributes of an individual component. Although any particular polymer can lack the chemical or structural dimensions of native tissue, polymers used in combination can take advantage of the varying strengths, bioactivities, and degradation rates of all its components. Second, some natural polymers are not amenable to electrospinning owing to molecular weight or solubility issues. Polyblend mixtures can help solubilize these materials, allowing their preparation in nanofibers.

Compared with copolymer and modified nanofibers, polyblend nanofibers offer an alternative approach that is simple, economical and does not waste biological materials during preparation. Copolymers, strands of different polymer segments, have been synthesized for electrospinning applications [10,11]. This is an appealing option, but the need for complicated synthetic schemes and the difficulty in modifying naturally derived polymers makes copolymer preparation difficult. Alternatively, synthetic nanofibers have been modified using physical coatings and chemical grafting to impart biorecognition sites, such as the RGD (Lys-Gly-Asp) peptide integrin recognition site, and other biochemical signatures that they lack [12]. Both copolymerization and physical coating provide new functionalities, but they can be laborious, inefficient and costly. In particular, the slow mass transfer of biomolecules within porous scaffolds limits surface modifications.

Component polymer types

Many different biocompatible polymers have been electrospun into nanofibers and are broadly classified as either synthetically or naturally derived. Their varying material properties make their use application-dependent. These key properties, including biodegradation, biocompatibility, mechanical strength, hydrophilicity (or hydrophobicity), are detailed in Figure 1 for the polymers most commonly used in electrospinning.

Synthetic polymers are widely used for synthesis of polyblend nanofibers owing to the diversity of their physicochemical properties, including hydrophilicity/hydrophobicity, surface charge and mechanical strength (modulus and yield strength). The most widely electrospun synthetic polymers include the glycolide- and lactide-based linear aliphatic polyesters, polylactic acid (PLA), polyglycolic acid (PGA) and their copolymer, polylactic-*co*-glycolic acid (PLGA). All of these materials are biocompatible, can be cheaply produced from reliable raw material sources and have been extensively used in soft tissue regeneration and, as carriers, for controlled drug delivery [13].

Although synthetic materials are strong, cheap and reliable, they do not share the biochemical signatures expressed in native fibers of the body. To form biomimetic nanofibers, an appealing approach has been to process naturally derived polymers into nanofibers [14,15]. Collagen, gelatin (a product of partially hydrolyzed collagen), fibrinogen, chitosan and alginate have all been purified and used as the starting polymer for nanofiber preparation. Many of these materials retain cell-binding sites

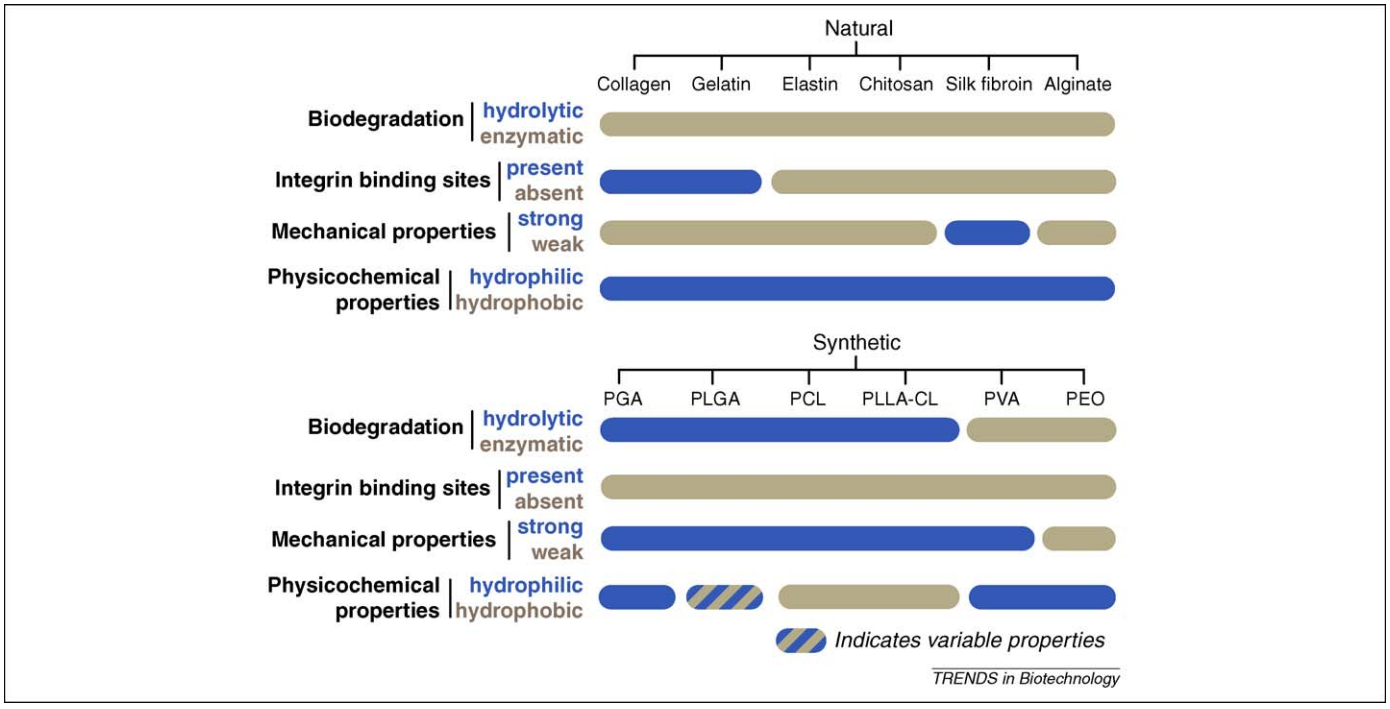

Figure 1. Commonly used natural and synthetic polymers for development of polyblend nanofibers with their key biological, mechanical and physicochemical properties.

and biomolecular signatures that can affect cell response to the nanomaterial or emulate natural tissue [16]. For example, chitosan and alginate are considered to be an excellent GAG proxy, and are biocompatible, biodegradable and biorenewable [17–21].

The harvesting and processing of natural polymers, however, is not as straightforward as synthetic polymer production. Sources and forms of animal-derived, natural polymers can significantly affect nanofiber formation. For example, different sources of collagen (calfskin versus placental) and isotypes (type I versus type III) have been shown to directly affect the physical properties, including architectural arrangement of the fibers [14]. In addition, these materials hold the risk of disease transmission and possible antigenicity [22]. Thus, the ability to generate nanofibrous matrices from non-animal sourced natural polymers, such as chitosan and alginate, might represent a significant advancement in overcoming the challenges in source and batch variability and provide virtually unlimited resources for development of tissue compatible scaffolds.

Synthetic polymer blends

Synthetic polyblend nanofibers have primarily been prepared for the facile modulation of fiber degradation rates. Many of these materials investigated are biodegradable, a feature that allows material engineers to design scaffolds that disintegrate as neo-tissue is formed or their drug payloads are released. Labile groups, such as esters, amides, ureas, urethanes, anhydrides and carbonates, are broken by either hydrolysis or enzymatic decay. This site-specific degradation reduces polymers to inoffensive degradation products, such as the nontoxic metabolites formed by PLA, PGA and PLGA, which are excreted as water and carbon dioxide. The speed of these degradation processes can vary (the hydrophilic PGA degrades in weeks, whereas the more hydrophobic PLA degrades over months and years), making different polymers suitable for diverse applications. The quickly degrading PGA and PLA are well suited for tissue engineering applications, whereas the slowly degrading PCL is more appealing for long-term implants. The degradation rates of electrospun nanofibers can be further tuned by using synthetic copolymers, such as PLLA–CL (PLA and PCL) and PLGA (PLA and PGA), with varying component ratios, or using polyblends of similar polymers. A list of common synthetic polyblend nanofibers along with their targeted applications is provided in Table 1.

Natural polymer blends

Natural polyblend nanofibers have been prepared for better emulation of the ECM. Several commonly used natural polyblend nanofibers and their targeted applications are provided in Table 1. Collagen–chitosan polyblend nanofibers [23] have been prepared to better mimic the ECM which is similarly composed of the fibrous proteins and GAGs (Box 2). Although fibrous proteins are costlier than natural polysaccharide polymers, they are typically easier to electrospin into nanofibers. Polysaccharides can be difficult materials for electrospinning because of gelation when their starting solutions are at concentrations below what is required for electrospinning [24,25]. Although natural materials represent the same biochemical materials found in native tissue, stringent chemical processing during isolation and purification can disrupt their innate structures, making nanofibers prepared from natural polymers weak. In addition, these polymers are normally distensible in water. Thus, natural polymer nanofibers are commonly strengthened with glutaraldehyde or carbodiimide-based crosslinking agents after syn-

Table 1. Synthetic and natural polyblends

Component polymers	Solvent	Fiber diameter (nm)	Targeted applications	Comments	Ref.
Synthetic–synthetic polyblends					
PLGA and PLGA	DMF/THF	~550	Cartilage reconstruction	PLGA with varying lactic acid:glycolic acid ratios were blended together	[38]
PCL and PLGA	DCM/DMF	280–8000	Peripheral nerve regeneration	Higher flexibility than traditional channel guides	[42]
PLGA and PLA–PEG	Aqueous	250–5000	Skin/cartilage regeneration and DNA therapy	DNA plasmids were incorporated into the polymer solution prior to nanofiber fabrication	[39]
PLA-based blend	DMF	50–500	Tissue engineering	Four-part blend of PLA, PLGA, PLA-PEO-PLA and lactide	[43]
Natural–natural polyblends					
Collagen and chitosan	HFP	434–691	Vascular and nerve tissue engineering	Without a crosslinker, these all-natural polymer-based nanofibers are unstable	[23]
Collagen and collagen	HFP	~390	Tissue engineering	Blends were prepared with collagen types I (calfskin) and III (human placenta)	[14]
Collagen and elastin	HFP	110–1120	Cardiovascular tissue engineering	Without a crosslinker, these all-natural polymer-based nanofibers are unstable	[26]
Silk fibroin and chitin	HFP	340–920	Tissue engineering	Phase separation between the polymers was observed	[27]

Polymers: PCL, polycaprolactone; PLGA, polylactic-*co*-glycolic acid; PEG, polyethylene glycol. **Solvents**: DCM, dichloromethane; DMF, dimethylformamide; THF, tetrahydrofuran; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol.

thesis. Polyblends of collagen–chitosan [23], collagen–collagen (varying source/type) [14] and collagen–elastin [26] have been crosslinked with glutaraldehyde to prevent dissolution. In fact, material sensitivity to water has been so significant, that it has been necessary to apply glutaraldehyde as a vapor instead of an aqueous crosslinker solution to prevent material disintegration during crosslinking. Although crosslinking can stabilize and strengthen nanofibrous structures, agents such as glutaraldehyde can increase the risk of material cytotoxicity and calcification *in vivo*. To avoid the use of toxic chemicals, natural polymers have been crosslinked with *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide chemistry or without the use of crosslinkers at all. For example, chitin–silk fibroin polyblend nanofibers have been processed with water vapor to prevent fiber swelling or dissolving in water [27]. Also, the thickness and orientation of the nanofibers in a scaffold, as well as porosity and pore size of the scaffold can significantly affect overall material strength. Collagen polyblends were prepared as parallel arrays of collagen "fibrils" to make the all-natural polymer scaffolds stronger than disordered fibrous mats of the same nanofibers [14].

Natural–synthetic polymer blends

Hybrid nanofibers prepared with combinations of natural and synthetic polymers have been more widely investigated than natural–natural and synthetic–synthetic polyblend nanofibers. This is primarily because nanofibers can be engineered to retain the mechanical strength and durability of a synthetic component and the biological functionality of a natural polymer. These combinations have yielded material systems tuned for use in vasculature (elastin–PLGA), cardiovascular (elastin–gelatin–PCL),

Box 2. Extracellular matrix (ECM) components

Structural proteins

Collagen: The majority of the protein content of the ECM comes from the collagen superfamily (>90%). These collagens (predominantly types I, II, III and IV) share a structural motif of three polypeptide chains that form a triple helical configuration. Interactions between these helices form larger fibril structures (~50–200 nm diameter). The orientation of collagen fiber architecture plays a critical role in tissue structure and function (aligned fibers in ligaments, spiral arrangement in smooth muscles).

Elastin: Found in large quantities in ligaments, tendons, skin and the heart, elastin molecules are formed by relatively unstructured polypeptide chains crosslinked together to form a rubber-like network. This material elastically stretches and shrinks reversibly owing to the uncoiling/recoiling of the individual protein molecules.

Specialized proteins

Adhesive proteins: Several filamentous cell adhesion matrix proteins including laminins and fibronectins share the interstitial spaces of the ECM. These glycoproteins (proteins modified with short carbohydrate residues) are involved in binding matrix proteins (e.g. collagen and fibrin), polysaccharides and surface adhesion receptors on the cell surface, called integrins. These long proteins can modulate cell shape, migration and adhesion as well as organize the ECM components.

Proteoglycans

Proteoglycans: Proteoglycans are a diverse subclass of glycoproteins that are modified with linear polysaccharide chains (glycosaminoglycans or GAGs). Their carbohydrate content (95%) typically outnumbers the protein content (5%) significantly. The arrangement of the sugar residues in these specialized GAGs varies depending upon the tissue type they occupy. ECM proteoglycans play a space-filling role, binding water molecules and cations between fibrilar components of the ECM. Additionally, the GAG chains also bind to ECM proteins, helping anchor cells to the ECM via surface-bound proteoglycans.

Mineral deposits (bone)

Apatite: In addition to the organic component of bone ECM (e.g. collagen type I and proteoglycans), there is a large mineral component in the form of apatite. This crystalline calcium phosphate plays a significant role in the mechanical strength of the bone and storage of calcium and phosphate ions of the body. These minerals include hydroxyapatite $Ca(PO_4)_6(OH)_2$, carbonate apatite $Ca_{10}(PO_4)_6CO_3$ and fluoroapatite $Ca_{10}(PO_4)_6F_2$. These minerals form plate-shaped apatite crystals with diameters of ~50 nm within the ECM.

Table 2. Mixed synthetic and natural polyblends

Natural polymers	Synthetic polymers	Solvent	Fiber diameter (nm)	Targeted applications	Comments	Ref.
Chitosan	PEO	Acetic acid/ DMF/DMSO	40–several microns	Wound dressings, drug delivery	Stable in water; surfactant used during fabrication for improved nanofiber structure	[24]
	PVA	TFA/DCM	120–470	Tissue engineering	PVA helped break up the rigid interactions between chitosan chains for electrospinning	[53]
	PCL	TFA/TFE	100–200	Peripheral nerve regeneration	Nanofibers showed high stability over protracted time spans	[40]
	PLLA–CL	HFP	124–426	Tissue engineering	Porosity increased with an increase in the weight ratio of chitosan:PLLA–CL used	[54]
Chitin	PGA	HFP	50–350	Human skin tissue engineering	PGA degradation was accelerated in the presence of chitin	[55]
Collagen	PCL	HFP	520	Vascular tissue engineering	High burst pressure strength and mechanical properties	[3]
	PCL	HFP	600–900	Human skin tissue engineering	Scaffolds were chemically crosslinked; increase in PCL content yielded stronger mechanical properties	[31]
	PEO	Aqueous	100–150	Wound dressings and tissue engineering	Tensile strength changed with variation in collagen:PEO ratio	[29]
	PCL	HFP	500–600	Peripheral nerve regeneration	Better axon guidance was observed in collagen/PCL polyblends vs. PCL-only fibers	[41]
	PLLA–CL	HFP	100–200	Vascular tissue engineering	Normal endothelial cell attachment and growth was observed	[56]
	PLLA–CL	HFP	120–520	Vascular tissue engineering	Collagen phase separation was observed in some preparations	[32]
Collagen and elastin	PCL	HFP	470–1600	Cardiovascular tissue engineering	PCL content enhances the mechanical properties of the nanofibers	[26]
	PLGA, PCL, PLLA, or PLLA–CL	HFP	470–770	Cardiovascular tissue engineering	Scaffolds were chemically crosslinked; relative concentration of collagen/ elastin/synthetic polymer was 45/15/40 (w/w/w)	[57,58]
Elastin, collagen or elastin and collagen	PEO	Aqueous	220–600	Tissue engineering	Nanofibers were chemically crosslinked; electrospinning solutions included no organic solvents	[30]
Gelatin	PLLA–CL	TFE	50–500	Human skin tissue engineering	Gelatin content increased hydrophilicity and decreased mechanical strength	[59]
	PCL	HFP or TFE	640–880 (HFP) 50–1000 (TFE)	Cardiovascular tissue engineering	PCL enhances the mechanical strength of the nanofiber	[26,60]
	PCL	TFE	2790–4630	Tissue engineering	Core-shell formation (PCL core; gelatin shell); Crosslinker employed	[36]
	PCL	TFE	160–232	Neural tissue engineering	Cells proliferated on both aligned and random nanofibrous mats	[4]
	Polyaniline	HFP	60–800	Cardiac, and neural tissue engineering	Scaffolds were chemically crosslinked; polyaniline and its blended nanofibers are electrically conductive	[61]
Gelatin and elastin	PLGA	HFP	~380	Vascular, cardiac and pulmonary tissue engineering	Nanofibers showed good stability without the need of crosslinkers	[62]
Silk fibroin	PLLA–CL	HFP	130–650	Vascular tissue engineering	Controllable degradation rate; silk fibroin content increases fiber hydrophilicity	[63]
	PEO	HFP or aqueous	700–800	Tissue engineering	Electrospinning solutions included no organic solvents	[28]
Alginate	PEO	Aqueous/DMSO	~75	Tissue engineering	Both ionic and covalent crosslinking improved structural properties	[19]

Table 2 (*Continued*)

Natural polymers	Synthetic polymers	Solvent	Fiber diameter (nm)	Targeted applications	Comments	Ref.
Dextran	PLGA	DMSO/DMF	~1000	Tissue engineering	Nanofibers were photo-crosslinked	[64,65]
Casein or lipase enzyme	PEO or PVA	TEA/water/buffer	100–500	General use of natural polymers	Scaffolds were chemically crosslinked; enzyme activity was retained	[66]

Polymers: PCL, polycaprolactone; PLGA, polylactic-*co*-glycolic acid; PEO, polyethylene oxide; PVA, polyvinyl alcohol; PLLA–CL, poly-L-lactide-*co*-ε-caprolactone; PGA, polyglycolic acid; PANi, polyaniline.
Solvents: DCM, dichloromethane; DMF, dimethylformamide; THF, tetrahydrofuran; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol; TEA, triethanolamine; aqueous indicates water-based solution.

skin (collagen–PCL) and nerve (chitosan–PCL) regeneration. Importantly, these materials do not require added processing after electrospinning, such as chemical crosslinking, to maintain adequate mechanical strength. An exhaustive list of natural–synthetic polymer blend nanofibers is provided in Table 2.

In addition to combining properties, polyblends have been used to facilitate the preparation of natural polymer fibers with polymers that are not amenable to electrospinning. For instance, casein (a milk protein), silk fibroin and chitosan are not readily prepared by electrospinning without rigorous processing with inorganic acids and neutral salts that can cause unwanted polymer degradation or damage. This is typically as a result of the strong intermolecular forces or three-dimensional structures of the native polymers. Silk fibroin can form insoluble β-sheet precipitates that prevent electrospinning of fine silk fibroin nanofibers. These polymers can be solubilized, however, with the addition of certain polymers. Polyethylene oxide (PEO) or polyvinyl acetate (PVAc) has been mixed with silk fibroin, alginate and chitosan to yield blends that can be readily prepared as nanofibers [19,24,28].

Many of the component polymers described so far have been dissolved in volatile solvents. These organics allow full polymer extension in solution and because of their low boiling points can dry during preparation. However, residual organic solvents in the nanofiber scaffold can be harmful when used *in vivo*. Alternatively, the hydrophilic polymer, PEO, has been used to solubilize natural polymers in aqueous solution, including silk fibroin [28], collagen [29,30], elastin and collagen–elastin [30].

Blend distribution

Typically, miscible polymers premixed prior to electrospinning yield a uniform phase distribution throughout the electrospun nanofibers (Figure 2), but this is not always the case. When polymer content reaches a certain threshold, local domains of unmixed polymer can form. This phase segregation was observed in electrospun collagen–PCL blends when the PCL component was raised to 30% [31] and in a collagen–PLCL polyblend where the components were mixed in equal parts [32]. It is believed that the demixing of the component materials might occur during the flight of the nanofibers in electrospinning, possibly as a result of the ionic character of the collagen. The resulting phase inhomogeneities can lead to weakened mechanical properties, particularly when the material size is reduced to the nanoscale. Similarly, the degree of crystallinity within the blend can affect the biodegradation rate of the fiber itself [33].

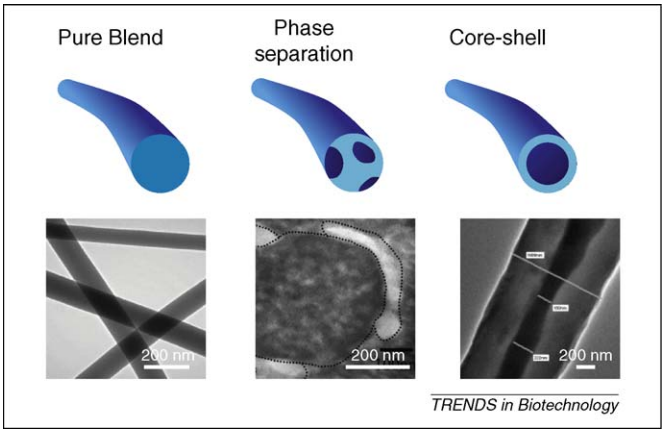

Figure 2. Internal structure of nanofiber polyblends. Premixing of multiple components used in nanofiber preparations typically forms uniform pure blends (left) {transmission electron microscope (TEM) image reproduced, with permission from Wiley-VCH Verlag GmbH & Co. KGaA, from Ref. [40]}, but separation of material phases might result by design (right) (TEM image, reproduced with permission from Wiley-VCH Verlag GmbH & Co. KGaA, from Ref. [34]) or as a result of processing parameters (middle) (TEM image, reproduced with permission from the American Chemical Society, from Ref. [32]). Shown above are the major types of electrospun nanofibers and possible phases that can result during synthesis.

Nanofibers have also been prepared with core-shell structures by a technique called coaxial electrospinning (Figure 2). In this method, two immiscible liquids are pushed through concentrically arranged needles that form a single outlet. As the liquids are pumped out of the needles the outer polymer sheaths the inner "core" material and the deposited polymer nanofiber has a core-shell structure [34]. This technique has been widely employed for the preparation of ceramic core-shell and hollow nanofibers [35], but has also been used with multipolymer formulations. For instance, PCL has been wrapped in a gelatin shell to form nanofibers with a mechanically strong core and a bioactive surface [36]. The core-shell nanostructure also has obvious implications for local drug delivery through selective protection and release of encapsulated payloads that will be discussed later. Although not strictly polyblends, coaxial fibers adapt multiple polymers for enhanced properties.

Polyblend nanofiber applications

The ability to tailor the physical and chemical properties of nanofibers using polymer blends allows engineers to prepare highly specific materials for individual applications. These areas of biomedical implementation are broadly

classified as either tissue engineering or therapeutic delivery, each requiring specific biological and mechanical properties.

Tissue engineering

Successful tissue replacement and regeneration relies on the ability of a mechanical scaffold to foster cellular ingrowth and rapid repopulation. This requires synthetic solutions to emulate the native ECM of various tissues including musculoskeletal tissue (bone, ligament cartilage), skin, nerve, vascular tissue, among others. Broadly, the ECM is the organization of fibrous proteins that surrounds and supports local cells as detailed in Box 2. The distribution and makeup of this protein–polysaccharide network ultimately controls cell shape, defines tissue architecture and helps regulate its physiological function.

To prepare nanofibers that can emulate the biochemical and topographical makeup of the ECM, nanofiber scaffolds have been prepared using the ECM proteins and polysaccharides or synthetic formulations that match their physical properties. Electrospinning allows the polymers to be prepared in hierarchical structures with unique alignment or stacking that matches the porous nature of the native tissue. In addition, electrospinning is a mild technique that does not degrade or aggregate electrospun proteins and other macromolecules [37]. This ensures that the bioactivity of natural polyamides (collagen, gelatin and elastin) and polysaccharides (chitin and chitosan) remain functional. These biomolecular footholds promote cell adhesion and migration and can activate secondary messenger systems that alter gene expression of local cells leading to phenotypic changes (proliferation, differentiation, mobilization). These cues can in effect cause changes to the composition of the ECM through remodeling by the host.

In addition to mimicking the biochemical and structural environment, certain tissue systems have additional demands. For instance, vascular tissue must accommodate the high pressure and flow, demonstrating a high resistance to shear stress. PCL–collagen blends have demonstrated the appropriate strength and elasticity to resist long-term stress [3] making them suitable substitutes. Likewise, several PLGA-based polyblends have been prepared with mechanical properties similar to those of skin and cartilage [38,39].

Nanofibers electrospun onto flat collection plates can form nonwoven fibrous mats that are well suited for relatively flat scaffolds, such as skin grafts and wound dressings. By electrospinning polyblends onto rotating spindles, nanofibers can be fabricated into large tubular constructs [32]. These scaffolds are well suited as nerve guides for peripheral nerve regeneration, where severed nerve endings cannot be repaired with sutures. Synthetic nerve guides can take the place of autografts, to redirect nerve growth across the critical gap. These materials have been prepared with PCL polyblends with chitosan [40], collagen [41] and PLGA [42], and have demonstrated strong mechanical properties, capable of being sutured to the nerve ends and maintaining structural stability *in vivo*.

If successful, tissue engineering induces the formation of neo-tissue necessitating the breakdown and clearance of implanted scaffolds. Synthetic polyesters such as PLGA and PCL allow controlled degradation through hydrolysis that voids the need for surgical retrieval after use [43]. Natural polymers are also biodegradable, but these materials undergo cell-induced proteolytic degradation leading to natural tissue remodeling. Therefore, these polymers will breakdown as cells mobilize into and adjacent to the scaffold making them well suited for tissue regeneration applications [16].

Therapeutic delivery

Nanofiber scaffolds have been identified as local drug delivery vehicles owing to their large surface area and controlled degradation. Polyblends have been loaded with drugs by one of three different incorporation strategies: (i) polymer–drug blends; (ii) encapsulation of water-soluble emulsions; and (iii) reservoirs inside core-shell structures.

Drugs have been incorporated into polyblend nanofibers by premixing the polymer solution with the therapeutic before electrospinning. This has been demonstrated with the two antibiotics: tetracycline hydrochloride (in polyethylene-co-vinyl acetate and PLA) [44] and cefoxitin sodium (in PLGA/PEG-b-PLA/PLA) [5]. Importantly, the addition of the drug can affect the physical properties of the electrospun nanofiber. The cefoxitin sodium caused a reduction in fiber diameter (250 vs. 350 nm) and morphological changes (less beading) as a result of, in part, the increased conductivity of the polymer solution (with the addition of an ionized drug molecule).

Water-soluble therapeutics has also been encapsulated in hydrophobic polymers by electrospinning aqueous dispersions within a polymer–organic solvent mixture [45]. Emulsion-based electrospinning has been used in polyblends to capture the hydrophilic model protein cytochrome *c* in PLLA–polyethylene imine and PLLA–polylysine nanofibers [37]. These polyblends could quicken the release rate of the protein by increasing the proportion of the hydrophilic polymer component. The use of emulsions has also been shown to alter the nanofiber properties during electrospinning. Sy *et al.* showed that by varying the relative ratio of aqueous and organic phases in a mixture, the viscosity (and subsequently the thinning) of the nanofiber is altered [46].

In addition to loading small molecules and proteins, functional nucleic acids have been successfully incorporated into the nanofibers for gene delivery. Luu *et al.* successfully introduced plasmid DNA into polymeric nanofibers yielding a biologically active blend scaffold [39]. The released DNA was able to induce cell transfection. It is believed that the DNA was largely carried near the surface of the nanofibers, which leads to observed burst release. By controlling the conformational properties of the nanofibers (e.g. fiber diameter, porosity and degradation rate), the release rate could be controlled [39]. To increase transcription efficiency, DNA has been condensed onto chitosan and incorporated into PLGA–hydroxyapatite nanofiber scaffolds (the hydroxyapatite enhances the mechanical strength of the structure) [47]. Chitosan complexes with DNA formed nanoparticles, which protected the nucleic acids from degradation. The DNA can be complexed with the nanofibers either by simple adsorption,

adsorption with chitosan–DNA nanoparticles or encapsulation of the nanoparticles prior to electrospinning. Again, electrospinning did not deactivate the DNA during encapsulation, while showing long-term release rates. The dual composite (hydroxyapatite and DNA) demonstrates an important opportunity for synergistic therapy [47].

In addition to blending the drug into the polymer mixture, a composite drug–polymer can be prepared by encapsulation of drugs or biomolecules by coaxial electrospinning, forming core-shell structures. This approach offers several advantages. First, the core-encapsulated material does not have to be electrospinnable. Instead, the drug, protein, nucleic acids or growth factors can be simply prepared in the core solution, protected from denaturing. Second, the controlled release of the material can be regulated by the degradation rate or porosity of the polymer shell. Core-shell systems have been used to carry antibiotics [48,49], bioactive proteins [50,51] and antioxidants (for wound healing) [49].

Concluding remarks and perspectives

The use of polyblend nanofibers in biomedical applications has gained increased attention in recent years. These polyblend nanofibers prepared by electrospinning mixtures of synthetic and natural polymers have demonstrated robust biochemical, structural and mechanical properties that cannot be achieved by any single polymer. Not only has this technique produced nanostructures with properties inherited from each of its constituent polymers, but it has permitted the use of natural polymers, including proteins and polysaccharides, that are otherwise difficult to prepare in fiber form. The electrospinning represents a simple, efficient and scalable method that is well suited to prepare clinically relevant materials.

There remains significant optimism about polyblend nanofibers and their potential impact on regenerative medicine and controlled drug delivery. The dimensions of these nanofiber blends mimic the dimensions of the natural fibrils of the ECM of the body. Researchers are beginning to assemble these nanofibers in arrangements that better emulate local tissue systems [52]. By modulating the fiber alignment and orientation, three-dimensional scaffolds can be formed that match the mechanical properties and microenvironments of varying tissue types (e.g. aligned ligament fibers versus spiral smooth muscle fibers). Fiber orientation of polyblends will play an increasing role in producing topographical cues that can direct cellular activity to regenerate functional tissues, such as tendons, nerves, corneal stroma, and muscle and heart tissue. For example, fiber orientation would be particularly important in directing axonal elongation in nerve regeneration and stem cell differentiation. New inroads are also being made into the integration of various types of biomolecules into polyblend solutions, which can stimulate cellular activity during tissue regeneration, induce stem cell differentiation or prevent infection simultaneously. Further tuning of nanofiber porosity and pore size will allow better nutrient/waste transport and cell mobility, and controlled drug release.

The major challenge in development of polyblend nanofibers will be to identify the operating parameters and polyblend mixing factors that control the physical and biological features of a hybrid polymer system. Bioactivity, phase distribution within the nanofiber, and degradation rate are all key features that must be defined on an application-by-application basis. The preparation of thick, highly aligned nanofibers for practical use will require an additional challenge to be met: fiber alignment decreases as a result of the residual charge of the fibers which increases as the thickness of the fibrous mat grows. Finally, strict *in vivo* testing, including performance and toxicity studies will have to be conducted before this new class of materials can be identified as suitable for human use.

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References

1 Jiang, W. *et al.* (2008) Nanoparticle-mediated cellular response is size-dependent. *Nat. Nanotechnol.* 3, 145–150
2 Gerardo-Nava, J. *et al.* (2009) Human neural cell interactions with orientated electrospun nanofibers in vitro. *Nanomed.* 4, 11–30
3 Lee, S.J. *et al.* (2008) Development of a composite vascular scaffolding system that withstands physiological vascular conditions. *Biomaterials* 29, 2891–2898
4 Gupta, D. *et al.* (2009) Aligned and random nanofibrous substrate for the in vitro culture of Schwann cells for neural tissue engineering. *Acta Biomater.* 5, 2560–2569
5 Kim, K. *et al.* (2004) Incorporation and controlled release of a hydrophilic antibiotic using poly(lactide-co-glycolide)-based electrospun nanofibrous scaffolds. *J. Control. Release* 98, 47–56
6 Liu, X. *et al.* (2005) Surface engineering of nano-fibrous poly(L-lactic acid) scaffolds via self-assembly technique for bone tissue engineering. *J. Biomed. Nanotechnol.* 1, 54–60
7 Hartgerink, J.D. *et al.* (2001) Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science* 294, 1684–1688
8 Li, D. and Xia, Y. (2004) Electrospinning of nanofibers: Reinventing the wheel? *Adv. Mater.* 16, 1151–1170
9 Liang, D. *et al.* (2007) Functional electrospun nanofibrous scaffolds for biomedical applications. *Adv. Drug Deliv. Rev.* 59, 1392–1412
10 Mo, X.M. *et al.* (2004) Electrospun P(LLA-CL) nanofiber: a biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation. *Biomaterials* 25, 1883–1890
11 Chung, S. *et al.* (2009) Nanofibrous scaffolds electrospun from elastomeric biodegradable poly(L-lactide-co-epsilon-caprolactone) copolymer. *Biomed. Mater.* 4, 15019
12 Kim, T.G. and Park, T.G. (2006) Biomimicking extracellular matrix: cell adhesive RGD peptide modified electrospun poly(D,L-lactic-co-glycolic acid) nanofiber mesh. *Tissue Eng.* 12, 221–233
13 Li, W-J. *et al.* (2002) Electrospun nanofibrous structure: a novel scaffold for tissue engineering. *J. Biomed. Mater. Res.* 60, 613–621
14 Matthews, J.A. *et al.* (2002) Electrospinning of collagen nanofibers. *Biomacromolecules* 3, 232–238
15 Li, M. *et al.* (2005) Electrospun protein fibers as matrices for tissue engineering. *Biomaterials* 26, 5999–6008
16 Dang, J.M. and Leong, K.W. (2006) Natural polymers for gene delivery and tissue engineering. *Adv. Drug Deliv. Rev.* 58, 487–499
17 Lee, K.Y. *et al.* (2009) Electrospinning of polysaccharides for regenerative medicine. *Adv. Drug Deliv. Rev.* 61, 1020–1032
18 Li, Z. *et al.* (2010) Feeder-free self-renewal of human embryonic stem cells in 3D porous natural polymer scaffolds. *Biomaterials* 31, 404–412
19 Bhattarai, N. and Zhang, M. (2007) Controlled synthesis and structural stability of alginate-based nanofibers. *Nanotechnology* 18, 455601
20 Bhattarai, N. *et al.* (2005) PEG-grafted chitosan as an injectable thermosensitive hydrogel for sustained protein release. *J. Control. Release* 103, 609–624

21 Li, Z. *et al.* (2005) Chitosan-alginate hybrid scaffolds for bone tissue engineering. *Biomaterials* 26, 3919–3928
22 Uhrich, K.E. *et al.* (1999) Polymeric systems for controlled drug release. *Chem. Rev.* 99, 3181–3198
23 Chen, Z.G. *et al.* (2010) Electrospun collagen-chitosan nanofiber: a biomimetic extracellular matrix for endothelial cell and smooth muscle cell. *Acta Biomater.* 6, 372–382
24 Bhattarai, N. *et al.* (2005) Electrospun chitosan-based nanofibers and their cellular compatibility. *Biomaterials* 26, 6176–6184
25 Bhattarai, N. *et al.* (2006) Alginate-based nanofibrous scaffolds: structural, mechanical, and biological properties. *Adv. Mater.* 18, 1463–1467
26 Heydarkhan-Hagvall, S. *et al.* (2008) Three-dimensional electrospun ECM-based hybrid scaffolds for cardiovascular tissue engineering. *Biomaterials* 29, 2907–2914
27 Park, K.E. *et al.* (2006) Biomimetic nanofibrous scaffolds: preparation and characterization of chitin/silk fibroin blend nanofibers. *Int. J. Biol. Macromol.* 38, 165–173
28 Jin, H.J. *et al.* (2002) Electrospinning Bombyx mori silk with poly(ethylene oxide). *Biomacromolecules* 3, 1233–1239
29 Huang, L. *et al.* (2001) Engineered collagen-PEO nanofibers and fabrics. *J. Biomater. Sci. Polym. Ed.* 12, 979–993
30 Buttafoco, L. *et al.* (2006) Electrospinning of collagen and elastin for tissue engineering applications. *Biomaterials* 27, 724–734
31 Powell, H.M. and Boyce, S.T. (2009) Engineered human skin fabricated using electrospun collagen-PCL blends: morphogenesis and mechanical properties. *Tissue Eng. Part A* 15, 2177–2187
32 Kwon, I.K. and Matsuda, T. (2005) Co-electrospun nanofiber fabrics of poly(L-lactide-co-epsilon-caprolactone) with type I collagen or heparin. *Biomacromolecules* 6, 2096–2105
33 Gross, R.A. and Kalra, B. (2002) Biodegradable polymers for the environment. *Science* 297, 803–807
34 Sun, Z. *et al.* (2003) Compound core-shell polymer nanofibers by co-electrospinning. *Adv. Mater.* 15, 1929–1932
35 Loscertales, I.G. *et al.* (2004) Electrically forced coaxial nanojets for one-step hollow nanofiber design. *J. Am. Chem. Soc.* 126, 5376–5377
36 Zhao, P. *et al.* (2007) Biodegradable fibrous scaffolds composed of gelatin coated poly(epsilon-caprolactone) prepared by coaxial electrospinning. *J. Biomed. Mater. Res. A* 83, 372–382
37 Maretschek, S. *et al.* (2008) Electrospun biodegradable nanofiber nonwovens for controlled release of proteins. *J. Control. Release* 127, 180–187
38 Shin, H.J. *et al.* (2006) Electrospun PLGA nanofiber scaffolds for articular cartilage reconstruction: mechanical stability, degradation and cellular responses under mechanical stimulation in vitro. *J. Biomater. Sci. Polym. Ed.* 17, 103–119
39 Luu, Y.K. *et al.* (2003) Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA-PEG block copolymers. *J. Control. Release* 89, 341–353
40 Bhattarai, N. *et al.* (2009) Natural-synthetic polyblend nanofibers for biomedical applications. *Adv. Mater.* 21, 2792–2797
41 Schnell, E. *et al.* (2007) Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-epsilon-caprolactone and a collagen/poly-epsilon-caprolactone blend. *Biomaterials* 28, 3012–3025
42 Panseri, S. *et al.* (2008) Electrospun micro- and nanofiber tubes for functional nervous regeneration in sciatic nerve transections. *BMC Biotechnol.* 8, 39
43 Kim, K. *et al.* (2003) Control of degradation rate and hydrophilicity in electrospun non-woven poly(D,L-lactide) nanofiber scaffolds for biomedical applications. *Biomaterials* 24, 4977–4985
44 Kenawy el, R. *et al.* (2002) Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic acid), and a blend. *J. Control. Release* 81, 57–64
45 Sanders, E.H. *et al.* (2003) Two-phase electrospinning from a single electrified jet: microencapsulation of aqueous reservoirs in poly(ethylene-co-vinyl acetate) fibers. *Macromolecules* 36, 3803–3805
46 Sy, J.C. *et al.* (2009) Emulsion as a means of controlling electrospinning of polymers. *Adv. Mater.* 21, 1814–1819
47 Nie, H. and Wang, C.H. (2007) Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA. *J. Control. Release* 120, 111–121
48 He, C.L. *et al.* (2009) Fabrication of drug-loaded electrospun aligned fibrous threads for suture applications. *J. Biomed. Mater. Res. A* 89, 80–95
49 Huang, Z.M. *et al.* (2006) Encapsulating drugs in biodegradable ultrafine fibers through co-axial electrospinning. *J. Biomed. Mater. Res. A* 77, 169–179
50 Zhang, Y.Z. *et al.* (2006) Coaxial electrospinning of (fluorescein isothiocyanate-conjugated bovine serum albumin)-encapsulated poly(epsilon-caprolactone) nanofibers for sustained release. *Biomacromolecules* 7, 1049–1057
51 Liao, I.C. *et al.* (2006) Aligned core-shell nanofibers delivering bioactive proteins. *Nanomed.* 1, 465–471
52 Yang, X. *et al.* (2009) Nanofiber enabled layer-by-layer approach toward three-dimensional tissue formation. *Tissue Eng. Part A* 15, 945–956
53 Ohkawa, K. *et al.* (2004) Electrospinning of chitosan. *Macromol. Rapid Commun.* 25, 1600–1605
54 Chen, F. *et al.* (2008) Electrospun chitosan-P(LLA-CL) nanofibers for biomimetic extracellular matrix. *J. Biomater. Sci. Polym. Ed.* 19, 677–691
55 Park, K. *et al.* (2006) Biomimetic nanofibrous scaffolds: preparation and characterization of PGA/chitin blend nanofibers. *Biomacromolecules* 7, 635–643
56 He, W. *et al.* (2005) Fabrication and endothelialization of collagen-blended biodegradable polymer nanofibers: potential vascular graft for blood vessel tissue engineering. *Tissue Eng.* 11, 1574–1588
57 Stitzel, J. *et al.* (2006) Controlled fabrication of a biological vascular substitute. *Biomaterials* 27, 1088–1094
58 Lee, S.J. *et al.* (2007) In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. *J. Biomed. Mater. Res. A* 83, 999–1008
59 Jeong, S.I. *et al.* (2008) Electrospun gelatin/poly(L-lactide-co-epsilon-caprolactone) nanofibers for mechanically functional tissue-engineering scaffolds. *J. Biomater. Sci. Polym. Ed.* 19, 339–357
60 Zhang, Y. *et al.* (2005) Electrospinning of gelatin fibers and gelatin/PCL composite fibrous scaffolds. *J. Biomed. Mater. Res. Part B Appl. Biomater.* 72, 156–165
61 Li, M. *et al.* (2006) Electrospinning polyaniline-contained gelatin nanofibers for tissue engineering applications. *Biomaterials* 27, 2705–2715
62 Li, M. *et al.* (2006) Co-electrospun poly(lactide-co-glycolide), gelatin, and elastin blends for tissue engineering scaffolds. *J. Biomed. Mater. Res. A* 79, 963–973
63 Zhang, K. *et al.* Fabrication of silk fibroin blended P(LLA-CL) nanofibrous scaffolds for tissue engineering. *J. Biomed. Mater. Res. A* (in press)
64 Jiang, H. *et al.* (2004) Optimization and characterization of dextran membranes prepared by electrospinning. *Biomacromolecules* 5, 326–333
65 Pan, H. *et al.* (2006) Interaction of dermal fibroblasts with electrospun composite polymer scaffolds prepared from dextran and poly lactide-co-glycolide. *Biomaterials* 27, 3209–3220
66 Xie, J. and Hsieh, Y-L. (2003) Ultra-high surface fibrous membranes from electrospinning of natural proteins: casein and lipase enzyme. *J. Mater. Sci.* 38, 2125–2133