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Electrospinning of Polymeric Nanofibers for Tissue Engineering Applications: A Review

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ABSTRACT

Interest in electrospinning has recently escalated due to the ability to produce materials with nanoscale properties. Electrospun fibers have been investigated as promising tissue engineering scaffolds since they mimic the nanoscale properties of native extracellular matrix. In this review, we examine electrospinning by providing a brief description of the theory behind the process, examining the effect of changing the process parameters on fiber morphology, and discussing the potential applications and impacts of electrospinning on the field of tissue engineering.

INTRODUCTION

ELECTROSPINNING HAS GAINED POPULARITY in the last 10 years due in large part to an increased interest in nanoscale properties and technologies. This technique allows for the production of polymer fibers with diameters varying from 3 nm to greater than 5 μm.[1] Potential applications of electrospinning include filtration membranes, catalytic nanofibers, fiber-based sensors, and tissue engineering scaffolds.[1–3]

One attractive feature of electrospinning is the simplicity and inexpensive nature of the setup; the typical electrospinning setup consists of a syringe pump, a high voltage source, and a collector (Fig. 1). During the electro spinning process, a polymer solution is held at a needle tip by surface tension. The application of an electric field using the high-voltage source causes charge to be induced within the polymer, resulting in charge repulsion within the solution. This electrostatic force opposes the surface tension; eventually, the charge repulsion overcomes the surface tension, causing the initiation of a jet. As this jet travels, the solvent evaporates and an appropriate collector can be used to capture the polymer fiber. Figure 2A shows a scanning electron microscopy image of an electrospun polymer mesh.[4,5] This approach has been used successfully to spin a number of synthetic and natural[6–10] polymers into fibers many kilometers in length.[3,5]

Recently, electrospinning has gained popularity with the tissue engineering community as a potential means of producing scaffolds. The objective of this review is to describe briefly the theory behind the technique, examine the effect of changing the process parameters on fiber morphology, and discuss the application and impact of electrospinning on the field of tissue engineering.

THEORY OF ELECTROSPINNING

The stable electrospinning jet was described in detail by Reneker and Chun as being composed of four regions: the base, the jet, the splay, and the collection.[5] In the base region, the jet emerges from the needle to form a cone known as the Taylor cone. The shape of the base depends upon the surface tension of the liquid and the force of the electric field; jets can be ejected from surfaces that are essentially flat if the electric field is strong enough. Charging of the jet occurs at the base, with solutions of higher conductivity being more conducive to jet formation.[5] Electric forces then accelerate and stretch the polymer jet, causing the diameter to decrease as its length increases. Additionally, solvents with high vapor pressures may begin to evaporate, causing a decrease in jet diameter and velocity.[5] In the next region, Reneker and Chun hypothesized that radial charge repulsions cause the jet

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to "splay" into many small fibers of approximately equal diameter and charge per unit length.[5] The final diameter of the electrospun fibers upon collection is dependent upon how many splays are created.[5]

Rutledge and co-workers have recently used high-speed photography with exposure times as low as 18 ns to demonstrate that the jet that appears to splay is actually a single, rapidly whipping jet.[11,12] At high electric fields after traveling a short distance, the jet becomes unstable, begins to whip with a high frequency, and undergoes bending and stretching.[11] The group modeled the behavior of the jet in terms of three instabilities: the classical Rayleigh instability and two "conducting" modes. The axisymmetric Rayleigh instability is dominated by surface tension and is suppressed at high electric fields or charge densities.[11] The conducting modes are independent of surface tension and are dominated by electric forces; one conducting mode is axisymmetric and the other is nonaxisymmetric (whipping instability).[11,13,14] Rutledge and co-workers examined the competition between these instabilities for various applied electric fields and the flow rate and determined the dominant mode.[11] They constructed operating diagrams that outlined the conditions at which whipping could be expected; their predictions agreed well with experimental results.[11]

PARAMETERS OF ELECTROSPINNING PROCESS

From the previous description of theory, it is clear that the electrospinning process can be manipulated by a number of variables. Doshi and Reneker classified the parameters that control the process in terms of solution properties, controlled variables, and ambient parameters.[4] Solution properties include the viscosity, conductivity, surface tension, polymer molecular weight, dipole moment, and dielectric constant. The effects of the solution properties can be difficult to isolate since varying one parameter can generally affect other solution properties (e.g., changing the conductivity can also change the viscosity). Controlled variables include the flow rate, electric field strength, distance between tip and collector, needle tip design, and collector composition and geometry. Ambient parameters include temperature, humidity, and air velocity. In this section, studies that investigate the effect of each parameter on electrospun fiber morphologies and sizes are highlighted.

Viscosity/concentration

Solution viscosity (as controlled by changing the polymer concentration) has been found to be one of the biggest determiners of fiber size and morphology when spinning polymeric fibers. The relationship between the polymer viscosity and/or concentration on fibers obtained from electrospinning has been studied in a number of systems,[15–25] including poly(DL-lactic acid) (PDLA),[15] poly(lactic-co-glycolic acid) (PLGA),[16] poly(ethylene oxide) (PEO),[9,17–19] poly(vinyl alcohol) (PVA),[20–23] poly(methyl methacrylate) (PMMA),[24] polystyrene,[25] poly(L-lactic acid) (PLLA),[26] gelatin,[6] dextran,[8] and collagen type I-PEO.[9] At low polymer concentrations, defects in the form of beading and droplets have been observed (Fig. 2B);[6,8,9,15,17,19,21,22,25,27–33] the process under these conditions was characteristic of electrospraying rather than spinning.[28] Additionally, the presence of junctions and bundles have been seen, indicating that the fibers were still wet when reaching the collector.[17] Increasing the solution viscosity by increasing polymer concentration yielded uniform fibers with few beads and junctions.[6,9,17,25] In some cases, increasing the concentration of a polymer solution can also affect its surface tension;[17] however, for polystyrene solutions with constant surface tension, beading still decreased with increased viscosity.[25] This result indicates that the variation in viscosity was responsible for the morphological change of the fibers.[25] For solutions that were too concentrated (and therefore too viscous), the droplet dried out at the tip before jets could be initiated, preventing electrospinning.[15,28,34]

Attempts have been made to quantify the minimum polymer concentrations and viscosities required to electrospin fibers. Koski *et al*. found that it was possible to spin PVA as long as $[\eta]c > 5$ where $[\eta]$ was the intrinsic viscosity and c was the concentration.[20] For PEO, it was found that solutions with $[\eta]c > 10$ allowed for spinning.[18] McKee *et al*. found that a concentration greater than the entanglement concentration, c_e, was required to spin linear and branched poly(ethylene terephthalate-co-ethylene isophthalate) (PET-co-PEI).[35] Solutions with concentrations 2-2.5 times the entanglement concentration yielded uniform, bead-free fibers.[35] Recently, Gupta *et al*. synthesized PMMA and determined the critical chain overlap concentration, c^*.[24] They determined that when the solution concentration was less than c^* there was insufficient chain overlap to form polymer fibers; at all conditions, droplet formation was observed.[24] In the semi-dilute region (concentrations between c^* and c_e), beading was occasionally observed. At concentrations approximately double c_e, uniform, bead-free fibers were obtained.[24]

The diameter of the fibers produced by electrospinning has been found to increase with increasing solution concentration.[6,17,35–37] For example, PLLA fibers with diameters of 100-300 nm were produced from 1 wt% solutions while 5 wt% solutions yielded 800-2400 nm fibers.[26] Additionally, the diameter of PVA fibers increased from 87 ± 14 nm to 246 ± 50 nm by increasing the PVA concentration from 6 to 8%.[21] It was found that an increased fiber diameter correlated directly to a decrease in the surface area of electrospun mats.[37]

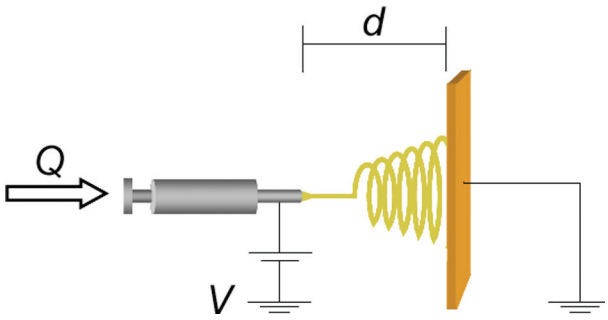

FIG. 1. Typical electrospinning setup. *Q*, flow rate; *d*, distance between plate and needle; *V*, applied voltage. (Color images are available online at www.liebertonline.com/ten).

Researchers have aimed to find a relationship between solution concentration and the fiber diameter. For example, it was found that increasing the concentration of gelatin yielded fibers with an increasing diameter according to a power law relationship.[6] For polyurethaneurea, it was found that fiber diameter was proportional to c^3 for solutions of a single polymer molecular weight.[28] McKee *et al*. found for PET-co-PEI solutions that the concentration could be normalized by c_e. They found that the fiber diameter scaled with $(c/c_e)^{2.6}$, regardless of polymer chain length and extent of branching.[35]

Electrospun fibers typically have a unimodal distribution; surprisingly, for PEO solutions spun at high concentrations, Deitzel *et al*. observed a bimodal distribution of fibers.[17] They found a secondary population of PEO fibers with a diameter approximately one third of the primary population, which they attributed to fiber-splaying events.[17] Demir *et al*. also found that polyurethane fibers electrospun at 12.8 wt% yielded fibers with three different sized diameters.[28]

Conductivity/solution charge density

It has been found that increasing the solution conductivity or charge density can be used to produce more uniform fibers with fewer beads present.[8,9,15,19,29,38,39] One approach to increasing solution conductivity has been through the addition of salt; for fibers spun from PEO,[19] collagen type I-PEO,[9] polyacrylic acid (PAA),[39] polyamide-6,[29] and PDLA,[15] uniformity increased and beading decreased upon the addition of salt. Pyridium formiate has also been used to increase conductivity of PLLA solutions and demonstrated a significant reduction in beading. It was hypothesized that this volatile salt additive would not remain in the fibers and therefore would not affect the properties of electrospun fibers.[26]

Conductivity has also been increased by the addition of alcohol to the solvent, resulting in smoother fibers of poly(hydroxybutyrate-co-valerate) (PHBV) with fewer beads present.[38] Likewise, the addition of tetrachloromethane, which reduced the solution conductivity, produced larger beads.[38] Cationic surfactants dodecyltrimethylammonium bromide and tetrabutylammonium chloride were added to polystyrene solutions, and it was found that a small amount (concentrations as low as 10^{-6} M) prevented bead formation.[40] The use of a nonionic surfactant, Triton X-405, did not completely prevent the formation of beads, so it was hypothesized that charged surfactants increased the solution conductivity and net charge density, causing an increase in the whipping instability yielding more uniform fibers.[40]

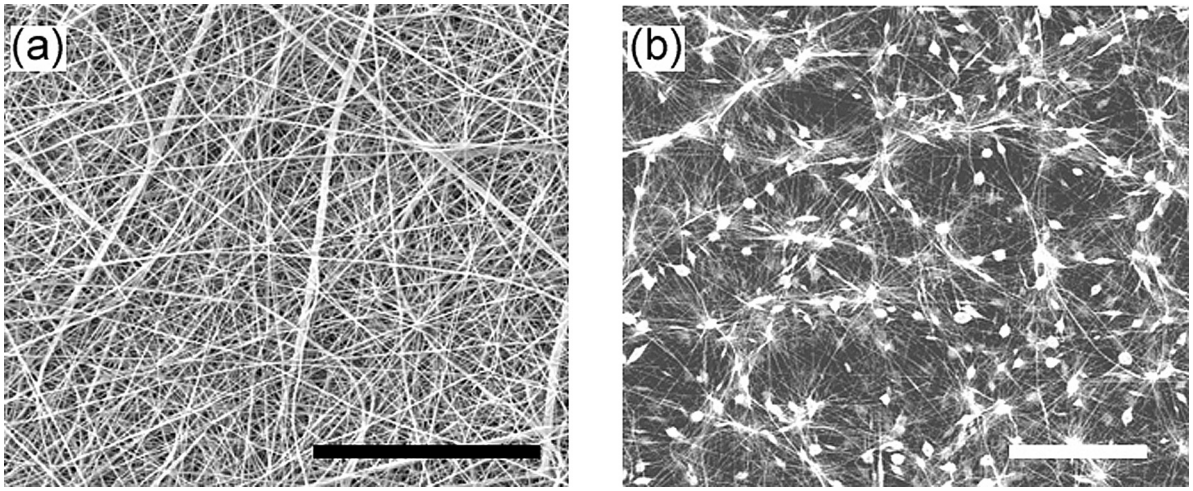

FIG. 2. (**a**) A random polymer fiber mesh produced by electrospinning a 9% PCL solution. (**b**) Electrospun mesh obtained using a 5% PCL solution; at low polymer concentrations defects in the form of beads and junctions are observed. Scale bars, 100 μm; spinning conditions: solvent = choloroform/methanol (3:1 by vol), voltage = 25 kV, distance to collector = 15 cm, flow rate = 6 mL/h.

The impact of the solution conductivity and charge density on the diameters of electrospun fibers was also studied. Zhang *et al*. showed that PVA fiber diameters were decreased from 214 ± 19 nm to 159 ± 21 nm when NaCl concentration was increased from 0.05 to 0.2% (spinning conditions: solvent = water, voltage = 5 kV, distance to collector = 10 cm, flow rate = 0.2 mL/h).[21] Zong *et al*. studied the effect of the addition of various salts (NaCl, KH_2PO_4, NaH_2PO_4) to PDLA solutions.[15] They found that salts with smaller ionic radii produced smaller fibers (~210 nm) while salts with larger ionic radii yielded larger ones (~1000 nm).[15] They attributed this difference to the higher charge density, and thereby mobility, of ions with smaller radii; the higher mobility resulted in increased elongational forces exerted on the fiber jet yielding a smaller fiber.[15] Additionally, the addition of proteins to dextran solutions yielded fibers with decreased diameters; since the proteins did not affect the solution viscosity, the variation in fiber diameter was attributed to changing the solution charge density.[8] In the same way, the addition of cationic surfactants was found to yield fibers with smaller diameters.[40] The addition of anionic surfactants has not been systematically investigated to date.

While increases in conductivity and charge density generally produced smaller fibers, for the spinning of PAA the reverse trend was observed: increases in conductivity yielded increases in the fiber diameter.[39] This difference could be attributed to the ionic groups inherent in PAA. Similarly, the addition of 1–5 wt% $MgCl_2$ to polyamide-6 increased the solution conductivity without impacting the viscosity or surface tension. This increase in conductivity also produced an increase in the fiber diameter.[29]

Surface tension

The impact of surface tension on the morphology and size of electrospun fibers has also been investigated. PHBV solutions with different surface tensions and similar conductivities were obtained using triethylbenzyl ammonium chloride, and it was found that beading was affected by the surface tension.[38] The addition of ethanol to PEO and PVA solutions lowered the surface tension.[19,21] In the case of the PEO, the solution containing ethanol exhibited less beading;[19] however, when ethanol was added to PVA solutions, beading was increased.[21] The difference in the effect of adding ethanol to these systems was attributed to the fact that it is a non-solvent for PVA and a solvent for PEO. Another approach to reducing the surface tension of polyurethaneurea solutions was to add polydimethylsiloxane, but no significant effect in fiber morphology was observed.[28]

Polymer molecular weight

Researches have examined the relationship between polymer molecular weight and the morphology and size of electrospun fibers. Gupta *et al*. synthesized PMMA varying in molecular weight from 12.47 to 365.7 kDa.[24] They determined c^* for each of these polymers and found that c^* decreased with increasing molecular weight. All polymers were then electrospun at identical conditions to isolate and ascertain the effect of molecular weight (spinning conditions: solvent = dimethylformamide, voltage = 10 kV, distance to collector = 15 cm, flow rate = 3 mL/h). Upon electrospinning, they found as the molecular weight increased, the number of beads and droplets was reduced. Uniform fibers were observed at concentrations that were 6-fold or more greater than c^*, regardless of polymer molecular weight.[24] Additionally, PMMA with a narrow molecular weight distribution gave uniform fibers at a lower concentration than those with larger molecular weight distributions.[24] For chitosan in acetic acid solutions, 30 kDa chitosan yielded fragile fibers with many beads present; polymer with a molecular weight of 398 kDa produced narrow fibers with rough surfaces.[7] For poly(N-isopropyl acrylamide), lowering the molecular weight produced smaller fibers that were more densely packed.[30] Varying the molecular weight of PEO from 600 to 4000 kDa during the spinning of PEO/chitosan solutions resulted in little difference in fiber diameter.[34] It was found that spinning polyamide-6 at various molecular weights produced fibers with diameters that were dependent upon the solution viscosity.[29]

Dipole moment and dielectric constant

Few studies have been performed to date to investigate the effect of dipole moment and dielectric constant on fiber properties. The spinning of polystyrene was studied in 18 different solvents, and the only solvents found to be used successfully had high values of dipole moment.[25] Solvents with a high dielectric constant were used to produce PMMA "cups."[41] Also, the productivity (number of fibers produced per unit time) of spinning polystyrene fibers was found to correlate with the dipole moment and dielectric constant.[42] To date, few studies have methodically examined the effect of dipole moment and dielectric constant on the resulting fiber morphology since these parameters are difficult to isolate.

Flow rate

Few studies have systematically investigated the relationship between solution feed or flow rate on fiber morphology and size. In general, it was found that lower flow rates yielded fibers with smaller diameters.[15] Flow rates that were too high resulted in beading since fibers did not have a chance to dry prior to reaching the collector.[21,38,42,43]

Field strength/voltage

One of the most studied parameters among the controlled variables is the effect of field strength or applied

voltage. At low voltages or field strengths, a drop is typically suspended at the needle tip, and a jet will originate from the Taylor cone producing bead-free spinning (assuming that the force of the electric field is sufficient to overcome the surface tension).[17] As the voltage is increased, the volume of the drop at the tip decreases, causing the Taylor cone to recede. The jet originates from the liquid surface within the tip, and more beading is seen.[17] As the voltage is increased further, the jet eventually moves around the edge of the tip, with no visible Taylor cone; at these conditions, the presence of many beads can be observed.[15,17]

Using laser diffraction, it has also been shown that increased voltages produces jets with larger diameters and ultimately lead to the formation of several jets.[28] The presence of beads and junctions at high voltages was found when spinning solutions of PEO,[17,19] PDLA,[15] bisophenol-A polysulfone,[43] chitosan,[7] and gelatin.[6] The correlation between fiber diameter and voltage was ambiguous. For PDLA[15] and PVA,[21] higher voltages yielded larger fiber diameters; however, when spinning silk-like polymer with fibronectin functionality[32] and bisophenol-A polysulfone,[43] the fiber diameter tended to decrease with increasing applied voltage.

Distance between tip and collector

Varying the distance between the tip and the collector has been examined as another approach to controlling the fiber diameters and morphology. It has been found that a minimum distance is required to allow the fibers sufficient time to dry before reaching the collector.[7] At distances that are either too close or too far, beading has been observed.[6,22] For the spinning of PVA,[21] gelatin,[6] chitosan,[7] and poly(vinylidene fluoride),[33] no significant effect of the distance between the tip and collector on the fiber size and morphology was observed. The spinning of silk-like polymer with fibronectin functionality fiber at closer distances produced flatter fibers while further distances gave rounder fibers.[32] For bisophenol-A polysulfone, closer distances between the tip and collector yielded smaller fibers.[43]

Needle tip design and placement

Several designs and configurations of needle tips have been investigated for the electrospinning process. For example, Li and Xia developed a coaxial, two-capillary spinneret (Fig. 3A).[44] Using feeds consisting of two immiscible liquids, they were able to produce hollow nanofibers (Fig. 3B).[44] They also used this spinneret to prepare blends of polymers.[45]

The use of multiple tips was investigated as a way to increase the throughput and production rate of electrospinning of PEO.[46] Multiple needle tips were also used to prepare blends of PVA and cellulose acetate. Using four tips and varying the number containing PVA and cellulose acetate allowed for fibers with various weight ratios of PVA and cellulose acetate to be produced.[47] Using two tips and a collector that could move transversely, mixes of PEO and polyurethane fibers were spun.[48] The transverse motion of the collector allowed for more uniform distribution of each polymer.[48]

Collector composition and geometry

A number of materials and geometries have been studied for the collection of electrospun polymeric fibers. Kim *et al*. collected PLLA and PLGA fibers on metal collectors, a water reservoir, and a methanol collector.[16] They found that smooth fibers were obtained using the metal collector. Collection on the surface of water caused the hydrophobic polymer fibers to shrink, while methanol caused swelling of the fibers.[16] Cellulose acetate was collected using copper mesh, aluminum foil, water, and paper.[36] It was found that the packing density was influenced by the conductivity of the collectors: the more conductive collectors dissipated the charge of the fibers. When this charge was not dissipated (non-conductive collectors), the fibers repelled one another, yielding a more porous structure.[36] Also, porous collectors, such as paper and copper mesh, produced a less-packed structure as compared to fibers collected on aluminum foil and water.[36] Poly(ethylene-co-vinyl alcohol) was even spun directly onto a human hand.[31]

Collectors with various geometries have also been designed and used. Rutledge and co-workers used two parallel plates when spinning their fibers in order to generate uniform electric fields.[12,49] Frame collectors were shown to yield aligned fibers with a conductive frame producing better alignment than a non-conductive one.[2] Also, an array of electrospun fibers has been produced using two conductive, collection rings.[50] The fibers were suspended between the rings, and fibers up to 10 cm long were obtained. The rotation of one of the collection rings allowed for the production of a multi-filament yarn.[50] PEO was also spun using a multiple field method in which the polymer jet passed through three parallel rings, each connected to an independent power supply.[51] This method produced smaller, bead-free fibers that collected in a more focused area.[51]

Fibers have also been collected using a rotating cylindrical drum collector rather than a stationary target (Fig. 3C); doing so has allowed for the alignment of the fibers (Fig. 3D).[10, 52] Better alignment was observed when this idea was extended to use a drum composed of copper wires that were grounded.[53] In another variation, a thin, steel pin was used as a counter electrode and was placed behind a rotating, non-conductive cylindrical collector; aligned fibers greater than 10 cm in length were obtained.[54] The rotating drum was also combined with the previously mentioned multiple field method to enable the

collection of fibers into thin strips or yarns.[51] Theron *et al*. employed a "tapered and grounded wheel-like bobbin" to collect and align nanofibers of PEO.[55] Their approach yielded fibers that were several hundred microns in length with diameters from 100 to 300 nm; braided fibers were also obtained.[55]

Ambient parameters

Few studies have been conducted to examine the effects of ambient parameters (i.e., temperature and humidity) on the electrospinning process. Mit-Uppatham *et al*. spun polyamide-6 fibers at temperatures ranging from 25 to 60°C.[29] They found that increasing the temperature yielded fibers with a decreased fiber diameter, and they attributed this decline in diameter to the decrease in the viscosity of the polymer solutions at increased temperatures. The humidity was varied by Casper *et al*. while spinning polystyrene solutions.[56] Their work showed that increasing the humidity resulted in the appearance of small circular pores on the surface of the fibers; increasing the humidity further lead to the pores coalescing.[56] Spinning has also been performed under vacuum in order to obtain higher electric fields; doing so produced fibers and yarns with larger diameters.[5]

In summary, we have highlighted studies that examine the effect of manipulating each parameter in the electrospinning process and the effect of that parameter on the resulting fiber morphology. Table 1 lists each parameter studied and its effect on the fiber morphology. However, it is difficult to isolate the effect of many of the parameters since they are interrelated. For example, changing the solution concentration/viscosity affects other solution properties, such as the conductivity and surface tension.

Additionally, though a large number of distinct polymers have been electrospun, there has been little systematic investigation of the conditions required for successful spinning. Typically, a trial-and-error approach has been employed in which the solution properties and spinning parameters are varied until uniform, defect-free fibers are obtained. Previously, it has been shown that a minimum concentration is required to electrospin; if there is insufficient chain overlap, uniform fibers will not be obtained. This minimum concentration is typically 5–10 times c^*; once this minimum concentration has been determined, varying other process parameters can be used to tune the resulting fiber morphology.

Overall, these studies highlight the potential of electrospinning for producing polymeric fiber meshes. By changing the solution and processing conditions, such as the polymer concentration, the properties of resulting mesh can be easily tuned. These meshes have tremendous potential as tissue engineered scaffolds, and the applications to date are discussed below.

TISSUE ENGINEERING SCAFFOLDS: SYNTHETIC EXTRACELLULAR MATRICES

As defined by Langer and Vacanti in 1993, tissue engineering is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function."[57] One aspect of tissue engineering has been the design of polymeric scaffolds with specific mechanical and biological properties similar to native extracellular matrix (ECM) in order to modulate cellular behavior. *In vivo*, a vast majority of the cells are in contact with the ECM, which is composed of a network of nanometer-sized proteins and glycosaminoglycans.[58] The intricate complexities of this spatial and temporal environment dynamically influence phenotypic and other cellular behavior by providing indirect and direct informational signaling cues.[59] For example, in bone, the presence of an organized collagen type I ECM for integrin binding is required for development of osteoprogenitor cells toward mature osteoblasts.[60] These types of interactions between cells and ECM can modulate cellular activities such as migration, proliferation, differentiation, gene expression, and secretion of various hormones and growth factors.[61] Thus, the more closely the *in vivo* environment (i.e., chemical composition, morphology, surface functional groups) can be recreated, the more likely the success of the tissue engineering scaffold.[62–64]

Tissue engineering scaffolds function as temporary ECMs until repair or regeneration occurs. A scaffold provides a 3-D framework for the cells to attach and develop *in vitro*.[65,66] Subsequently, the cell/scaffold construct can be implanted into a defect site for tissue repair and regeneration.[66] Although the desired characteristics of a scaffold vary slightly with the tissue trying to be recreated, there are general properties that are desirable. First and foremost, the scaffold should be biocompatible, meaning that it will integrate with the host tissue without eliciting a major immune response.[65,67] The scaffold should also be porous with a high surface-volume ratio to allow for cell attachment and in-growth, as well as exchange of nutrients during *in vitro* or *in vivo* culture.[65,66] Furthermore, the porous nature of the scaffold will allow for angiogenesis upon implantation in a defect site (for vascularized tissues). Also, because the scaffold acts as a temporary support for the cells to adhere and proliferate, it should mimic native ECM both architecturally and functionally.[65,68] Finally, a tissue engineering scaffold should be biodegradable so that a second surgery is not required to remove the implant.[68] The rate of degradation should coincide or at least be controllable to mimic the rate of neo-tissue formation.[67]

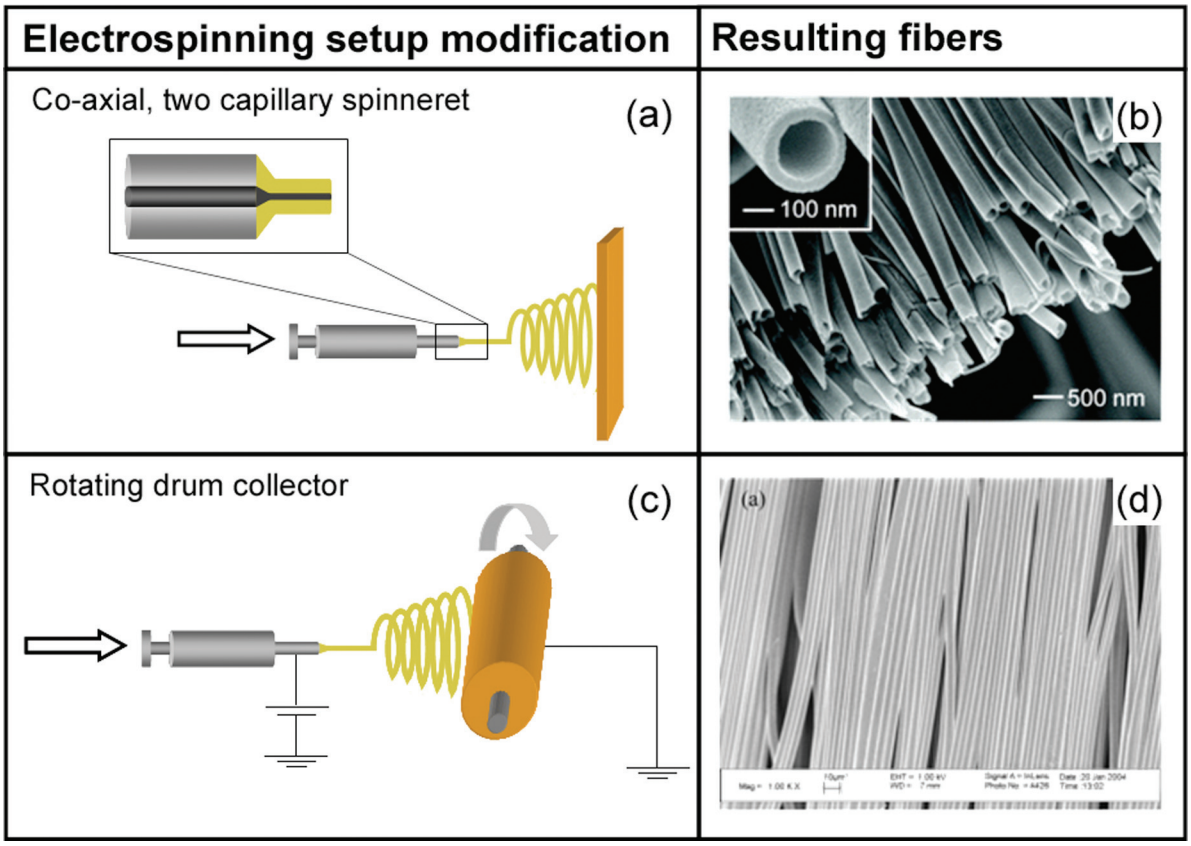

FIG. 3. Modifications of the typical electrospinning setup used to produce meshes with unique morphologies. (**a**) A co-axial, two capillary spinneret can be used to electrospin hollow nanofibers shown in **b**. A rotating drum collector (**c**) can be used to produce aligned fibers (**d**). Adapted (**a**) and reproduced (**c**) from Li and Xia. Direct fabrication of composite and ceramic hollow nanofibers by electrospinning. Nano. Lett. 4, 933, 2004. (**d**) Reproduced with permission from Chew et al. Sustained release of proteins from electrospun biodegradable fibers. Biomacromolecules **6,** 2017, 2005. (Color images are available online at www.liebertonline.com/ten).

CELLULAR RESPONSES TO NANOPHASE ELEMENTS

An important aspect of native ECM is its nanoscale components, such as collagen. It follows that tissue engineering scaffolds should also contain nanophase elements. Studies have shown that nanometer-sized elements can affect cellular behavior.[69,70] For example, osteoblast and osteoclast activity were increased on spherical nanophase alumina particles, which resembled the structure of hydroxyapatite crystals found in bone.[70] The nanofiber architecture has also been shown to selectively promote osteoblast proliferation and differentiation in carbon nanofibers.[71] Nanogrooved surfaces can induce contact guidance of human corneal epithelial cells, causing them to elongate and align their cytoskeleton along these topological features.[72] Highly porous PLLA scaffolds with nanoscale pores created using a liquid-liquid phase separation have been used for culture of neural stem cells and were shown to have a positive effect on neurite outgrowth.[73] Because the fiber diameters of nanofiber scaffolds are orders of magnitude smaller than the size of cells, cells are able to organize around the fibers[58] or spread and attach to adsorbed proteins at multiple focal points.[74]

ELECTROSPINNING FOR TISSUE ENGINEERING APPLICATIONS

Compared to self-assembly and phase separation techniques, electrospinning provides a simpler and more cost-effective means to produce scaffolds with an inter-connected pore structure and fiber diameters in the sub-micron range. The field of tissue engineering has sought to capitalize upon these features for the production of 3-D scaffolds. The following sections high-

Table 1. Effect of Changing Electrospinning Process Parameters on the Resultant Fiber Morphology

Process parameter	*Effect on fiber morphology*	*References*
Viscosity/concentration	• Low concentrations/viscosities yielded defects in the form of beads and junctions; increasing concentration/viscosity reduced the defects • Fiber diameters increased with increasing concentration/viscosity	6, 8, 9, 15–38
Conductivity/solution charge density	• Increasing the conductivity aided in the production of uniform bead-free fibers • Higher conductivities yielded smaller fibers in general (exceptions were PAA and polyamide-6)	8, 9, 20, 26, 30, 32, 37, 39–41
Surface tension	• No conclusive link established between surface tension and fiber morphology	19, 21, 28, 38
Polymer molecular weight	• Increasing molecular weight reduced the number of beads and droplets	24, 29, 30, 34
Dipole moment and dielectric constant	• Successful spinning occurred in solvents with a high dielectric constant	25, 41, 42
Flow rate	• Lower flow rates yielded fibers with smaller diameters • High flow rates produced fibers that were not dry upon reaching the collector	15, 21, 38, 42, 43
Field strength/voltage	• At too high voltage, beading was observed • Correlation between voltage and fiber diameter was ambiguous	16, 22, 25, 26, 28, 30, 32
Distance between tip and collector	• A minimum distance was required to obtain dried fibers • At distances either too close or too far, beading was observed	6, 7, 21, 22, 32, 33, 43
Needle tip design	• Using a coaxial, 2-capillary spinneret, hollow fibers were produced • Multiple needle tips were employed to increase throughput	44, 46–48
Collector composition and geometry	• Smoother fibers resulted from metal collectors; more porous fiber structure was obtained using porous collectors • Aligned fibers were obtained using a conductive frame, rotating drum, or a wheel-like bobbin collector • Yarns and braided fibers were also obtained	2, 10, 12, 19, 23, 27, 49–51, 53–55
Ambient parameters	• Increased temperature caused a decrease in solution viscosity, resulting in smaller fibers • Increasing humidity resulted in the appearance of circular pores on the fibers	5, 29, 56

light the use of electrospun scaffolds for tissue engineering applications.

Synthetic polymer scaffolds

There has been a surge in the use of electrospinning techniques to create nanofiber scaffolds for tissue engineering. The most typical method of collecting the electrospun fibers is on a grounded, collecting plate. Because of the randomness of the instable fiber jet, a highly porous, nonwoven fibrous sheet with a large surface-volume ratio is collected.[75] Electrospun PLGA fiber mats have been shown to have a porosity greater than 90%.[62] This material is ideal for tissue engineering scaffolds because the high surface area allows for a high percentage of cellular attachment, as well as for multiple focal adhesion points on different fibers due to nano-sized fiber diameters.[7] Additionally, fibers in the nanometer and submicron range more closely resemble the size scale of extracellular components.[75,76] The versatility of the technique has allowed for spinning of a very diverse set of synthetic and biological materials.[1,2] Innovative designs for fiber collection broaden the library of available scaffolds and increase the likelihood of successful engineering of specific tissue types. Furthermore, functional modifications to electrospun nanofiber matrices can improve a polymer's biocompatibility and cytocompatibility.

Electrospun nanofiber scaffolds are capable of supporting a wide variety of cell types. Human umbilical vein endothelial cells attached better and exhibited more proliferation when seeded onto 50:50 poly(L-lactic acid-co-ε-caprolactone) (PLCL) fibers with a diameter of 300 nm compared to 7 μm fibers. Cells attached to microfibers were rounded in shape and non-proliferative, whereas on nanofibers, the cells were spread and anchored on multiple fibers.[7] Mouse fibroblasts seeded on PLGA nanofibers adhered and spread according to fiber orientation.[62] Similar results were reported by smooth muscle and endothelial cells seeded onto hybrid scaffolds of 75:25 PLCL.[63]

The ECM like properties of electrospun nanofibers have been shown to affect the phenotypic behavior of a

variety of cell types. NIH 3T3 fibroblasts and normal rat kidney cells seeded onto polyamide nanofibers rearranged their actin cytoskeleton to a more *in vivo*-like morphology.[77] Breast epithelial cells on the same surface underwent morphogenesis to form multicellular spheroids. Fetal bovine chondrocytes seeded on nanofiber poly(ε-caprolactone) (PCL) scaffolds were able to maintain the chondrocytic phenotype during 3 weeks of culture, specifically upregulating collagen type IIB expression, which is indicative of a mature chondrocyte phenotype.[75] These studies demonstrate that nanofiber scaffolds are not only cytocompatible but can also be used to stimulate and encourage cell proliferation and phenotypic behavior. To date, the use of such scaffolds has been investigated for the engineering of mainly cartilage, bone, ligament/tendon, and vascular tissues.

Cartilage and bone engineering strategies have increasingly incorporated marrow stromal cells (MSC) because of their ability to differentiate into multiple cell lineages. Electrospun scaffolds have been shown to support the attachment and proliferation of MSCs.[78] Bone marrow-derived human MSCs seeded onto nanofiber PCL scaffolds are able to differentiate into adipogenic, chondrogenic, or osteogenic lineages based upon the culture media selected.[79] Marrow stromal cells seeded on electrospun PCL and in the presence of transforming growth factor-β (TGF-β) differentiated into a chondrocytic phenotype at levels comparable to traditional pellet cultures. Moreover, the constructs displayed a zonal morphology with a layer of cartilaginous matrix composed of collagen type II, cartilage proteoglycan link protein, and aggrecan.[80]

For bone tissue engineering, Yoshimoto *et al*. seeded MSCs from neonatal rats on electrospun PCL and cultured this construct in a rotating bioreactor; matrix mineralization and collagen type I deposition throughout the construct occurred after 4 weeks.[81] *In vivo* studies with similar constructs showed multi-layers of osteoblast-like cells, globular accretions, a woven bone-like appearance, and the presence of osteocyte-like cells embedded in mineralized matrix after explantation from the omenta of rats.[82]

Lee *et al*. used a rotating cylindrical target to align polyurethate nanofibers for tissue engineering ligament.[39] They discovered that, when exposed to mechanical strain, human ligament fibroblasts exhibited a spindle shape along aligned polyurethane fibers and secreted more ECM than when attached to a random fiber configuration.[39] Dalton *et al*. used yarns produced by the collection of nanofibers between dual rings as potential scaffolds for tissue engineering of tendons and muscles or as medical sutures.[50]

Novel fiber-collecting methods have been employed for tissue engineering of other tissue types. Ramakrishna *et al*. used a rotating drum collector for the alignment of electrospun fibers for vascular tissue engineering.[63] Human coronary artery smooth muscle cells attached and migrated along the axis of aligned PLCL (75:25) while expressing a spindle-like contractile phenotype; furthermore, the organization of the cytoskeleton inside these cells were parallel to the direction of the nanofibers.[83]

Fiber alignment was also conducive for cardiomyocyte attachment and growth; when seeded onto electrospun PLLA scaffolds, they exhibited fiber-guided filipodia-like protrusions and developed into sarcomeres.[84] A contractile cardiac graft was created by Shin *et al*. from cardiomyocytes seeded onto an electrospun PCL mesh that was spun and collected across a wire ring.[85] The passive load of this structure helped to condition the cardiomyocytes, which expressed cardiac-specific proteins such as α-myosin, connexin43, and cardiac troponin I, after 14 days of culture. Research is underway to extend this method to create implantable cardiac grafts.

Natural polymer scaffolds

Natural polymers are often used because of their enhanced biocompatibility and biofunctional motifs.[86] Collagen, for example, is often used as a scaffold for cells since it makes up a major component of the extracellular matrix. Furthermore, incorporation of collagen and other biological components such as alginate,[87] hyaluronic acid,[88] and starch[89] into synthetic polymers can improve the overall cytocompatibility of a scaffold. Thus, electrospinning of various biological substances, such as collagen, silk, fibrinogen, and chitosan, for biomedical applications has been investigated. Compared to synthetic polymers, electrospinning of biological materials is less versatile because a suitable solvent that does not compromise its integrity has to be used.[90]

A collagen nanofiber mesh scaffold may have the advantage of not only resembling the size scale but also the chemical and biological function of ECM. The spinning of collagen types I and III is feasible using 1,1,1,3,3,3 hexafluoro-2-propanol (HFP) as a solvent.[10,91] Collagen fiber diameters of 100 nm (type I) and 250 nm (type III) were achieved; these fibers possessed the typical 67 nm banding pattern observed in native collagen.[10] Culture of aortic smooth muscle cells under dynamic conditions led to proliferation and infiltration into the collagen network. By coating electrospun collagen with PCL, Venugopal *et al*. produced a scaffold with mechanical properties similar to skin and with the ability to support the attachment and proliferation of human dermal fibroblasts for dermal tissue engineering.[92]

Elastin and fibrinogen are other proteins found in the body that have been electrospun into nanofiber matrices. Elastin is a major component of the arteries and lungs, imparting resiliency and elasticity to these tissues.[93] Huang *et al*. developed a method to electrospin a syn-

thetic peptide sequence of elastin (Val-Pro-Gly-Val-Gly)$_4$(Val-Pro-Gly-Lys-Gly) mixed in water.[94] The ultimate tensile strength of dried nanofiber meshes was 35 MPa with a Young's modulus of 1.8 GPa. Through hydration and peptide cross-linking, it is possible to modulate these values to better mimic material properties of blood vessels.

The plasma serum protein fibrinogen is part of the wound healing and blood-clotting cascade. In order to spin human or bovine fibrinogen, it was solubilized in minimum essential medium/HFP (10:90).[95] A linear relationship between the fiber diameter (80–700 nm) and fibrinogen concentration (0.083-0.167 g/mL) was observed. A fibrinogen nanofiber mesh can be utilized as a biomedical gauze for wound healing.[96] Additionally, a fibrinogen scaffold could be conducive for cartilage repair, as fibrin glue has been suggested as a candidate material for neocartilage formation.[97]

Interestingly, Fang and Reneker were able to spin actual DNA fibers.[98] Other natural molecules that have been successfully spun include silk, chitosan, and dextran. Silk fibrin is derived from silkworm and has good biocompatibility, biodegradability, and minimal inflammatory response when implanted *in vivo*.[99,100] The silk nanofibers were able to support bone marrow stromal cell attachment and growth, despite the presence of PEO in the spinning solution.[100] The preparations of chitosan and dextran have also been investigated, and although their cytocompatibilities were not tested, these materials possess material and functional properties that could prove useful in tissue engineering strategies.[8,34]

Composite scaffolds

Composite scaffolds can also be created using electrospinning. For example, by sequentially spinning different polymer solutions, a scaffold with layers can be created. Each layer can be tailored for specific cell adhesion and could be potentially beneficial for zonal articular cartilage or arterial vessel repair.[48] Boland *et al*. have demonstrated smooth muscle cell infiltration into a multi-layered scaffold of collagen types I and III and elastin when cultured in a rotary cell culture system.[91] Alternatively, two or more polymer solutions can be spun concurrently, resulting in a scaffold with mixed types of fibers. Collagen types I and III could be spun in this manner to create a scaffold that better mimics their *in vivo* ratios.[10] Another strategy is to use a polymer that degrades faster than the other, thereby increasing the microvoid spaces for tissue in-growth.[48] By co-spinning solutions of PCL and gelatin, bigger pore sizes were achieved upon gelatin degradation, resulting in an increase in rabbit bone marrow cell migration.[90] However, the reported migration depth of the attached bone-marrow stromal cells was still only about 100 μm. The incorporation of gelatin to increase the pore size could be important since the small mesh sizes of electrospun scaffolds may be a limitation for tissue engineering applications.

In many of the *in vitro* studies described, the attached cells formed a monolayer at the top of the scaffold, but there is little evidence to suggest that cells migrated into the depths of the scaffold, especially since the pore sizes are small compared to the size of cells.[48] It has been suggested that cells cannot migrate through pores smaller than 10 μm.[101] In order for these nanofiber scaffolds to be functionally useful as tissue engineering scaffolds, cultivation of cells into a 3-D framework is critical.[81] However, dynamic culture conditions and *in vivo* studies have shown that there is cellular penetration and matrix deposition throughout nanofiber constructs although the mechanisms by which this occurs is not clear.[82,91,101] It is posited that in these conditions, cells are able to push against the fibers during their migration paths and thus optimize pore sizes for further cellular infiltration.[91,102]

Another type of composite scaffold developed has sought to incorporate carbon nanotubes into the electrospinning process as a means to reinforce polymeric fibers. Multi-walled carbon nanotubes were incorporated into PEO[81,103] and poly(acrylonitrile) nanofibers (PAN).[104] The PAN fibers were spun using a moving collector, and, at high concentrations, the nanotubes were aligned. It was found that the reinforced polymer fibers had an increase in tensile modulus by 144% at 20 wt% nanotubes and tensile strength increased by 75% at 5 wt% nanotubes.[105] Therefore, it may be possible to tailor the mechanical properties of nanofiber scaffolds to resemble that of the target tissue.

Functionalized scaffolds

A common strategy in tissue engineering is functionalization of a scaffold in order to increase biocompatibility or induce specific biological responses from attached cells. For example, prolonged *in vivo* activity of TGF-β2 was observed when it was bound to fibrillar collagen via poly(ethylene glycol) (PEG) compared to admixed samples.[106] Functionalization of electrospun fibers is also feasible and is typically carried out either by post-processing methods to conjugate molecules to the surface of nanofibers or by incorporating the bioactive factor into the spinning solution.

A novel, surface-modified scaffold was created for support of primary rat hepatocytes to illustrate the feasibility of covalently attaching cell-adhesive molecules on nanofibers. PAA was grafted onto poly(ε-caprolactone-co-ethyl ethylene phosphate) (PCLEEP) allowing for conjugation of galactose ligand, which mediates hepatocyte adhesion. Hepatocytes cultured on these galatosylated PCLEEP nanofiber scaffolds formed 20–100 μm spheroid aggregates that engulfed the nanofibers.[107]

Recently, the conjugation of bone morphogenetic pro-

tein-2 (BMP-2) on chitosan nanofibers for bone regeneration was described by Park *et al*.[108] BMP-2 conjugated to the surface of chitosan membranes increased osteoblastic cell attachment and retained bioactivity for up to 4 weeks. Furthermore, the BMP-2 coated surface of the chitosan membranes resulted in better proliferation, alkaline phosphatase activity, and calcium deposition of osteoblastic cells compared to BMP-2 adsorbed membranes.

Casper *et al*. incorporated low molecular weight heparin (LMWH) into nanofibers of PEO and PLGA by mixing it directly into the spinning solution.[109] By labeling the heparin with a fluorescent dye, the researchers were able to use multiphoton microscopy to show that heparin was distributed both throughout individual fibers, as well as the thickness of the mesh. Incorporation of PEG-LMWH resulted in improved heparin retention in the fibers and could theoretically allow slower release of attached growth factors that bind to heparin, such as basic fibroblast growth factor, vascular endothelial growth factor, heparin-binding epidermal growth factor, and TGF-β. Thus, this approach represents a simple way to deliver growth factors through incorporation of a growth-factor binding molecule into electrospun fibers.

Drug delivery carriers

The effective delivery of therapeutic agents toward alleviating medical conditions is one aspect of polymeric biomaterials design. Polymeric drug delivery systems are advantageous because they can be controlled to deliver drugs efficiently to a localized area.[110] The nuances of such a system may have profound implications on tissue engineering: nanofiber drug delivery systems may provide insight into the direct incorporation of bioactive growth factors into scaffolds. Additionally, drug delivery systems can be combined with implantable tissueengineering scaffolds to prevent infection while repair and regeneration occur. The discussion below describes the approaches to modulate the release of drugs from nanofiber scaffolds, with an emphasis on antibiotic delivery.

The high surface area–volume ratio of electrospun scaffolds allows the efficient delivery of a loaded drug. Various drugs have been successfully introduced (i.e., maintain their structure and bioactivity) into nanofiber scaffolds, which can potentially be used as drug delivery vehicles.[71,111] One method to incorporate therapeutic drugs into nanofibers involves solubilizing the drug into the polymer solution to be spun.[112] Using this method, a loading efficiency of 90% into PDLA nanofibers was reported for the antibiotic drug Mefoxin.[15] Covalent conjugation to polymers represents another method to modulate drug release.[113] Jiang *et al*. used a PLGA/PEG-*g*-chitosan (PEG-*g*-CHN) blend for delivery of ibuprofen.[114] The interactions between ibuprofen and chitosan in the physical blend attenuated ibuprofen release rates by limiting its diffusion. Moreover, conjugation of ibuprofen to PEG-*g*-CHN prior to spinning reduced the release rate even further.

It has also been suggested that the high porosity of nanofibers allows for rapid diffusion of degradation byproducts.[114] Burst release of Mefoxin in PDLA nanofibers was observed in the first 3 h and complete release occurred within 48 h.[15] Such a release profile may be suitable for prevention of postoperative infections. However, the burst release may also be indicative of the drug being attached only on the surface of the nanofibers. This was observed by Luu *et al*. who successfully incorporated intact plasmid DNA into PLGA and PLLA-PEG block copolymers that, upon release, was active over a 20 day period.[115] However, the DNA was localized on the nanofiber surfaces, leading to burst release within 2 h. This approach could serve as a potential platform for successful gene delivery. A tubular fiber structure, in which a bioactive molecule is encased within the nanofibers, could be a more effective platform for drug and gene delivery systems.[2] By changing solution components, it might be possible to encapsulate DNA into the fibers by micelle formation and thus obtain slower release kinetics.[115] More research is required to understand the mode of drug incorporation in electrospun nanofibers; for example, the high surface-volume ratio of nanofiber scaffolds may contribute to an increased burst release delivery effect.

Different release rates may be obtained by simply varying the fiber diameter or loading dosage. Zeng *et al*. used triethylbenzyl ammonium chloride and sodium dodecyl sulfate in PLLA solutions to create more uniform and smaller fiber diameters; the release of rifampin from these fibers in the presence of proteinase K followed zero order kinetics.[116] Also, blends of biodegradable polymers with different degradation rates can be used to control drug release. Release kinetics of tetracycline hydrochloride changed from burst to a more sustained release when 50:50 blends of PLLA:poly(ethylene-co-vinyl acetate) (PEVA) were used compared to either PLLA or PEVA alone.[112] However, the polymer choice may be dependent on the desired drug to be released and vice versa. Doxorubicin hydrochloride, a hydrophobic drug, was not well dispersed within electrospun PLLA fibers until it was made lipophilic.[116]

SUMMARY

Although electrospinning was first described over 70 years ago, attention to the technique has increased dramatically within the past 10 years, due in large part to the rising interest in nanoscale properties and materials. The tissue engineering community has begun to capital-

ize on the inherent nanoscale nature of electrospun polymeric fibers as potential scaffolds to mimic native ECM. Electrospun matrices are able to support the attachment and proliferation of a wide variety of cell types; moreover, the cells are able to maintain their phenotypes on these nanofiber scaffolds. Using innovative collectors and spinning techniques, scaffolds with aligned fibers, different compositions, improved mechanical properties, varying degradation rates, or functional moieties can be produced. Nevertheless, despite the comprehensive experimental and theoretical studies illustrating the ability to control fiber formation, concerns with fiber diameter uniformity still need to be addressed. Precise control of fiber morphology will be necessary for improved scaffold designs that better recreate the functions of native extracellular matrix. The generation of designer scaffolds with clinically relevant dimensions and the homogeneous distribution of cells within them will also need to be addressed for tissue engineering applications.

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