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Electrospun Nanofibers in Tissue Engineering

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1. Introduction

The field of tissue engineering and regenerative medicine is a fast growing scientific field. Many diseases and injuries that result in the loss of organ or tissue functions currently lack treatments which restore those functions and the patient to a desirable quality of life. Many current treatments could greatly benefit from incorporation of bioengineered organs and tissues, and investigation into this field shows great promise for modern medicine. Tissue engineering hypothesizes that by incorporating appropriate cells in the context of a three-dimensional scaffold and then implanting the cell-scaffold construct into an injury or defect, that the cells and scaffold will provide both active and passive healing properties. Various scaffolding systems exist and are generally made from natural and/or synthetic polymers. Scaffolds can be fabricated by many methods including solvent casting and particulate leaching, melt molding, rapid prototyping, phase separation, and many others (Yang et al., 2001). Of particular interest to the subject of nanofibers is electrospinning, which has seen widespread use in the field of tissue engineering due to the ease of use, scalability, adaptability, and capacity to form fibers on both the micro and nano scale (Sill & von Recum, 2008). In constructing scaffolds for tissue engineering it is ideal to provide cells with an environment which closely resembles their native extracellular matrix (ECM). The spinning of nanofibers allows for a connected and porous scaffold which can mimic the ECM of many tissue types structurally, chemically, and mechanically.

The ECM is a largely proteinaceous cellular environment that varies greatly between tissue types. In most tissue types the ECM is composed of a highly interconnected network of proteins such as collagen and elastin, and proteoglycans such as perlecan (Lodish et al., 2008). These molecules link together to form a functional environment in which cells live, move, receive and transmit signals, and which provides structural support to the cells, tissue, and organ as a whole. The composition of molecules in the ECM has a strong influence on the structural properties of the tissue which it helps compose. For example, in tissues which must stretch and bend, such as the muscle of the heart, elastin is a necessary ECM component which lends elasticity to the tissue when found in sufficient quantities (Lodish et al., 2008). Similar examples may be found in all tissue types, exemplifying the common theme of structure supplying function found throughout biology. The ECM is approximately nano scale and fibrous, though the fiber orientation depends on the tissue type. For example, dermis has randomly oriented fibers so as to provide structural support when stretched in different directions, while ligaments have a highly directional ECM so as to provide support in the direction of stress.

Cells respond to and adjust their environment in a variety of ways. Important to tissue engineering is the fact that the composition of the ECM can inform the cell as to what tissue type it is in, and hence can influence development and differentiation (Behonick & Werb, 2003). This fact is highly important for tissue engineering because rarely, if ever, are scaffolds seeded with terminally differentiated cells. Usually, a stem cell with the capacity to differentiate into a desired cell type is used, and thus control of the pathway of differentiation is important to the tissue engineer. Cells can be differentiated by the addition of soluble bioactive factors such as growth factors, but the effect of the physical environment on cellular differentiation also has a strong influence, and thus choosing scaffold properties which mimic the native ECM is desirable in order to trigger cellular differentiation toward a target cell type. During development of the chondrocyte, for example, fibronectin is deposited and then binds to cell surface receptors, triggering condensation of mesenchymal precursor cells into cell masses (Behonick & Werb, 2003). This deposited ECM will eventually come to form the boundaries between cartilage and other tissue types, just as ECM deposited in other tissues and organs establish their own boundaries. Some ECM proteins can further influence cellular development by binding growth factors and morphogens that are secreted by surrounding cells or that diffuse from a distance, providing chemical as well as physical influences on surrounding cells (Behonick & Werb, 2003).

2. The process of electrospinning

While many other scaffold fabrication methods are used in tissue engineering applications, few yet provide scaffolds with the critical similarities to the ECM that electrospinning of nanofibers can provide. Many fabrication processes allow for the inclusion of naturally derived materials such as collagen into the scaffold, but some, such as melt molding, require high temperatures which may denature proteins. Others, such as solvent casting and particulate leaching, provide scaffolds with very high porosity but potentially low interconnectivity, and may not allow for easy shaping (Yang et al., 2001). Production of nanofibers by electrospinning however provides excellent interconnectivity and control of porosity through adjustment of fiber size (Pham et al., 2006). This interconnectivity can allow for integration of cells into the scaffold if the pore size is large enough (Ju et al., 2010) and also allows for dissolution of soluble factors and nutrients through the scaffold. These properties closely mimic natural properties of the ECM and the parameters of the electrospinning process can be controlled so as to adjust to features of specific tissue types. For example, arteries are composed of a layer called the intima; an endothelial cell (EC) layer lining the lumen which sits on a basal lamina. Surrounding this layer is the tunica media which is a layer of connective tissue and smooth muscle cells (SMC). Ju et al. showed that it was possible to create a bilayered vascular scaffold which supported EC growth on an electrospun intimal layer and SMC growth on an electrospun media layer. These properties make electrospinning an incredibly versatile scaffold fabrication process, which is why it has been used for tissue engineering applications ranging from bone and blood vessels to tendons and nerve conduits.

Electrospinning in tissue engineering has come to prominence in the previous two decades. Used in many disciplines, electrospinning is a relatively simple and inexpensive scaffold fabrication process. It relies on a high voltage direct or alternating current source to charge a polymer solution or melt contained in a syringe. In tissue engineering, a solution is typically

used in order to avoid high temperatures and additional equipment. Opposite the syringe is a grounded fiber collector to which the charged polymer is attracted as the polymer solution is pumped out at a controlled rate. At a critical voltage, the buildup of like charges in the solution near the tip of the syringe leads to a self-repulsive force greater than the surface tension of the liquid which causes the solution to erupt into the four distinct regions observed by Reneker and Chun (1996). These four regions form a polymer current, which terminates on the grounded collector, releasing its charge and forming solid polymer fibers. The first region is the base where the polymer jet emerges from solution and tapers away to form the Taylor cone, which points in the direction of the grounded collector (Greiner & Wendorff, 2007). Following this region is the jet which traverses much of the distance between the syringe and the collector and in which the polymer strand stretches and the solvent largely evaporates, causing the fiber diameter to decrease and solidify. The third region is often called the splay and was originally thought to be composed of many strands which bifurcate off from the main strand of the jet. However, the splay was actually shown to consist of a single rapidly whipping strand of polymer which forms an opening spiral pattern as it approaches the collector. The fourth region is the grounded collector where the polymer releases its charge and any remaining solvent evaporates, leaving only thin polymer fibers.

2.1 Electrospinning parameters

A primary benefit offered by electrospinning is the wide range of parameters which can be controlled (Table 1). These parameters can be further subdivided into three categories: processing parameters, solution parameters and ambient parameters. Processing parameters include applied voltage, distance from the syringe to the collector, flow rate, needle gauge and shape, collector shape, movement, and construction. It has been shown that increasing voltage tends to cause a recession of the Taylor cone and an increase in beading of the fibers in a poly(ethylene oxide) (PEO) and water solution (Deitzel et al. 2001). Others have seen bead formation only at low voltages, with a decrease in bead formation and increase in fiber diameter with increasing voltage in some polymer solutions and still other effects in different polymer solutions, leading to the conclusion that optimal conditions vary between different polymer/solvent systems (Sill & von Recum, 2008). The effect of flow rate on fiber morphology has not been studied intensively, but Megelski et al. (2002) have found that increasing flow rate tends to increase pore size, but may lead to beading effects due to the inability of the solvent to evaporate before it reaches the collector. The distance from the tip to the collector also influences fiber morphology. Decreasing the jet length has been found to increase fiber diameter as well as increase bead formation (Doshi & Reneker, 1995; Megelski et al., 2002). An extremely important aspect of electrospinning in tissue engineering is the geometry of the scaffold. This can be manipulated by the shape of the collector as well as rotational and translational movement. Flat electrospun mats can be formed by spinning onto a flat collector, while tubular shaped constructs can be formed by spinning onto a cylindrically shaped collector undergoing rotational, and optionally translational, movement. Fibers can also be spun such that they are aligned which can be useful depending on the application (Theron et al., 2001; Xu et al., 2004).

Solution parameters include polymer concentration, polymer molecular weight, solution conductivity, solution volatility, and solution surface tension. Polymer concentration has a great influence on the electrospinning process, affecting both fiber morphology and other parameters such as surface tension (Sill & von Recum, 2008). A sufficient amount of polymer

must be dissolved in the solvent in order for fiber formation to occur as the polymer strands must have sufficient chain overlap in order to physically link and form fibers. In general, increasing polymer concentration typically increases fiber diameter (Gupta et al., 2005). Surface tension is affected by polymer concentration, solvent, and addition of other chemical entities. It has been shown to have varying effects on fiber diameter (Pham et al., 2006) and may affect the critical voltage at which the spinning process begins.

Ambient parameters have not been studied extensively, but it has been seen that temperature may influence fiber diameter by changing the viscosity of the polymer jet (Pham et al., 2006). Humidity may also affect fiber morphology, especially in polymers which are dissolved in aqueous solution, as excessive humidity may prevent solvent evaporation. While, these parameters may not be easily manipulated in many common lab settings, they must be controlled as much as possible in order to produce consistent results.

Fabrication parameters	Effect on fiber morphology
Solution concentration/viscosity	↓ – bead formation, ↑ – larger fibers
Conductivity/solution charge density	↑ – uniform bead-free fibers, smaller fibers
Surface tension	No conclusive link
Polymer molecular weight/viscosity	↑ – reduced the number of beads
Dipole moment and dielectric constant	Successful spinning
Flow rate	↓ – smaller fibers, ↑ – larger fibers
Field strength/voltage	↓ – larger fibers, ↑ – smaller fibers
Distance between needle tip and collector	↓ – smaller fibers, ↑ – larger fibers
Needle tip design and diameter	↑ needle diameter – larger fibers
Collector geometry	Controlled fiber orientation, construct shape
Ambient parameters (temperature and humidity)	↑ temperature – ↓ viscosity, ↑ humidity – appearance of circular pores on the fibers

Table 1. Fabrication parameters for controlling the configuration of electrospun fibers.

3. Uses of electrospun nanofibers in tissue engineering

Electrospun fibers have been used in practically all areas of tissue engineering. Tissue engineering itself is a diverse field which aims to restore or replace the functions of tissues and organs damaged by injury and disease. In this section we will focus our topic by selecting tissue types which have been studied sufficiently using electrospun nanofibers.

3.1 Osseous tissue

The engineering of bone tissue presents unique problems due to the mechanical properties of hard tissue and the incorporation of minerals and inorganic molecules. With the exception of the ossicles, bones provide either structural or protective support, functioning in motion, maintenance of shape, formation of hinges and shields to protect vital organs. Bones also function as a reservoir for marrow, containing and protecting the hematopoietic stem cells (HSC) which produce all of our red and white blood cells. The focus of this section will be on the engineering of osseous tissue, which is the hard, calcified material stereotypically thought of as "bone". Osseous tissue is divided into two subtypes, compact and spongy, which are synonymous with cortical and cancellous bone. Compact bone is

more dense than spongy, and tends to be stronger, more dense, and less vascular. Spongy bone contains marrow and is found inside compact osseous tissue and at the ends of long bones. Electrospun nanofiber meshes do not, on their own, possess mechanical properties similar to that of osseous tissue, but they have nonetheless been studied extensively in bone tissue engineering for their ability to support cell growth and differentiation into osteogenic phenotypes. A key cellular phenotype is the osteoblast, which is the cell type that lays down the ECM of osseous tissue and is thus the target cell type for recruitment and differentiation in bone reconstruction. Also found in osseous tissue are osteocytes and osteoclasts, which function in tissue maintenance and resorption respectively (Plotkin et al., 1999). All aforementioned cell types are found in mature osseous tissue, but tissue engineers focus on using osteoblasts because of their production of new ECM is essential for restructuring an electrospun mesh into a tissue capable of functioning as bone.

Several broad categories of nanofibers have been used in osseous tissue engineering: synthetic polymers such as poly(ε-caprolactone) (PCL), poly(L-lactic acid) (PLLA) and their copolymers, natural polymers such as collagen, composites of natural and synthetic polymers, inorganic materials such as bioactive glass and hydroxyapatite (HA), and composites of inorganic materials with synthetic and natural polymers (Jang et al., 2009). Results have shown that fibers incorporating materials naturally found in the ECM tend to be more effective at stimulating desired growth of cells than purely synthetic polymers, which is likely due the increased cell binding moieties found on natural materials. Nevertheless, studies using purely synthetic polymers have yielded useful information regarding the effect of surface topology on osteogenic differentiation. Badami et al. (2006) have shown that osteoprogenitor cell populations cultured on PLA, poly(ethylene glycol) (PEG) and their copolymers, with mean fiber diameters from 140 nm to 2.1 μm, had cell densities equal to or greater than cell populations cultured on glass plates in the presence of osteogenic culture medium. However, cell densities were actually observed to be lower when osteogenic medium was removed, illustrating the importance of proper chemical signaling, via ECM and soluble factors, in the growth and differentiation of all cells. An example of this is a study conducted using human mesenchymal stem cells (MSC), which are capable of differentiation into osseous tissue lineages, grown on electrospun silk fibroin and PEO, mixed with bone morphogenic protein-2 (BMP-2), HA nanoparticles or both. A synergistic effect was seen in calcium production per unit DNA (μg Ca/ng DNA) when both BMP-2 and HA nanoparticles where present in the scaffolds (Li et al., 2006). From this it can be concluded that providing cells with both chemical cues in the form of growth factors and a like-native substrate in the form of a nanofiber matrix incorporating HA is superior to either factor alone. This reaffirms the idea that in order to trigger stem cell differentiation toward a desired lineage, the native environment of that lineage must be reconstructed as closely as possible. Many attempts have been made at this reconstruction, including some interesting combinations of post-electrospinning mineralization. One successful attempt utilized sequential addition of acetic acid, $CaCl_2$, and H_3PO_4 onto electrospun collagen and electrospun poly(lactide-*co*-glycolide) (PLGA) nanofibers to create mineralized scaffolds for use in osseous tissue engineering (Liao et al., 2008). Their process resulted in noticeably mineralized nanofibers, effectively changing the surface topography of the matrix. Collagen nanofibers tended to have a more uniform distribution of mineralization than PLGA, and the mineralization tended to form where fibers crossed. Examination of the mineral phase formed during this process showed that the scaffolds were largely coated with HA and brushite, making this process an attractive method for post-electrospinning fiber modification in bone tissue engineering.

Another study illustrating the idea of post-electrospinning mineralization utilized a combination of electrospinning poly(lactide-*co*-ε-caprolactone) (PLCL)/gelatin and electrospraying HA nanoparticles (Gupta et al., 2009). Polymer solutions were electrospun onto glass coverslips taped to a rotating mandrel while a HA solution was electrosprayed concurrently onto the fibers. In comparison to fibers created using PLCL/gelatin/HA blends, the morphology of concurrently electrospun and electrosprayed fibers showed significantly more mineralization and topological heterogeneity. Additionally, when human fetal osteoblast cells were cultured on the PLCL/gelatin/HA electrosprayed scaffolds they showed higher alkaline phosphatase activity, an osteoblast marker, than PLCL/gelatin/HA blended scaffolds. These results not only reiterate the need for creating ECM mimicking scaffolds, but also illustrate that the development of new scaffold fabrication methods are still highly important in the field of tissue engineering.

Of particular interest in the area of adapting electrospinning for new techniques is the growing field of gene therapy. The ability to force expression of target genes in cells has recently garnered much attention, and a prime example in bone tissue engineering is the incorporation of a BMP-2 plasmid into PLGA/HA electrospun scaffolds (Nie & Wang, 2007). This example is especially interesting because it uses three different methods of DNA incorporation, giving rise to three different release profiles. Transfection by plasmid vectors is advantageous because, unlike viral vectors, there is little risk of incorporation of the DNA into the genome. Plasmid DNA was incorporated into the scaffolds by binding naked DNA onto the PLGA/HA nanofiber surface, binding DNA/chitosan nanoparticles to fiber surface, and incorporating DNA/chitosan nanoparticles into the fibers themselves. The addition of nanoparticles into the polymer solution significantly changed fiber morphology from nano scale to micro scale. Of significant interest, however, are the release profiles of DNA from the fibrous scaffolds. A fast, burst release occurred when naked DNA was added to the fiber surface, with 50% release after only 1-2 days. A slower controlled release observed when DNA/chitosan nanoparticles were added to pre-electrospun fiber surfaces, with 50% release after about 3-9 days, depending on the HA content. Incorporating DNA/chitosan nanoparticles into the fibers themselves further delayed release, with 50% release occurring after approximately 18-31 days. The differences in release profiles can be explained by examining the physical condition of the DNA. Naked DNA is capable of simple diffusion out of the scaffolds because it is small compared to the fibers and pore size, and can change shape. DNA encapsulated in chitosan nanoparticles however cannot diffuse freely due to their ~100 nm diameter size, and so these particles take longer to diffuse out. DNA/chitosan nanoparticles electrospun inside PLGA/HA fibers require fiber degradation to occur for release and thus their release is the slowest. Human MSC were found to grow on the scaffolds, though not as well as on tissue culture plates. *In vitro* expression of BMP-2 was used to measure transfection efficiency, and it was found that DNA/chitosan nanoparticles bound to the outside of nanofibers had the highest transfection rates, but also the highest toxicity. However, significant transfection was seen in the groups where nanoparticles were incorporated into the fibers, and this group also had the highest viability. These results are significant as they show the ability of nanofibers to act as more than passive substitutes for the ECM; they can also become active manipulators of cellular expression and differentiation.

3.2 Cartilage

Cartilage damage can result from chronic wear, injury and disease and plays a strong role in osteoarthritis. Of primary concern is articular cartilage that is found in the pivoting areas of

joints. Articular cartilage is largely avascular and is comprised of mostly collagen, elastin, and proteoglycans, with a relatively low cell density. As a result, lesions in articular cartilage do not heal well, if at all, frequently lead to joint pain, and may ultimately lead to irreversible damage in larger areas of cartilage and the underlying bone (Hunziker, 2002). Many tissue engineering approaches to cartilage regeneration have been examined, including the use of hydrogels, collagen sponges, gelatin microspheres and other scaffolding systems (Stoop, 2008). Different types of electrospun nanofibers have been examined as well, ranging from pure synthetics to composites and natural polymers.

Attempts to use a purely synthetic approach to engineer cartilage have employed PLGA nanofibers and primary porcine chondrocytes to test for cell viability, proliferation, and maintenance of a chondrocyte-like phenotype (Shin et al., 2006). The maintenance of chondrocyte phenotype was given particular attention, as chondrocytes tend to de-differentiate during culture. Cells were grown on both electrospun PLGA nanofiber mats and PLGA membranes were used as a control. By measuring the DNA and glycosaminoglycan (GAG, a common ECM component) content it was seen that the nanofiber scaffolds supported a significantly higher cell number and greater ECM synthesis than membranes. Intermittent hydrostatic pressure was used to condition the cellularized scaffolds and was seen to greatly improve both cell proliferation and GAG synthesis over unconditioned electrospun scaffolds. These results show again the great effect that mechanical stimulation and scaffold topography can have on cell phenotype and proliferation.

While chondrocytes are commonly used for cartilage tissue engineering, they remain difficult to harvest due to the low density found in native cartilage. MSC are capable of differentiating into many cell lineages, and have been found to change into chondrogenic lineages when grown in high densities in cell pellets (Li et al., 2005). However, cell pellets do not possess good mechanical properties for implantation, and so the authors sought to use PCL nanofiber scaffolds to support MSC proliferation and differentiation into a chondrocyte lineage. Cells were grown on PCL nanofiber mats or as cell pellets formed by centrifuging, and both methods used either chondrogenic media or media containing TGF-β1 to induce differentiation to chondrocyte lineages. By examining the expressed RNA and rate of GAG synthesis the authors found that the 3D nature of the nanofiber scaffolds caused a significantly higher degree of differentiation toward the desired phenotype, showing that the alternate cell source, i.e. bone marrow derived MSC, can be successfully differentiated into chondrocyte-like cells for potential *in vivo* use. An interesting incorporation of nanofibers into a tissue engineered construct is the use of nanofibers not as a scaffold unto itself, but as a topological modification to a microfiber scaffold. Thorvaldsson et al. (2008) showed that it was possible to coat single PLLA microfibers with PCL nanofibers, giving additional ECM-like physical characteristics to the scaffold to aid the growth of chondrocytes. The results of this study show qualitatively that the addition of a nanofiber topography onto a microfiber scaffold may increase chondrocyte adhesion and provide a structure closer to the native ECM than either nanofibers or microfibers alone can provide.

3.3 Blood vessels

Tissue engineering of blood vessels is primarily focused on development of small diameter vascular grafts. Small diameter arteries, arteries of less than 5 mm in diameter, include the coronary arteries and many peripheral arteries found in the arms and legs. Both coronary artery disease and peripheral vascular disease are caused by atherosclerosis and can lead to

serious complications including ischemic heart failure in the case of coronary artery disease and ischemic ulcers and tissue death leading to amputation in the case of peripheral vascular disease. Currently, autologous veins are the first choice for grafting when arterial bypass is necessary. However, this procedure is not optimal because it necessitates two surgeries, one to harvest the donor vein and one for implantation, and can lead to donor site morbidity. Additionally, an autologous graft may not be available due to prior surgery, disease, or trauma. Synthetic materials such as poly(ethylene terphthalate) (Dacron®) and expanded poly(tetrafluoroethylene) (ePTFE) have proven highly successful for large diameter grafts, but perform poorly in small diameter grafts due to thrombosis and anastomotic hyperplasia (Chan-Park et al., 2009). A non-autologous graft would thus be of great socioeconomic value and has been the subject of much investigation. Electrospinning lends itself well to the construction of vascular scaffolds due to the ease of shaping tubular constructs using rotation and translational motion.

One of the advantages of tissue engineering blood vessels is the relative simplicity of vascular structures. While blood vessels do possess the extremely high level of complexity found in all cells, not to mention the additional level of complexity created by a layered tissue structure, intercellular communication, and specific types of ECM, they are nonetheless significantly less complex structures than organs such as the kidney and brain. Two primary characteristics which tissue engineered vascular grafts must possess are proper mechanical properties to withstand physiological conditions and a confluent endothelialized lumen to resist thrombosis. Efforts to determine potential scaffold materials have been examined using electrospinning of synthetic/natural composites (Lee et al., 2007). Scaffolds were electrospun using 15% elastin, 45% collagen and 40% synthetic polymer, either PLGA, PLLA, PCL or PLCL, dissolved in 1,1,1,3,3,3-hexa-iso-fluoropropanol (HFIP) and crosslinked after electrospinning. It was found that mechanical properties differed between scaffolds containing different synthetic polymers. A time course study of scaffold behavior in culture medium conditions showed that scaffold deformation occurred in all scaffold types, but significant decreases in patency was observed in PLGA/collagen/elastin and PLCL/collagen/elastin scaffolds after 28 days, while PLLA/collagen/elastin and PCL/collagen/elastin maintained their patency. The scaffolds were also shown to support cell adhesion and growth, indicating that they could potentially be fully cellularized. These data show that it is possible to tailor the mechanical properties of vascular scaffolds by selecting the appropriate quantities and types of synthetic and natural polymers. It also highlights the potential decrease in patency of the scaffold when exposed to physiological conditions, which could lead to graft failure. Further studies were conducted to show that a PCL/collagen graft has native-like mechanical properties of compliance, burst pressure, and suture retention strength (Lee et al., 2008) (Figure 1). These properties are necessary for a vessel to have proper flow conductance, withstand physiological pressures, and be surgically implanted without risk of failure. Scaffolds were constructed by electrospinning 1:1 mass ratio of PCL/collagen to form scaffolds with an internal diameter of 4.75 mm. Burst pressure was measured by increasing fluid pressure inside the scaffold until failure occurred and was found to be approximately 4915 mmHg, significantly higher than any blood pressure experienced *in vivo*. The scaffolds had better suture retention strength than the accepted standard, and compliance comparable to native blood vessels. When the scaffolds were subjected to flow conditions they maintained structural integrity well, losing 14% of their ultimate tensile strength after 4 weeks compared to 3% in static conditions. Additional studies were performed using electrospun PCL/collagen nanofibers to test for

growth of EC and SMC growth and proliferation. These results clearly show that it is possible to create electrospun nanofiber scaffolds with mechanical properties required of implantable grafts which are capable of supporting EC and SMC adhesion and proliferation (Lee et al., 2008).

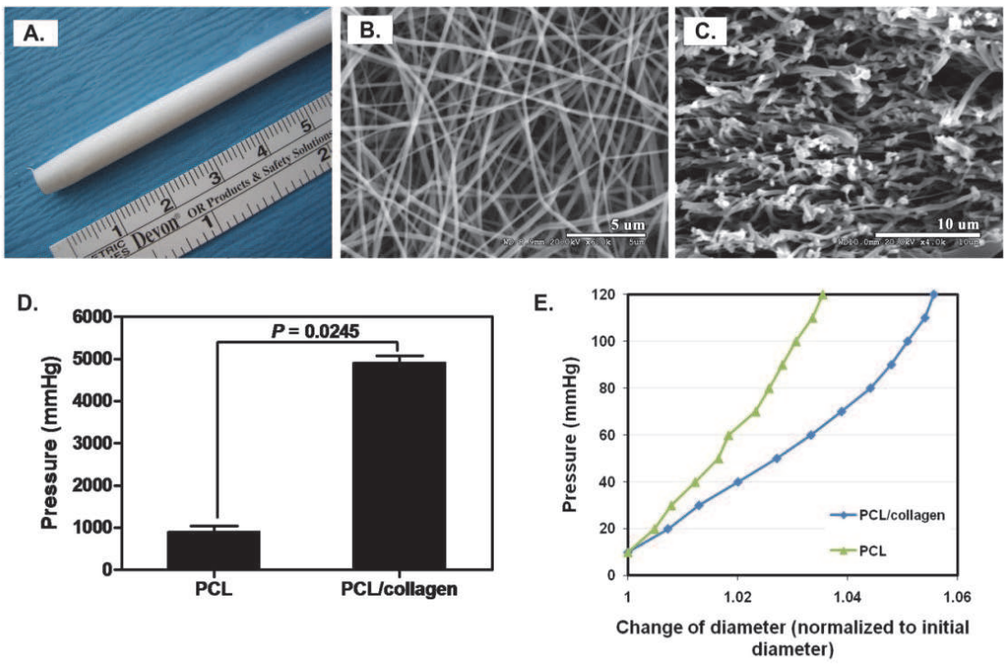


Fig. 1. (A) The gross appearance and SEM images of electrospun PCL/collagen composite scaffolds: (B) surface (×6K) and (C) cross-sectional (×4K) morphologies. (D) Burst pressure strength of electrospun PCL/collagen scaffolds compared to electrospun PCL scaffolds (*P<0.05). (E) Compliance curves for electrospun PCL/collagen scaffolds compared to electrospun PCL scaffolds. Reproduced with permission from all authors, *Biomaterials*, 2008, **29**, 19, 2891. © Elsevier 2008 (Lee et al., 2008).

In a second study, these PCL/collagen scaffolds were tested in an animal model to determine ability of EC and SMC seeded grafts to function *in vivo* (Tillman et al., 2009). Scaffolds were seeded with sheep primary EC and SMC and bioconditioned for nine days to acclimate the cells to *in vivo* flow conditions. A subset of scaffolds was then tested for platelet adhesion by an *ex vivo* system in sheep to flow blood through the scaffolds. Scaffolds without an endothelialized lumen were seen to have significantly more platelet adhesion than endothelialized scaffolds, suggesting that thrombus formation would be significantly less likely to occur when scaffolds are seeded with EC and preconditioned for flow. A second subset of scaffolds was used in a rabbit aorto-iliac bypass surgery and left *in vivo* for 4 weeks. In addition, the grafts showed little to no loss of patency over the course of implantation in rabbits as measured by ultrasonography. Tensile strength of the grafts was reduced after a 1-month explant, though remained comparable to native arteries. This study is an exciting example of the potential of nanofibers in tissue engineering. While much longer term animal and ultimately human studies will be needed, these results show that it is possible to create nano-structured vascular grafts capable of being cellularized and implanted into living animals to replace the function of native vessels (Tillman et al., 2009).

Further studies have examined the potential to create vascular grafts which combine both nano and microfibers to allow for better integration of SMC and development of multiple SMC layers (Ju et al., 2010). An internal layer of PCL/collagen nanofibers was electrospun to give fibers approximately 270 nm in diameter for the purpose of supporting a luminal EC monolayer. Surrounding this layer, PCL/collagen fibers with diameters of approximately 1 μm, 2.4 μm and 4.45 μm in different groups were added in order to increase the pore size and thus the capacity of the outer layer to support SMC ingrowth. Scaffolds were found to support EC on the luminal nanofiber layer and SMC growth on the outer microfiber layer. It was also found that SMC integration was significantly increased when 2.4 μm and 4.45 μm fibers were used, as these fiber sizes provide pore sizes large enough for cells to grow into, a feature not generally observed with pure nanofibers. These data illustrate that combinations of micro and nano scale structures can provide scaffold properties not observed when only one or the other is used alone (Ju et al. 2010).

3.4 Nerve

Tissues of the nervous system are often damaged by traumatic injury. Damaged nerves are slow to regenerate, and damage to the central nervous system or large nerves in the peripheral nervous system often never heal. This is evidenced by the inability of para- and quadriplegic sufferers to regain function of their extremities after spinal cord injury and permanent loss of function or sensation of an extremity due to a peripheral nerve injury. Of the two main divisions of the nervous system, tissue engineers have primarily focused on the regeneration of the peripheral nervous system. As such, successful approaches to nerve regeneration have typically incorporated a tubular scaffold which provides both an artificial ECM and directionality through which the peripheral nerve can grow. This regrowth has not been seen to exceed a few centimeters, but remains a great success of tissue engineering to date.

In order to determine appropriate substrates for nerve tissue regrowth it is necessary to test the growth of neuronal or other cells that have the capacity to differentiate into neurons. One study examined the behavior of C17.2 mouse nerve stem cells on electrospun PCL/gelatin nanofiber scaffolds with both random and aligned fiber orientations (Ghasemi-Mobarakeh et al., 2008). It was found that not only did PCL/gelatin fibers support cell growth better than PCL only fibers, but that when fibers were aligned the stem cells had neurite outgrowth along the direction of the fibers, suggesting that fiber alignment may be beneficial in directing new growth of nerve cells. However, sources of nerve stem cells may not be available in human adults so alternative sources will likely be necessary. MSC have been examined for their potential to differentiate into neural lineages on PLCL/collagen nanofiber scaffolds (Prabhakaran et al., 2009). PLCL/collagen scaffolds alone are not sufficient to induce differentiation into a neural lineage. It was necessary to induce this differentiation over the course of 35-37 days using a cocktail of various growth factors while the cells grew on the scaffolds. Cells grown on the PLCL/collagen scaffolds and treated with the growth factor cocktail were qualitatively found to have neuronal-like phenotypes to a greater extent than control groups, and also to have higher growth rates. This suggests that PLCL/collagen nanofibers have a beneficial effect on culturing a population of neuronal-like cells from MSC, thus making them a potentially useful source of cells for neural tissue engineering.

Nanofibers have also been used to form tubular conduits for regrowth of damaged nerves *in vivo*. One study examined the ability of a tubular construct made of both micro- and

nanofibers to promote growth of rat sciatic nerve after a 10 mm section of nerve was removed (Panseri et al., 2008). PCL microfibers and PLGA-*co*-PCL nanofibers were electrospun separately into flat sheets. The microfibers were then deposited around a copper wire to create the lumen of the conduit, and nanofibers were deposited around the microfibers to create a tight mesh barrier. The experimental group had sections of sciatic nerve removed and the conduit sutured in place between the two dissected ends. The control group had their nerves either transected or had equal sized sections removed without the addition of the conduits. The injuries were left unrepaired in the control group. Re-connection of nerves in the control group did not occur in any subjects, as expected. Approximately 40% of conduits collapsed as a result of displacement and body movement. However, in all rats that did not suffer conduit collapse tissue regrowth and neurite outgrowth was observed inside the conduit. Markers of neuronal cells were observed through the length of the conduits as well as myelinated axons. Autophagy occurred in a set of animals tested, but those which did not experience it experienced increasing sensitivity in the experimental limb over the four month trial, showing a steadying increase in nerve function. The majority of the treated animals were also seen to experience motor function in the treated limb after four months. These results highlight the capacity of nanofibers to act as a simple barrier to assist in significant regeneration of peripheral nervous tissue and function.

3.5 Skin

The development of engineered skin is another goal of tissue engineering. There are numerous skin injuries, especially burns, which create large surface area wounds that require skin coverage. Currently, the primary mode of treatment is autologous skin grafting, but other regenerative medicine technologies are beginning to be employed. This is because autologous treatment frequently requires multiple graft procedures for large wounds and always results in donor site morbidity. Moreover, in extreme cases, donor sites are not always available for grafting. Tissue engineering would like to develop an off-the-shelf engineered skin or wound dressing that would prevent the need to use donor sites for grafting and that would preclude the need for multiple surgeries. An in depth discussion of normal skin anatomy and physiology is outside the scope of this chapter, but for more details the reader is referred to Moore & Dalley's Clinically Oriented Anatomy among other sources (Wong & Chang, 2009; Moore & Dalley, 1999; Ross et al., 2003). Skin is composed of three layers: epidermis, dermis, and hypodermis. The epidermis is avascular and composed of squamous epithelial cells call keratinocytes. There is a basal layer of keratinocytes that are stem cells which divide asymmetrically. After a cell division, one cell will remain in the basal layer while the other will be pushed upward. As the keratinocytes progress from the basal layer to the apical layer of the squamous epithelium, they produce an increasing amount of keratin. Once the cells reach the surface, they are filled with keratin and are eventually sloughed off. The epidermis provides the barrier function of the skin. Below the epidermis is the dermis. The dermis is loose connective tissue that consists of large amounts of ECM, namely type I and III collagen and elastin, although there are other components. The primary cell type of this layer is the fibroblast which maintains the dermis. Other structures in the dermis include blood vessels, lymphatics, glands, cutaneous nerves, and hair follicles. The hypodermis is primarily loose connective tissue with lots of adipose tissue. This layer of skin serves primarily as an energy storage site and as insulation (Moore & Dalley, 1999; Ross et al., 2003; Wong & Chang, 2009).

Electrospinning has been used as an approach for developing scaffolds for engineered skin and wound dressings, although less than other scaffold strategies (Priya et al., 2008). Nevertheless, several investigators have used electrospinning for skin tissue engineering. Of these, most of the reports have been *in vitro* evaluations (Kumbar et al., 2008; Powell & Boyce, 2009; Sun et al., 2005; Yang et al., 2009; Zhou et al., 2008). Sun et al. (2005) used electrospun polystyrene (PS) as a model system for investigating what effect the spatial cues of an electrospun system would be on engineered skin formation. They also evaluated the effect of culturing cells in an air-liquid interface (a common culture technique for skin culture) compared to submerged scaffolds. The electrospun PS scaffolds had fiber diameters of approximately 10 μm. They found that when keratinocytes, fibroblasts, and EC were co-cultured, they could self organize without any extra cues other than the three-dimensional electrospun scaffold and the air-liquid interface.

One study used electrospun PLGA as wound dressings for skin wounds (Kumbar et al., 2008). The study investigated the effect of fiber size on fibroblasts. The authors investigated scaffolds with fiber diameters ranging from 150 nm to 6000 nm to evaluate scaffold-seeded fibroblast behavior in terms of proliferation, morphology, and gene expression. They found that fibroblasts proliferated best and had spread morphology in the fiber range of 350 nm to 1100 nm. Moreover, scaffolds in this range had a higher expression of type III collagen. All the scaffolds showed fibroblast expression of type I collagen and elastin, but scaffolds in the 350-1100 nm range did not show a great difference from other fiber sizes tested, i.e. type I collagen and elastin did not show a fiber-dependent trend in expression. Finally, all scaffolds exhibited tensile moduli within the range of normal human skin (Kumbar et al., 2008).

Another study investigated the electrospinning of carboxyethyl chitosan/poly(vinyl alcohol) (PVA) scaffolds for future use as skin wound dressings (Zhou et al., 2008). Chitosan has good biological properties such as biodegradability, biocompatibility, is antibacterial, and promotes wound healing. Thus, the authors wanted to find a way to electrospun N-carboxyethyl chitosan in an aqueous solution (typically materials dissolved in aqueous medium are more difficult to electrospin due to the low volatility of water). Upon initial attempts, they were not able to electrospin chitosan, however upon blending with various ratios of PVA, they were able to form fibers. These fibers had average diameters ranging from 131 nm to 456 nm. After fabrication, the scaffolds were crosslinked with glutaraldehyde vapor so that they would not dissolve upon wetting. The results showed that the scaffolds containing chitosan could be formed by electrospinning in an aqueous solution, that the scaffolds could be crosslinked to prevent re-dissolving, and that the scaffolds were cytocompatible when tested with mouse fibroblasts. Thus, the study concluded that scaffold might be useful for skin regeneration (Powell & Boyce, 2009).

Powell & Boyce (2009) used electrospun collagen and PCL blends as scaffolds for forming engineered skin. They added PCL to electrospun collagen in order to improve its handling properties, i.e. to minimize size reduction upon wetting and increase mechanical strength. Upon comparing different mass percentages of PCL blended with collagen to PCL alone and collagen alone they found that mechanical properties were not significantly improved over collagen alone until there was at least 10% PCL in the scaffold. However, the scaffold containing 10% PCL had the lowest elongation. These scaffolds were co-cultured with fibroblasts and keratinocytes using an air-liquid interface. Fibroblasts were seeded 24 hr before keratinocytes. When evaluating the mechanical properties of seeded scaffolds at

later time points, few differences between groups were found. The main finding was that the 30% PCL scaffold had the weakest mechanical properties. As the PCL content increased, the scaffolds were less able to accommodate the seeded cells showing a lower density of fibroblasts and un-stratified dermal and epidermal layers without a basal cell layer. In contrast, scaffolds that contained 10% PCL or less had well stratified dermal and epidermal layers with the presence of a basal cell layer. The investigators postulated that at the higher PCL concentrations, the fibroblasts were unable to form a confluent layer to accommodate the keratinocytes which thus resulted in an unorganized system. This unorganized system accounted for the lower mechanical properties observed. Interestingly, the 100% PCL scaffold had similar mechanical properties to the lower PCL content scaffolds even though it did not form well-organized engineered skin. The authors attributed this to the PCL itself resisting most of the load. Surface electrical capacitance, which is a measure of the hydration of the epidermal layer, was also measured. As the keratinocyte layer started to keratinize, it started to dry and act as a barrier which is a natural function of skin. The authors found that scaffolds with a lower percent of PCL had better capacitance values with the pure collagen scaffold and 1% PCL scaffolds approaching capacitance values similar to normal skin. Finally, the authors showed that adding PCL to electrospun collagen reduces the amount of scaffold contraction after wetting, although except for the 30% PCL and 100% PCL scaffolds, this reduction was relatively modest (Powell & Boyce, 2009).

Yang et al. (2009) fabricated electrospun scaffolds consisting of PLGA and type I collagen with high porosities and large pore sizes. The fiber diameter of the scaffolds was approximately 1 μm regardless of collagen concentration (collagen concentrations used were 0%, 5%, 10%, and 30%). To improve pore size by controlling fiber deposition density, a mandrel that consisted of metal rods parallel to one another that inserted into a ring at either side was used. Thus, the mandrel had the basic shape of a cylindrical mandrel, but instead of having a full surface it only had spokes of metal. This allowed fibers to deposit between the metal rods at a lower density than they would on a solid surface thus increasing the porosity and pore size of the scaffolds. This mandrel was dubbed the metal frame cylinder. By increasing the percentage of collagen in the scaffolds, the pore size and porosity decreased. These scaffolds had pore sizes in the range of 90-130 μm. The scaffolds with 30% collagen had the highest human dermal fibroblast cell attachment and viability. The seeded fibroblasts also produced more type I collagen on scaffolds containing higher concentrations of collagen. Finally, the addition of collagen decreased the mechanical properties of the electrospun scaffolds. Nevertheless, the authors concluded that adding collagen to the electrospun scaffolds improved cell attachment, proliferation, type I collagen production, and that the high porosity in the scaffolds allowed for fibroblasts to migrate into the scaffold making them good candidates for tissue engineering in general (Yang et al., 2009).

Even though there are several studies investigating electrospun scaffolds for skin tissue engineering, to our knowledge there have yet to be any publications that investigate the use of these scaffolds *in vivo*. In contrast, numerous tissue engineering strategies that employ non-electrospun scaffolds have been studied *in vivo* (Priya et al., 2008). Regardless, electrospinning has become a simple and economical method to generate scaffolds that mimic the ECM of tissues. Thus, electrospun scaffolds have even found use in skin tissue engineering. While the work presented here is promising, in order for it to

be clinically translatable, animal studies will be necessary. The type of study will depend on the intended application of the engineered skin, but most likely the initial studies would investigate the ability of electrospun scaffolds to facilitate skin regeneration in a wound excision model. If these attempts are successful, then investigations should focus on using engineered skin constructs to treat burn wounds which are much more challenging than excisional wounds due to the large degree of necrosis and inflammation in a burn wound. As with any technology, animal studies should start in smaller animals such as rodents and progress to more clinically relevant models such as pigs. Perhaps future studies will investigate the use of electrospun scaffolds for engineered skin constructs *in vivo*.

3.6 Ligaments and tendons

Tendon and ligament injuries are common. Often, clinical repair of damaged tendons or ligaments require autografting from a donor site which, as with any autograft, results in donor site morbidity. Thus, there is much interest in developing engineered tendon and ligament for the repair of these injuries such that donor site morbidity can be avoided. The structure of tendons and ligaments, while fairly complex, is primarily composed of sparse specialized fibroblastic cells in an abundant type I collagen matrix. Tendon and ligament tissue engineering have been attempted for some time. It was initially thought that these tissues would be simple to engineer due to their low cell content and vascularity (which is also why tendons and ligaments frequently do not heal well (Strauss et al., 2007, as cited in Sahoo et al., 2010b), however, this has not proven to be true. While there have been a plethora of studies that try to engineer both tendon and ligament, few have used electrospinning as the fabrication method for their scaffold. As will be shown, this is primarily due to the lack of strength of most electrospun scaffolds.

Lee et al. (2005) used electrospun polyurethane (PU) to study the effect of fiber alignment and cyclic loading on the ECM generation of human ligament fibroblasts. Electrospinning was used in order to mimic the "fine fibrous structure of connective tissues." Electrospun mats were created where the PU fibers were either aligned or randomly oriented. The aligned fibers had an average diameter of 657 nm. The scaffolds were then seeded with human ligament fibroblasts. In addition to seeding, some scaffolds were exposed to cyclic strain. For aligned fibers, strain was applied in both the direction parallel to alignment and perpendicular to alignment. Not surprisingly, the aligned scaffolds had higher stiffness and strength. On the aligned structures, the seeded cells were oriented in the direction of alignment and produced more collagen than on the randomly oriented scaffolds. Cyclic stretching increased collagen content in both the scaffolds that were stretched parallel to the fiber orientation and those stretched perpendicular to the fiber orientation, although stretching in the parallel direction had the highest collagen. Stretching of randomly oriented scaffolds did not increase collagen content over either the aligned or randomly oriented statically seeded control groups. Thus, the authors concluded that aligned nanofiber scaffolds are a biomimetic scaffold that can recapitulate an environment similar to the native ligament (Lee et al., 2005). As has been illustrated previously in Section 3, aligned fibers, which are easy to form with electrospinning, have proven to be beneficial topographical cues for various tissue types.

Sahoo et al. have published a series of studies investigating the development of a composite scaffold for ligament tissue engineering consisting of a knitted component

covered with electrospun fibers (Sahoo et al., 2006, 2007, 2010a, 2010b). Sahoo et al. (2006) combined electrospinning with knitted scaffolds in order to take advantage of a knitted scaffold's strength and the high surface-to-volume ratio and ECM scale of electrospun fibers. They electrospun PLGA nanofibers onto knitted PLGA scaffolds. The electrospun fibers had diameters ranging from 300-900 nm. Bone marrow-derived MSC were chosen as their cell source because ligament fibroblasts are scarce and have low proliferation rates whereas MSC are easy to obtain, have good proliferative properties, can differentiate into various mesenchymal lineages, and produce ECM with type I collagen. When these scaffolds were seeded with a cell suspension of MSC and compared to knitted scaffolds without electrospun fibers that had cells seeded in a fibrin gel matrix, the scaffolds with electrospun fibers had better proliferation and higher expression of type I collagen, decorin, and biglycan (Sahoo et al., 2006). Sahoo et al. (2007) compared knit PLGA and PLLA scaffolds coated with PCL, electrospun PLGA fibers, or collagen to woven fibers. They found that while woven fibers and knitted PLGA coated with PCL had better strength than the other groups, they had poor cell attachment and proliferation. Knitted PLGA with electrospun PLGA fibers had the best cell proliferation. Knitted PLLA scaffolds did not fair as well in terms of cell proliferation and attachment. Thus, the best scaffold in terms of biological properties was a knitted PLGA scaffold with electrospun PLGA fibers. However, these scaffolds degraded rapidly *in vitro* and lost their properties within 3-4 weeks. Thus, the authors proposed that a different material suck as silk would be better to use as the knitted scaffold material (Sahoo et al., 2007). Sahoo et al. (2010a) published one study describing the incorporation of basic fibroblast growth factor (bFGF) into electrospun PLGA fibers which was released over the course of 1 week. bFGF was used because it is known to stimulate MSC to proliferate, self-renew, and differentiate into fibroblastic cells (Sahoo et al., 2010a). Bovine serum albumin, used as a carrier protein, and bFGF were blended with PLGA and dissolved in HFIP. Fibers were electrospun from this solution. The authors showed that the fibers contained bFGF and that bFGF was indeed released from the fibers over the course of a week. Released bFGF was bioactive, promoted cell proliferation and differentiation to a fibroblastic state, increased expression of tendon/ligament specific proteins, and increased the deposition of collagen and tenascin-C (Sahoo et al., 2010a). As a continuation of this work, Sahoo et al. incorporated the bFGF-releasing PLGA nanofibers with a knitted silk scaffold (Sahoo et al., 2010b). The knitted silk scaffold provided better mechanical strength with *in vitro* degradation than previous scaffolds fabricated by this group. Moreover, after 3 weeks in culture with MSC, the bFGF-releasing group had higher strength than unseeded or bFGF-negative scaffolds. The bFGF-releasing group also had a higher stiffness, although it was not statistically significant. Finally, the authors showed that these novel scaffolds promoted and facilitated MSC attachment, proliferation and tenogenic differentiation. Expression of ECM proteins type I collagen, type III collagen, biglycan and fibronectin was increased on the bFGF-releasing group compared to other groups after 14 days. Interestingly, at 7 days, the expression of the collagen proteins was lower on the bFGF-releasing scaffolds than on the bFGF-negative scaffolds, but was higher at 14 days. The authors hypothesized that this was due to the fact that the bFGF initially promoted the proliferation of undifferentiated MSC, and then later their tenogenic differentiation (Sahoo et al., 2010b). Thus, Sahoo et al. in their series of studies have developed interesting scaffolds that incorporate electrospun fibers to serve as a biomimetic topographical cue and as a depot for growth factor release (Sahoo et al. 2006,

2007, 2010a, 2010b). This set of studies demonstrates that electrospun fibers can be used as an adjunct to other scaffolds to improve ECM mimicry and as a depot for drug or growth factor release.

Vaquette et al. (2010) used a similar approach to Sahoo et al. In this study, aligned PLCL fibers were electrospun (although in this case they were microfibers and not nanofibers) onto knit silk scaffolds. They showed that these scaffolds had stress-strain behavior similar to native ligament, that rat MSC, when seeded on the scaffolds, proliferated on the scaffolds and aligned in the direction of the fibers. They also found that MSC seeded on these scaffolds produced type I and III collagens in higher amounts than MSC cultured on tissue culture plates. The authors concluded that they created scaffolds with similar properties to ligaments that would be suitable for use in a bioreactor and neo-ligament formation in the future (Vaquette et al., 2010).

Another study used electrospun nanofibers as a scaffold for rotator cuff repair (Moffat et al., 2009). In this study, Moffat et al. compared the culture of human rotator cuff tendon fibroblast culture on aligned versus unaligned electrospun PLGA scaffolds. The authors found that aligned scaffolds were stronger and stiffer than unaligned scaffolds. Additionally, fibroblasts cultured on the aligned scaffolds attached along the axis of alignment whereas on unaligned scaffolds they were randomly oriented. Fibroblasts on aligned scaffolds had higher expression of α2 integrin suggesting that aligned matrices better mimic native collagen than unaligned matrices (this is because α2 integrin complexes with β1 integrin in order to bind to collagen). Moreover, type I and type III collagens were produced by fibroblasts on both aligned and unaligned scaffolds, but the collagens deposited on the aligned scaffold were done so in the direction of alignment. Finally, the aligned scaffolds maintained mechanical properties better than unaligned scaffolds when they were acellular or seeded. More importantly, despite the decrease in mechanical properties due to hydrolytic degradation of PLGA, the scaffolds maintained physiologically relevant mechanical properties over the 14 day-culture period compared to those known in the literature for rotator cuff tendons. Thus, the authors suggest that aligned nanofiber scaffolds have potential for tendon regeneration, especially rotator cuff tendons (Moffat et al., 2009). This is another example of where electrospun scaffold mimic the ECM of native tissue.

In summary for tendon and ligament tissue engineering, electrospinning has primarily been used as an adjunct to other fabrication techniques. This is because electrospun scaffolds are typically mats and relatively thin and so do not exhibit the strength needed to serve as scaffolds for these tissue types. It certainly may be possible to use electrospun mats to start the development of tissue engineered tendons or ligaments, for instance by utilizing cyclic stretching in a bioreactor, but in general, they seem unlikely to serve as a material-only replacement due to their lack of strength. For this reason, nearly all of the studies described above combined electrospinning with another fabrication technique (in these cases knitted scaffolds) to take advantage of the nanofiber architecture of spun fibers and the strength and porosity of knitted scaffolds (Ouyang et al., 2003 as cited in Sahoo et al. 2006; Sahoo et al., 2006). The exception is the study by Moffat et al. which claimed that physiologically relevant mechanical properties for rotator cuff tendons were maintained using electrospun PLGA scaffolds alone (Moffat et al., 2009). Perhaps using aligned fibers will improve the ability of electrospun scaffolds to be used alone instead of in a composite. Moreover, rotator cuff tendons are broad and flat which makes an electrospun membrane alone more suitable for this application than for other tendon applications where tendons are more rounded.

3.7 Ligament/tendon-bone interfaces

More recently, researchers have started to investigate ways to form composite tissues which will be important in linking tissues that interact with one another. An example of this is the ligament/tendon to bone interface. These interfaces are particularly relevant when considering the clinical repair of torn or ruptured tendons or ligaments such as the rotator cuff and anterior cruciate ligament (ACL). Both of these injuries are common in the United States with 75,000 rotator cuff repairs (Vitale et al. as cited in Moffat et al., 2009) and 100,000 ACL reconstruction surgeries performed annually (American Academy of Orthopaedic Surgeons, as cited in Spalazzi et al., 2008).

Two groups have used electrospun scaffolds in attempt to engineer this tissue interface (Spalazzi et al., 2008; Li et al., 2009). Spalazzi et al. (2008) used electrospun PLGA to create a mechanoactive scaffold by taking advantage of the contraction that occurs in PLGA upon wetting. They hypothesized that applying a compressive load to a tendon would promote its metaplasia into a fibrocartilage-like tissue. This would be useful because tendon autografts are frequently used to repair anterior cruciate ligaments, however, when tendons are used alone instead of bone-patellar tendon-bone (BPTB) grafts, they do not integrate with the bone. This is because unlike BPTB grafts, tendon autografts do not contain an attached segment of bone. Thus, to aid integration with bone, the authors wanted to find a way to cause tendons to remodel towards a fibrocartilage phenotype. To accomplish this, aligned nanofibers were fabricated that were then either wrapped around patellar tendon alone, or first around a cylindrical scaffold composed of sintered PLGA microspheres that was then wrapped around the tendon. After the scaffolds were wetted, the electrospun fibers naturally contracted which resulted in compressive loading of the tendon, or in case of the composite cylinder-electrospun scaffold, the compression of the cylindrical scaffold and subsequent compression of the patellar tendon. DNA content, histology, GAG content, and expression of fibrocartilage specific markers type II collagen, aggrecan, and TGF-β3 were evaluated. In the case of the electrospun scaffold wrapped around the tendon alone, remodeling occurred by 5 days as evidenced by dense matrix with high cellularity, but the tendon had recovered its normal crimp pattern by 14 days. When the cylindrical-electrospun composite scaffold (or mesh+collar scaffold) was used, the increase in density and cellularity remained at 14 days. In the mesh+collar scaffold compressed tendons collagen fiber size decreased and remained parallel to the long axis of the tendon whereas non-loaded tendon had a loss of collagen organization by 14 days. GAG content was higher in the mesh+scaffold loaded tendons than uncompressed tendon by day 1 although the unloaded tendon had higher DNA content. Finally, after 24 hours, the mesh+scaffold compressed tendons had increased type II collagen, aggrecan, and TGF-β3 expression compared to day 0 and compared to unloaded controls. Thus, the authors proposed that these scaffolds could be used clinically by sliding tendon autografts through the mesh+scaffold "collars" before using them to repair anterior cruciate ligaments. After placement, the mesh+scaffold would contract, transferring a compressive load to the tendon causing tendon metaplasia to fibrocartilage. Moreover, because the scaffolds are made of PLGA, they would degrade leaving behind re-organized tissue. Finally, the authors also proposed that the scaffolds could be further modified to provide growth factors to aid in the metaplasia. All of this is hypothesized to ultimately result in tendon integration with bone and thus improve outcomes of ACL repair with tendon autografts that lack a bone component (Spalazzi et al., 2008).

Li et al. (2009) used electrospun scaffolds in which a gradient of calcium phosphate was formed to mimic tendon-to-bone interfaces. Electrospun PLGA or PCL scaffolds were plasma treated or coated with gelatin respectively and a gradient of calcium phosphate mineral was applied along the length of the scaffold using ten times simulated body fluid. A mineral gradient was formed in these scaffolds as evidenced with SEM and decreasing mineral content was observed when moving from one end of the scaffold to the other. Moreover, the gradient had functional consequences in that it created a mechanical property gradient across the scaffold where the mineralized side had a higher modulus than the non-mineralized side and there was a gradual decrease in modulus from the mineralized end to the non-mineralized end. Finally, MC3T3 cells, a preosteoblastic cell line, were seeded on the scaffolds and showed preferential attachment and spreading on the more mineralized regions of the scaffolds. The cell density of these cells decreased with decreasing mineral content. Thus the authors proposed that these scaffolds may be useful for engineering tendon-bone interfaces (Li et al., 2009).

As tissue engineering moves forward, interfaces between different tissue types will become increasingly important. The first study described above uses electrospinning as a potential an adjuvant to current clinical therapies to improve bone-tendon integration and thus clinical outcomes (Spalazzi et al., 2008). The second study demonstrates the electrospinning can be used as a technique to facilitate the engineering of these transitions. Here, the authors create a mineral gradient along the length of an electrospun scaffold in order to facilitate the formation of the bone-to-tendon transition and promote rotator cuff tendon repair in future studies (Li et al., 2009). Thus, electrospun nanofibers will likely continue to find novel uses in the field of composite tissue engineering.

3.8 Skeletal muscle and muscle-tendon junctions

When skeletal muscle tissue loses function due to a congenital condition or an acquired condition such as a traumatic injury or tumor excision, there is no effective clinical treatment for replacing that function (Moon et al., 2008). Tissue engineering once again offers the opportunity to one day develop clinical treatments to repair damaged skeletal muscle and restore function. Our laboratory has used electrospinning to develop scaffolds for skeletal muscle tissue engineering (Choi et al., 2008). Electrospun scaffolds were made of a blend of PCL and collagen to investigate the effect that aligned fibers would have on skeletal myotube formation. Fibers were aligned by increasing the rotation speed of a plate mandrel. The aligned-fiber scaffolds enhanced skeletal muscle cell alignment and myofiber length compared to randomly oriented electrospun scaffolds. This suggested, as has already been pointed out in Section 3, that aligned-fiber scaffolds may be advantageous for organizing tissue engineered tissues, in this case skeletal muscle. The scaffolds from this study may one day be useful for forming functional tissue that could be implanted into a patient (Choi et al., 2008).

More recently, there has been increasing interest in composite tissue engineering, which as described in the previous section will become increasingly important as the field of tissue engineering advances. Specifically, we have begun to investigate scaffolds that would be appropriate for muscle-tendon junction tissue engineering (Ladd et al., 2010). Muscle and tendon tissue have different design requirements for scaffolds, but we wanted to create a single scaffold that could accommodate both tissue types. To do this, we decided to design scaffolds that mimic the trends in mechanical properties observed in

native MTJ. Our working hypothesis is that a scaffold that mimics these trends would promote the formation of MTJ tissue. We employed a co-electrospinning fabrication technique to begin developing such scaffolds. We showed that by using PCL and PLLA blended with type I collagen, that we could develop scaffolds with regional differences in mechanical properties and that these differences mimicked the trends observed in native MTJ (Ladd et al., 2010). Figure 2 showing the result of co-electrospinning to create a scaffold with regional variations in mechanical properties that mimic the trends observed in native muscle-tendon junction. While our working hypothesis remains to be tested, this is another example of where electrospinning is being used to create novel scaffolds for tissue engineering.

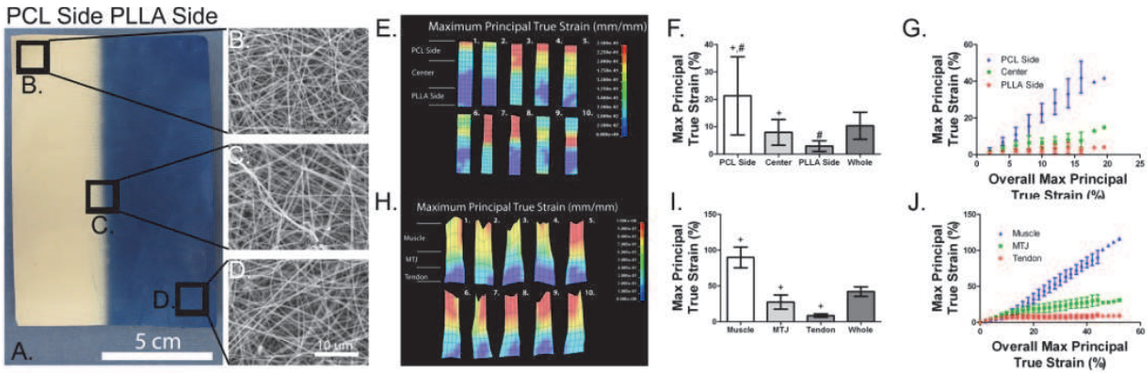


Fig. 2. (A) Image showing the three regions of the scaffold: PCL side, Center, and PLLA side with methylene blue dye added. (B-D) SEM images from the different regions of scaffold showing fiber morphology and diameter (all images ×4k). (B) PCL side (550±98 nm), (C) center (504±93 nm), (D) PLLA side (452±40 nm). (E) Color maps of the true strain profiles of the scaffolds when undergoing uniaxial tensile testing. Maximum principal strain is displayed and the scale is from 0 to 0.25 mm/mm strain. (E-G) The quantification (E) by showing the differences in max strain from each region. Maximum principal true strain for the regions of the scaffold at each overall strain interval for the whole scaffold. (H-J) Analagous data for native MTJ tissue tested in the same manner. Note that (H) has a scale from 0 to 1 mm/mm strain. From these figures, it is clear that the scaffolds mimic the trends in mechanical properties observed in native tissue. Matching symbols indicate statistical difference from one another. Significance was defined as *P<0.05. Reproduced with permission from all authors, *Biomaterials*, 2010, **32**, 6, 1549. © Elsevier 2010 (Ladd et al., 2010).

In summary, electrospinning provides a unique fabrication technique that could prove to be useful for both skeletal muscle tissue engineering and composite tissue engineering. The ability to form and control the orientation of nanofibers allows scaffolds to incorporate topographical cues to help align skeletal myofibers. The ability to incorporate multiple material types into a single scaffold allows one to create scaffolds with regional variations in mechanical properties that may be useful for MTJ tissue engineering and also may prove useful for other types of composite tissue engineering. Undoubtedly, more work is needed to create clinically useful applications despite its successful use in the laboratory setting. Nevertheless, electrospinning remains as a useful tool in the tissue engineer's armamentarium.

4. Current challenges with using electrospinning in tissue engineering

As with any fabrication method, there are advantages and disadvantages to using electrospinning as a means of scaffold fabrication for tissue engineering. Many of the advantages have already been highlighted in previous sections such as the mimicking of native ECM and the ability to adapt the technique for versatile applications. Here, we discuss several limitations of the electrospinning and strategies to work towards overcoming them.

An inherent limitation to using electrospun nanofibrous scaffolds in tissue engineering applications is the small pore size. Nanofiber scaffolds typically result in pore sizes on the sub-micron to few micron scale. Because all mammalian cells range in size from several micrometers to several hundred micrometers in size, these cells typically cannot penetrate the scaffold. This poses a problem for tissue engineers because we often aim to form fully cellularized three dimensional tissue constructs. There have been attempts to improve the pore size of electrospun nanofiber scaffolds. The two primary methods are to co-electrospin a set of sacrificial fibers or adding porogens (Baker et al., 2008; Nam et al., 2007). Sacrificial fibers are fibers that are co-electrospun with the main scaffold component, but that are easily dissolved after fabrication leaving behind larger pores. For instance, Baker et al. (2008) used PEO, a water soluble polymer, as the sacrificial component of electrospun scaffolds. After dissolving the PEO, the pore size of the electrospun scaffolds increased. Porogens, primarily salt, have also been added to electrospun scaffolds as they are being electrospun (Nam et al., 2007). Typically the porogens are dropped onto a mandrel or collecting plate while the fibers are being deposited. Then, after fabrication, the porogens can be removed by dissolution. These approaches have improved pore size and allowed for improved cellular infiltration. However, these approaches may compromise the mechanical properties and dimensional stability of the scaffold when the fibers dissolve and collapse. Microintegration is another approach developed by Stankus et al. (2006) for producing a highly cellularized construct. However, this process may limit homogeneity, sterility, and cell survival, due to the long-term processing times required.

Another inherent limitation of electrospinning is scaffold thickness, especially when spinning onto a cylindrical mandrel. Electrospun scaffolds tend to be thin, typically less than one millimeter in many cases (Inoguchi et al., 2006; Lee et al., 2008). Scaffolds with thicknesses greater than one millimeter are possible to fabricate, but are less commonly seen in the literature (Pham et al., 2006b). Moreover, in our experience, achieving millimeter or greater thicknesses requires the use of a large volume of polymer solution. The inability to obtain thicker scaffolds may be dictated by the strength of the electric field. When spinning is initiated, there is an electrostatic attraction from the charged needle tip to the grounded mandrel. As spinning continues and fibers are deposited on the mandrel in layers. We presume that the layers act as an insulator, thus preventing sufficient current flow from the newly deposited fibers to the grounded mandrel and resulting in a buildup of charge on the outer fibers. This charge buildup would decrease the electrostatic force the charged polymer feels from the grounded mandrel, resulting in the fibers depositing on the mandrel with less efficiency, an increased attraction of the fibers for other objects, and a decrease in mat density due to loose fiber packing. In other words, the fiber deposition efficiency decreases as fibers are deposited on the mandrel and thus in order to obtain thicker scaffolds, one must use a large volume of polymer solution. McCullen et al. (2010) attempted to increase scaffold thickness using multi-layering electrospun scaffolds with a collagen gel for bone

tissue engineering. After fabricating multi-layered electrospun scaffolds, micro-scaled pores were created by a laser ablation technique. Thus, they were able to maintain the unique nanofibrous structure that supports cellular interactions and to fabricate micro-scaled features within the scaffold.

An additional limitation of electrospinning is the inability to precisely control where the fibers are deposited (Teo & Ramakrishna, 2005). As fibers whip towards a collecting plate or mandrel, they deposit randomly unless there is some other force to cause them to orient differently. To try to overcome this limitation, researchers have used large cylindrical mandrels with high rotation speeds to cause fiber alignment (Choi et al., 2008; Moffat et al., 2009) or used parallel electrodes to apply an electric field that would force the fiber to align across the plates or in other orientations (Li et al., 2003; Teo & S Ramakrishna, 2005; Li et al., 2005; Yang et al., 2009). Still, electrospinning would be an even more powerful technique if one could precisely control where the polymer jet would land such that computer-assisted design of electrospun scaffolds would be possible. This would allow researchers to easily create nanofibers and create a much broader range of scaffold geometries and thicknesses. Unfortunately, no one has discovered a way to do this to date. The closest anyone has come to approaching this ability was in a study by Kameoka et al. (2003) where they created a scanning tip electrospinning source that used an arrow-shaped tip, polymer droplet, and high voltage to create a Taylor cone and spin fibers. They were able to control deposition to a region of about 5 mm in size. While some researchers have found creative ways to make electrospun scaffolds of differing geometries (besides tubes and sheets), the inability to precisely control the deposition location of the polymer jet limits the variety of possible geometries.

Most researchers tend to have electrospinning systems which still require a manual setup, i.e. manual positioning of the needle tip from the collector. Moreover, often humidity and temperature are not well-controlled and these ambient parameters can have an effect on the fibers. Because these parameters are often not precisely controlled, there is an increase in the variation of properties and fiber morphologies from scaffold to scaffold. In order to improve the repeatability and homogeneity between spinning batches, modern technology should be incorporated into electrospinning systems to more precisely control these parameters. For instance, without much difficulty, a computer-controlled spinning apparatus that is self-contained to control temperature and humidity and that precisely controls air gap distances, mandrel rotation speed, voltage, and spinneret flow rate could be developed. Such a system would allow for more consistent scaffold fabrication and serve as an improved system to continue to investigate how these spinning parameters affect final fiber morphology.

5. The future of electrospinning in tissue engineering and regenerative medicine

Electrospinning has evolved as a powerful tool in various tissue engineering applications. This fabrication technology provides the ability to control biomaterial composition, fiber diameter, fiber alignment, geometry, and drug/protein incorporation into a scaffold. Nano-scaled fibers generated by electrospinning are able to improve the cellular interactions of a wide variety of cell types; moreover, the cells are able to maintain their phenotypic and functional characteristics on nano-scaled fibrous scaffolds. Additionally, a growing body of evidence demonstrates that nano-to micro-scaled topography plays an important role in controlling the adhesion, proliferation, differentiation, and survival of cells in culture.

Therefore, electrospun nano-scaled fibrous scaffolds can serve as a tool for studying the topographical aspects of cellular interactions that would lead to improved tissue formation. Furthermore, these electrospun scaffolds can be functionalized by adding biochemical and mechanical cues to enhance cellular interactions for tissue engineering applications. However, specific interactions between cells and electrospun scaffolds functionalized through surface modifications and bioactive factor incorporation are still poorly understood. Understanding of the specific cues that enhance cell adhesion, proliferation, and guidance of cells seeded on a scaffold, as well as cues that could affect host cell infiltration, differentiation, and vascularization in *in vitro* and *in vivo*, is crucial for the advancement of tissue engineering applications.

The most significant challenges that prohibit the expansion of electrospinning applications are increasing scaffold thickness and pore size. If these two problems were to be solved, electrospun nanofibers would prove to be even more useful than they are currently. Electrospun scaffolds can also be used as a component of a composite scaffold, similar to those developed by Sahoo et al. (Sahoo et al., 2006; Sahoo et al., 2007; Sahoo et al., 2010a; Sahoo et al., 2010b) or incorporated with hydrogel scaffolds to create layered structures. More detailed understanding of how the process parameters interact with one another, especially the electric field, may provide ways to better control fiber deposition. Thus, basic research into the physics of electrospinning will be essential in overcoming the challenges outlined above. For example, in a recent study by Ziabari et al. the simultaneous effects of solution concentration, spinning distance, applied voltage, and flow rate on mean fiber diameter and standard deviation of fiber diameter were investigated (Ziabari et al., 2010). Perhaps with more basic research, we will be able to apply electrospun nanofibers as a controlled fiber jet such that the fibers can be precisely deposited with micron-scale or sub-micron accuracy. Such a capability would be immensely useful for tissue engineering scaffold design.

6. Acknowledgments

This work was supported by the Department of Energy (DE-FG02-09ER64711), Telemedicine and Advanced Technology Research Center (TATRC) at the U.S. Army Medical Research and Material Command (USAMRMC) through award W81XWH-07-1-0718, and Orthopaedic Trauma Research Program (USAMRAA OTRP07-07128091) of Department of Defense.

7. References

Badami, A.S. et al., 2006. Effect of fiber diameter on spreading, proliferation, and differentiation of osteoblastic cells on electrospun poly(lactic acid) substrates. *Biomaterials*, 27(4), pp.596-606.

Baker, B.M. et al., 2008. The potential to improve cell infiltration in composite fiber-aligned electrospun scaffolds by the selective removal of sacrificial fibers. *Biomaterials*, 29(15), pp.2348-2358.

Behonick, D.J. & Werb, Z., 2003. A bit of give and take: the relationship between the extracellular matrix and the developing chondrocyte. *Mechanisms of Development*, 120(11), pp.1327-1336.

Chan-Park, M.B. et al., 2009. Biomimetic control of vascular smooth muscle cell morphology and phenotype for functional tissue-engineered small-diameter blood vessels. *Journal of Biomedical Materials Research. Part A*, 88(4), pp.1104-1121.

Choi, J.S. et al., 2008. The influence of electrospun aligned poly(ε-caprolactone)/collagen nanofiber meshes on the formation of self-aligned skeletal muscle myotubes. *Biomaterials*, 29(19), pp.2899-2906.

Deitzel, J.M. et al., 2001. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer*, 42, pp.261-272.

Doshi, J. & Reneker, D.H., 1995. Journal of Electrostatics. *Journal of Electrostatics*, 35(2-3), pp.151-160.

Ghasemi-Mobarakeh, L. et al., 2008. Electrospun poly(ε-caprolactone)/gelatin nanofibrous scaffolds for nerve tissue engineering. *Biomaterials*, 29(34), pp.4532-4539.

Greiner, A. & Wendorff, J.H., 2007. Electrospinning: a fascinating method for the preparation of ultrathin fibers. *Angewandte Chemie,* 46(30), pp.5670-5703.

Gupta, P. et al., 2005. Electrospinning of linear homopolymers of poly(methyl methacrylate): exploring relationships between fiber formation, viscosity, molecular weight and concentration in a good solvent. *Polymer*, 46, pp.4799-4810.

Gupta, D. et al., 2009. Nanostructured biocomposite substrates by electrospinning and electrospraying for the mineralization of osteoblasts. *Biomaterials*, 30(11), pp.2085-2094.

Hunziker, E.B., 2002. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis and Cartilage,* 10(6), pp.432-463.

Inoguchi, H. et al., 2006. Mechanical responses of a compliant electrospun poly(L-lactide-*co*-ε-caprolactone) small-diameter vascular graft. Biomaterials, 27(8), 1470-8.

Jang, J.-H., Castano, O. & Kim, H.-W., 2009. Electrospun materials as potential platforms for bone tissue engineering. *Advanced Drug Delivery Reviews*, 61(12), pp.1065-1083.

Ju, Y.M. et al., 2010. Bilayered scaffold for engineering cellularized blood vessels. *Biomaterials*, 31(15), pp.4313-4321.

Kameoka, J. et al., 2003. A scanning tip electrospinning source for deposition of oriented nanofibres. *Nanotechnology*, 14(10), pp.1124-1129.

Kumbar, S.G. et al., 2008. Electrospun poly(lactic acid-*co*-glycolic acid) scaffolds for skin tissue engineering. *Biomaterials*, 29(30), pp.4100-4107.

Ladd, M.R. et al., 2010. Co-electrospun dual scaffolding system with potential for muscle-tendon junction tissue engineering. *Biomaterials*, 32(6), pp.1549-1559.

Lee, C.H. et al., 2005. Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. *Biomaterials*, 26(11), pp.1261-1270.

Lee, S.J. et al., 2007. In vitro evaluation of electrospun nanofiber scaffolds for vascular graft application. *Journal of Biomedical Materials Research, Part A*, 83(4), p.999–1008.

Lee, S.J. et al., 2008. Development of a composite vascular scaffolding system that withstands physiological vascular conditions. *Biomaterials*, 29(19), pp.2891-2898.

Li, C. et al., 2006. Electrospun silk-BMP-2 scaffolds for bone tissue engineering. *Biomaterials*, 27(16), pp.3115-3124.

Li, D., Wang, Y. & Xia, Y., 2003. Electrospinning of Polymeric and Ceramic Nanofibers as Uniaxially Aligned Arrays. *Nano Letters*, 3(8), pp.1167-1171.

Li, W.-J. et al., 2005. A three-dimensional nanofibrous scaffold for cartilage tissue engineering using human mesenchymal stem cells. *Biomaterials*, 26(6), pp.599-609.

Li, X. et al., 2009. Nanofiber scaffolds with gradations in mineral content for mimicking the tendon-to-bone insertion site. *Nano Letters*, 9(7), pp.2763-2768.

Liao, S. et al., 2008. Processing nanoengineered scaffolds through electrospinning and mineralization suitable for biomimetic bone tissue engineering. *Journal of the Mechanical Behavior of Biomedical Materials*, 1(3), pp.252-260.

Lodish, H. et al., 2008. *Molecular Cell Biology* 6th ed., New York, New York: W. H. Freeman and Company. pp.820-833.

McCullen, S.D. et al., 2010. In situ collagen polymerization of layered cell-seeded electrospun scaffolds for bone tissue engineering applications. Tissue Engineering. Part C, 16(5), pp.1095-1105.

Megelski, S. et al., 2002. Micro- and nanostructured surface morphology on electrospun polymer fibers. *Macromolecules*, 35(22), pp.8456-8466.

Moffat, K.L. et al., 2009. Novel nanofiber-based scaffold for rotator cuff repair and augmentation. *Tissue Engineering. Part A*, 15(1), pp.115-126.

Moon, D.G. et al., 2008. Cyclic mechanical preconditioning improves engineered muscle contraction. *Tissue Engineering. Part A*, 14(4), pp.473-482.

Moore, K.L. & Dalley, A.F., 1999. *Clinically Oriented Anatomy* 4th ed., Baltimore, MD: Lippincott Williams & Wilkins. pp.12-14.

Nam, J. et al., 2007. Improved cellular infiltration in electrospun fiber via engineered porosity. *Tissue Engineering*, 13(9), pp.2249-2257.

Nie, H. & Wang, C.-H., 2007. Fabrication and characterization of PLGA/HAp composite scaffolds for delivery of BMP-2 plasmid DNA. *Journal of Controlled Release* 120(1-2), pp.111-121.

Pham, Q.P., Sharma, U. & Mikos, A.G., 2006. Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Engineering*, 12(5), pp.1197-1211.

Plotkin, L.I. et al., 1999. Prevention of osteocyte and osteoblast apoptosis by bisphosphonates and calcitonin. *Journal of Clinical Investigation*, 104(10), pp.1363-1374.

Powell, H.M. & Boyce, S.T., 2009. Engineered human skin fabricated using electrospun collagen-PCL blends: morphogenesis and mechanical properties. *Tissue Engineering. Part A*, 15(8), pp.2177-2187.

Prabhakaran, M.P., Venugopal, J.R. & Ramakrishna, Seeram, 2009. Mesenchymal stem cell differentiation to neuronal cells on electrospun nanofibrous substrates for nerve tissue engineering. *Biomaterials*, 30(28), pp.4996-5003.

Priya, S.G., Jungvid, H. & Kumar, A., 2008. Skin tissue engineering for tissue repair and regeneration. *Tissue Engineering. Part B, Reviews*, 14(1), pp.105-118.

Reneker, D. H., & Chun, I., 1996. Nanometre diameter fibres of polymer, produced by electrospinning. *Nanotechnology*, 7, pp.216-223.

Ross, M.H., Kaye, G.I. & Pawlina, W., 2003. Integumentary System. In *Histology A Text and Atlas*. Baltimore, MD: Lippincott Williams & Wilkins, pp. 400-433.

Sahoo, S. et al., 2006. Characterization of a novel polymeric scaffold for potential application in tendon/ligament tissue engineering. *Tissue Engineering*, 12(1), pp.91-99.

Sahoo, S., Cho-Hong, J.G. & Siew-Lok, T., 2007. Development of hybrid polymer scaffolds for potential applications in ligament and tendon tissue engineering. *Biomedical Materials (Bristol, England)*, 2(3), pp.169-173.

Sahoo, S. et al., 2010a. Bioactive nanofibers for fibroblastic differentiation of mesenchymal precursor cells for ligament/tendon tissue engineering applications. *Differentiation; Research in Biological Diversity*, 79(2), pp.102-110.

Sahoo, S., Toh, Siew Lok & Goh, J.C.H., 2010b. A bFGF-releasing silk/PLGA-based biohybrid scaffold for ligament/tendon tissue engineering using mesenchymal progenitor cells. *Biomaterials*, 31(11), pp.2990-2998.

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. *Journal of Biomaterials Science. Polymer Edition*, 17(1-2), pp.103-119.

Sill, T.J. & von Recum, H. a, 2008. Electrospinning: applications in drug delivery and tissue engineering. *Biomaterials*, 29(13), pp.1989-2006.

Spalazzi, J.P. et al., 2008. Mechanoactive scaffold induces tendon remodeling and expression of fibrocartilage markers. *Clinical Orthopaedics and Related Research*, 466(8), pp.1938-1948.

Stankus, J.J. et al., 2006. Microintegrating smooth muscle cells into a biodegradable, elastomeric fiber matrix. *Biomaterials*, 27(5), pp.735-744.

Stoop, R., 2008. Smart biomaterials for tissue engineering of cartilage. *Injury*, 39 Suppl 1, pp.S77-87.

Sun, T. et al., 2005. Self-organization of skin cells in three-dimensional electrospun polystyrene scaffolds. *Tissue Engineering*, 11(7-8), pp.1023-1033.

Teo, W.E. & Ramakrishna, S, 2005. Electrospun fibre bundle made of aligned nanofibres over two fixed points. *Nanotechnology*, 16(9), pp.1878-1884.

Theron, A., Zussman, E. & Yarin, A., 2001. Electrostatic field-assisted alignment of electrospun nanofibres. *Nanotechnology*, 12, pp.384-390.

Thorvaldsson, A. et al., 2008. Electrospinning of highly porous scaffolds for cartilage regeneration. *Biomacromolecules*, 9(3), pp.1044-1049.

Tillman, B.W. et al., 2009. The in vivo stability of electrospun polycaprolactone-collagen scaffolds in vascular reconstruction. *Biomaterials*, 30(4), pp.583-588.

Vaquette, C. et al., 2010. Aligned poly(L-lactic-*co*-ε-caprolactone) electrospun microfibers and knitted structure: a novel composite scaffold for ligament tissue engineering. *Journal of Biomedical Materials Research. Part A*, 94(4), pp.1270-1282.

Wong, D.J., Chang, H.Y. & Biology, E., 2009. Skin tissue engineering. *StemBook*, pp.1-9.

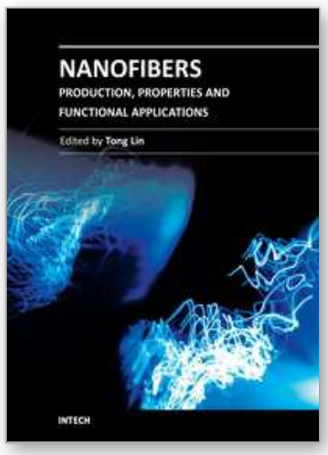
Xu, C., 2004. Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials*, 25(5), pp.877-886.

Yang, S. et al., 2001. The design of scaffolds for use in tissue engineering. Part I. Traditional factors. *Tissue Engineering*, 7(6), pp.679-689.

Yang, Y. et al., 2009. Electrospun Composite Mats of Poly[(D,L-lactide)-*co*-glycolide] and Collagen with High Porosity as Potential Scaffolds for Skin Tissue Engineering. *Macromolecular Materials and Engineering*, 294(9), pp.611-619.

Zhou, Y. et al., 2008. Electrospun water-soluble carboxyethyl chitosan/poly(vinyl alcohol) nanofibrous membrane as potential wound dressing for skin regeneration. *Biomacromolecules*, 9(1), pp.349-354.

Ziabari, M., Mottaghitalab, V. & Haghi, A.K., 2010. A new approach for optimization of electrospun nanofiber formation process. *Methodology*, 27(1), pp.340-354.



Nanofibers - Production, Properties and Functional Applications
Edited by Dr. Tong Lin

ISBN 978-953-307-420-7
Hard cover, 458 pages
Publisher InTech
Published online 14, November, 2011
Published in print edition November, 2011

As an important one-dimensional nanomaterial, nanofibers have extremely high specific surface area because of their small diameters, and nanofiber membranes are highly porous with excellent pore interconnectivity. These unique characteristics plus the functionalities from the materials themselves impart nanofibers with a number of novel properties for advanced applications. This book is a compilation of contributions made by experts who specialize in nanofibers. It provides an up-to-date coverage of in nanofiber preparation, properties and functional applications. I am deeply appreciative of all the authors and have no doubt that their contribution will be a useful resource for anyone associated with the discipline of nanofibers.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Mitchell R. Ladd, Tanner K. Hill, James J. Yoo and Sang Jin Lee (2011). Electrospun Nanofibers in Tissue Engineering, Nanofibers - Production, Properties and Functional Applications, Dr. Tong Lin (Ed.), ISBN: 978-953-307-420-7, InTech, Available from: http://www.intechopen.com/books/nanofibers-production-properties-and-functional-applications/electrospun-nanofibers-in-tissue-engineering

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