We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

24

Biomimetic Architectures for Tissue Engineering

Jianming Li, Sean Connell and Riyi Shi
Purdue University
USA

1. Introduction

Within the human body, there is a vast array of uniquely arranged biologic structures. The elegance of these geometries is only matched by their equally varied functional sophistication. The harmony at which all components operate is truly awe-inspiring and from an engineering perspective, daunting to replicate. Yet in depth analysis of body tissues reveals a unique story. Many complex hierarchal structures can be deconstructed into simple recurring forms. Two ubiquitous geometries native to soft tissue are fibrillar networks and thin walled tubules. For instance, much of the extracellular matrix (ECM) that lends mechanical support to cells and tissues are fibrillar in nature. Vessels involved in either fluid transport or filtration are also high aspect ratio tubes of varying diameters. Furthermore, fibrillar and tubular themes are even found in hard tissues such as bone and cartilage.

In this chapter, we describe how researchers are synthetically recreating three-dimensional matrix analogs for regenerative medicine. We first highlight the complexities and nuances of real tissue and discuss the challenges in designing, fabricating and implementing biomimetic scaffolds for implantation. Applications of state of the art research pertaining to soft tissues and stem cell work will also be examined. We finally address current technological shortcomings and provide strategies for recreating function-specific tissue/organ systems with appropriate biophysical parameters

1.1 Structure-Function Relationships

The unique architectures found in biology have been evolutionarily shaped to perform particular tasks and this marriage between form and function is well manifested in the human body. For example, the layout of the nervous system is closely tied to cellular specialization. Neurons that perform signal integration are endowed with complex, multi-dendritic processes, while transmission neurons have axons that span several meters in length in some mammals. A similar undercurrent is observed in the circulatory system, where the biconcave geometry of red blood cells is optimized to facilitate oxygen exchange, mobility in a fluid medium and clotting (Wang, Pan et al. 2009; Wang, Gao et al. 2009). Injury or pathology can affect cell morphology, inducing problematic physiologic abnormalities. In the discussion of blood, conditions such as sickle cell anemia can alter flow dynamics and cause unwanted blood clots.

The forces at work that determine cell shape are also evident in the extracellular matrix (Figure 1). Mechanically mediated adaptation occurs frequently in the musculoskeletal system. For instance, bone undergoes a structural remodeling process in the presence or absence of loading (Wolffe's law). Recent studies into bone's nanostructure also reveal heterogeneous patterns that may impart load dispersal properties (Ortiz 2009). The axial hierarchal organization of supporting ligaments and tendons also reflect their functional role. Much like cables, these aligned elastic tissues are well suited to bearing tensile loads (Frank 2004). We emphasize that biologic structure-function relationships exist at all length scales- even down to the nanometer level of DNA and proteins.

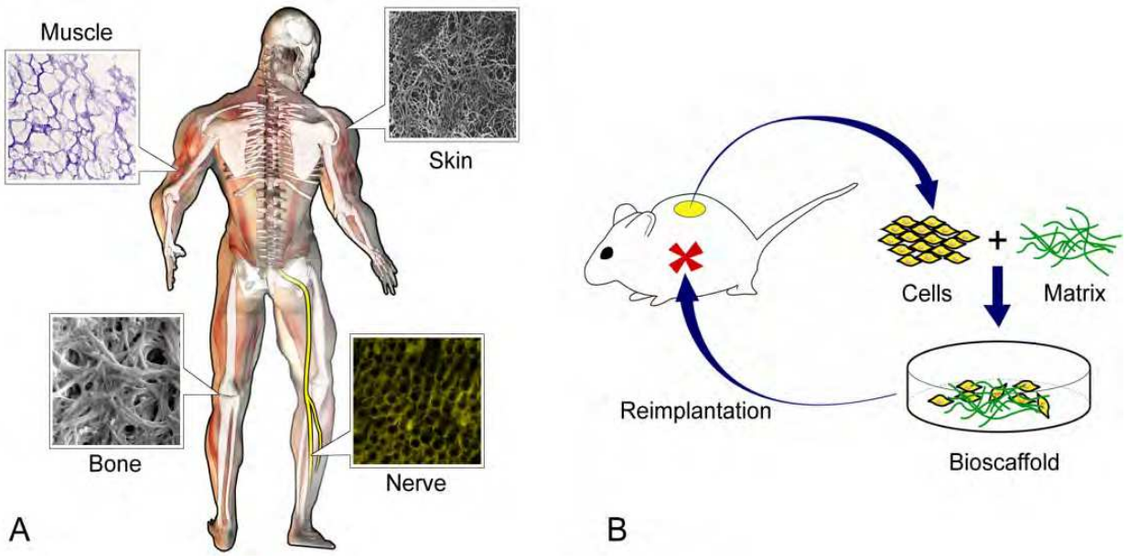

Fig. 1. (A) Many body tissues depict fibrillar and tubular thematic elements. Muscle and skin cross sections reprinted with permission (Singelyn, DeQuach et al. 2009) and (Chen, Ho et al. 2004), respectively. Bone micrograph courtesy of O. Akkus. (B) Tissue engineering aims to restore function by repair, replacement or regeneration of damaged tissue. Cellular therapies involve harvesting healthy cells (adult or stem cells), culturing in a matrix and subsequent reimplantation into the damaged site. Alternatively, acellular (cell free) biomatrices may also be used to promote endogenous healing and remodelling.

1.2 The Role of Architecture in Tissue Engineering

Like most machines, components of the body eventually fail. Disease states, mechanical insult or normal wear may initiate the process of tissue degradation. The goal of tissue engineering is the restoration of lost function. Recently, cellular therapies have drawn significant interest, with stem cell research at the forefront of biomedical innovation. The pluripotent nature of stem cells makes them attractive candidates for regenerating all organ types. In contrast, acellular treatments designed to promote endogenous wound healing are also tenable strategies. However, regardless of the therapeutic regime it has become evident that matrix architecture plays an active role in tissue remodeling. The matrix contributes to how a cell transduces input from the external physical environment into biochemical signals that dictate cell response. A wealth of evidence has shown that cells grown in 3-D culture

systems demonstrate altered morphologies and gene expression compared to traditional 2-D platforms. Affecting the cell's physical environment may be used to ultimately control cell behavior and fate (Sands and Mooney 2007; Ingber 2008). Thus, the structure-function relationships that govern normal physiology are equally instrumental during the repair process. This dogma underpins tissue engineered replacements: restorative devices should emulate the natural order of the body.

Interestingly, while there are countless architectural schemes *in vivo*, two elements found in high frequency are fibrils and tubules. Most body tissues are hierarchal fibrillar or tubular arrangements. It is the variation in size, organization and composition of these simple building blocks that dictates the wide range of observed mechanical and biophysical properties (Figure 2). Replicating these diverse structures from the macroscopic to the nanoscale level is a significant scientific undertaking. However, advancements in micro and nanofabrication have paved the path for constructing biologic analogs beginning at the molecular level. In the following sections, we discuss unique manufacturing methods capable of producing ECM-like architectures. Specifically, we emphasize emergent manufacturing techniques such as electrospinning, phase separation and nanoscale self-assembly. These methods provide invaluable tools for developing the next generation of biointegrative implants.

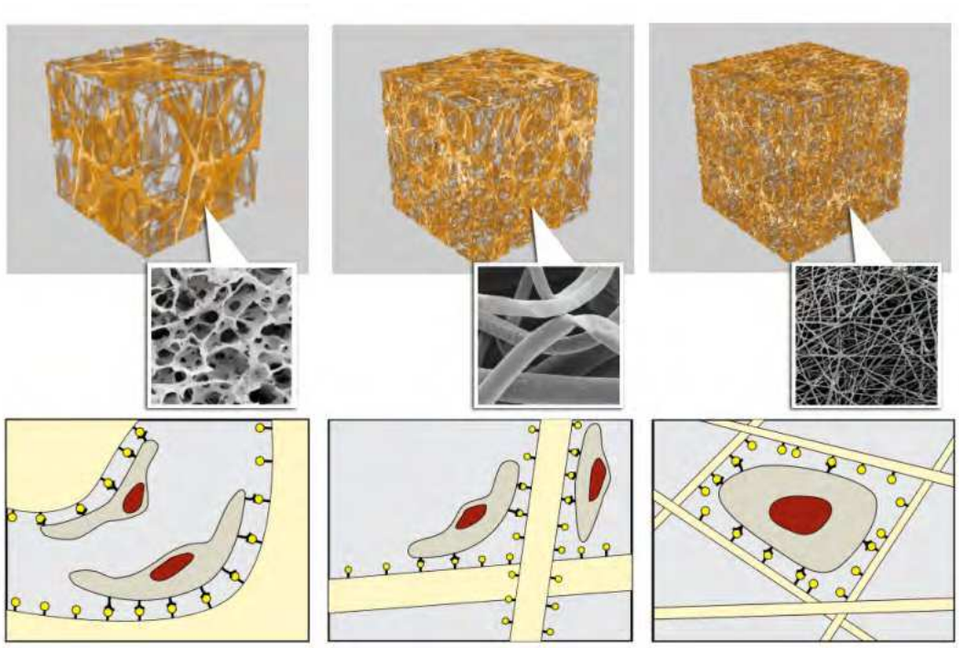

Fig. 2. The role of length scales on cellular behavior. As geometric features become smaller, changes in cell morphology and fate can be observed. Micrometer based lengths induce more 2-D (planar) geometries, while cells are more spatially interactive on 3-D nanoscaled meshes. Adapted from (Stevens and George 2005).

1.3 Emulating Nature: Fabrication of Micro and Nanoscale Architectures

1.3.1 Electrospinning

Electrospinning is a fabrication technique that utilizes electrostatic forces to draw continuous fibers from a viscoelastic medium. The process of electrospinning is quite archaic, dating back to 1902. However, in the mid 1990's electrospinning was resurrected in the laboratory as many researchers recognized its utility in the growing nanoengineering discipline (Doshi and Reneker 1995). Since then, a multitude of materials ranging from synthetic and natural polymers, ceramics, semiconductors, biomacromolecules and even cell suspensions have been electrostatically spun (Pham, Sharma et al. 2006; Jayasinghe, Irvine et al. 2007). Resultant fibers can be as large as 10μm or as small as 5nm. The material versatility, ease of manufacture, combined with fibers within biologically relevant length scales makes electrospinning a highly attractive method for scaffold production.

The fundamental principle guiding electrospinning is uniaxial stretching of a fluid. Figure 3 describes a typical laboratory configuration for electrospinning. A high voltage (5-30kV) is applied to a dissolved polymer solution. The repulsive force in the fluid accumulates until at a critical point, the electrostatic repulsion overcomes surface tension and a bead erupts from the spinneret (tip). The exiting jet soon enters a dynamic instability phase, which is marked by a chaotic swirling motion. Continued elongation of the jet coincides with solvent evaporation and the fluid stream eventually lands on the grounded collector plate. At this point, the charges dissipate and the fiber solidifies/cures.

The dynamics of the spray jet is a well studied phenomenon and computational models have been made to describe the thinning event. Variables such as viscosity, conductivity, voltage, tip to collector distance and humidity can have an effect on fiber morphology. Details for these can be found elsewhere (Rutledge and Fridrikh 2007). Since electrospinning generates shear stresses within the thinning jet, large scale alignment at the molecular level can be achieved (Stephens, Fahnestock et al. 2005).

One of the primary challenges facing electrospinning is the control of architecture. Since the spinning process is predominately chaotic, the final mat of fiber is unwoven and randomly oriented. However, multiple strategies have been devised to fabricate hierarchal or oriented fibrils. These include focusing of the falling jet with external electric fields or modifications to the collecting plate. For example, one technique used for obtaining aligned fibers is the placement of a rotating disc/drum as the collector (Figure 3). Electrospun fibers are deposited on the edge of the disc, with alignment being parallel to the edge width. Electrospinning may also be used to coat various shapes such as tubes for cardiovascular applications. Alternatively, Xia and co-workers developed a unique split plate collector system to create aligned fibers over a span of several centimeters (Li, Wang et al. 2004). If alternating split plates are used, the ground can be cycled between each electrode pair, creating layer by layer stacked mats of orthogonal alignment (Xie, Macewan et al. 2009).

Others have also used field assisted approaches to create braided fibers (Theron, Zussman et al. 2001). These particular structures have promise in use for axially loaded tissue such as ligaments and tendons. Coaxial spinning with two concentric spinnerets has also shown potential for creating composite fibers (Jiang, Hu et al. 2005; McCann, Marquez et al. 2006). In this process a hybrid fiber with a different core material can be produced. The core can be retained or selectively dissolved to fashion microtubular entities.

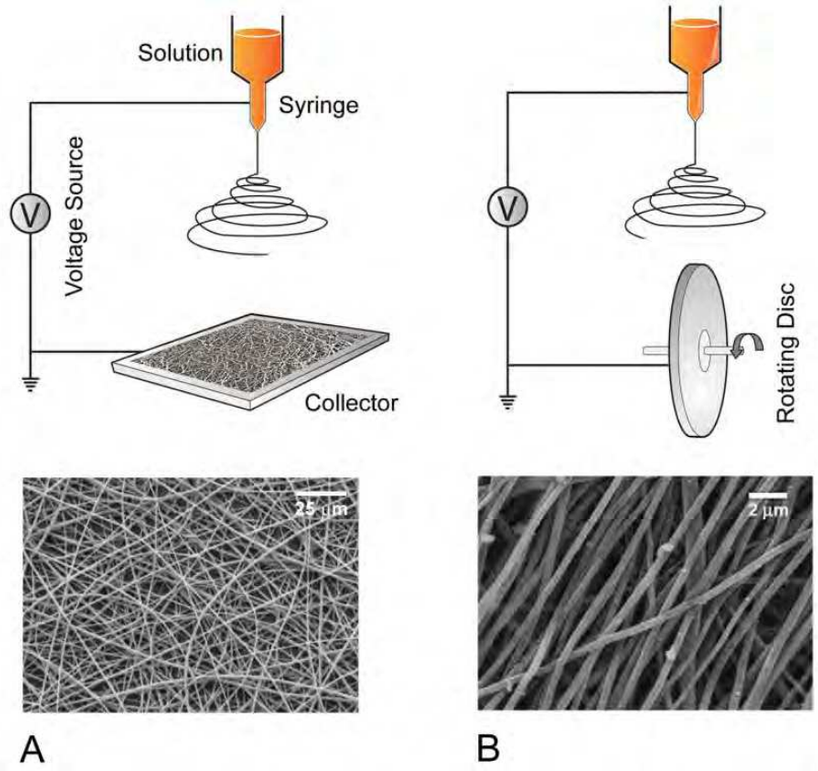

Fig. 3. (A) Schematic depiction of the electrospinning process. A charged solution is drawn from the tip and the residual random fibers collect on a grounded plate. (B) A spinning disc technique commonly employed to create aligned electrospun fibers. Fibers aggregate on the disc edge. Corresponding random and aligned fibers produced from shown setup. Reproduced with permission from (Prabhakaran, Venugopal et al. 2009) and (Lee, Bashur et al. 2009).

1.3.2 Phase Separation and Selective Dissolution

Phase separation is a unique process that has the capacity to form both fibrillar and tubular constructs. There are multiple variations to phase separation, but a common modality is solid-liquid phase extraction. One novel strategy involves creating a thermal gradient to separate two constituent phases. For example, Ma and Zhang used this method to manufacture nanofibrous foams and networks (Ma and Zhang 1999). In this process, the parent polymer was first dissolved in a solvent. The solution was then placed in a refrigerator to gel. Solvent exchange was carried out with distilled water and the subsequent compound frozen and freeze dried. Resultant fibrillar architectures showed fibril diameters between 50-500nm. Local porosity was high, reaching 98% in some cases. Fiber matrix properties were controlled primarily by the gelation temperature. The same group also manipulated this technique to form microtubular arrays. In this case, a dissolved polymer solution was laterally insulated and placed on a cold source. The uniaxial thermal gradient induced a directional precipitation of the dissolved polymer (Figure 4). The solid-liquid separated solution was subsequently freeze dried. Stokols and Tuszynski also used a uniaxial thermal gradient with agarose to form linear channels (Stokols and Tuszynski 2004). These channels were continuous for up to 1cm and had pore sizes (channel diameter)

on the order of 30-100□m. These structures swelled in the presence of water due to hydrophilicity and resembled endogenous myotubes and endoneurial tubes.
Recently, Li et al. devised an inexpensive method to form single and aggregate high aspect ratio tubules with sacrificial sugar (Li, Rickett et al. 2009). Initially, sugar filaments formed from melt spinning were coated with a degradable polymer (PLLA). After polymer curing, the sugar core was dissolved with water, leaving a thin continuous tubular membrane. The final tubules also showed nanoscopic pores which are desirable for nutrient and waste diffusion. Multiple tube aggregates were made by fusion. The use of "cotton candy" as a core template was also employed by Bellan et al. for simulated microvasculature (Bellan, Singh et al. 2009). In this case, PDMS polymer was poured around long sugar filaments. The sugar was dissolved with water, leaving a solid PDMS block with embedded microtubes. The winding tubes were capable of supporting fluid media at physiologic flow rates.

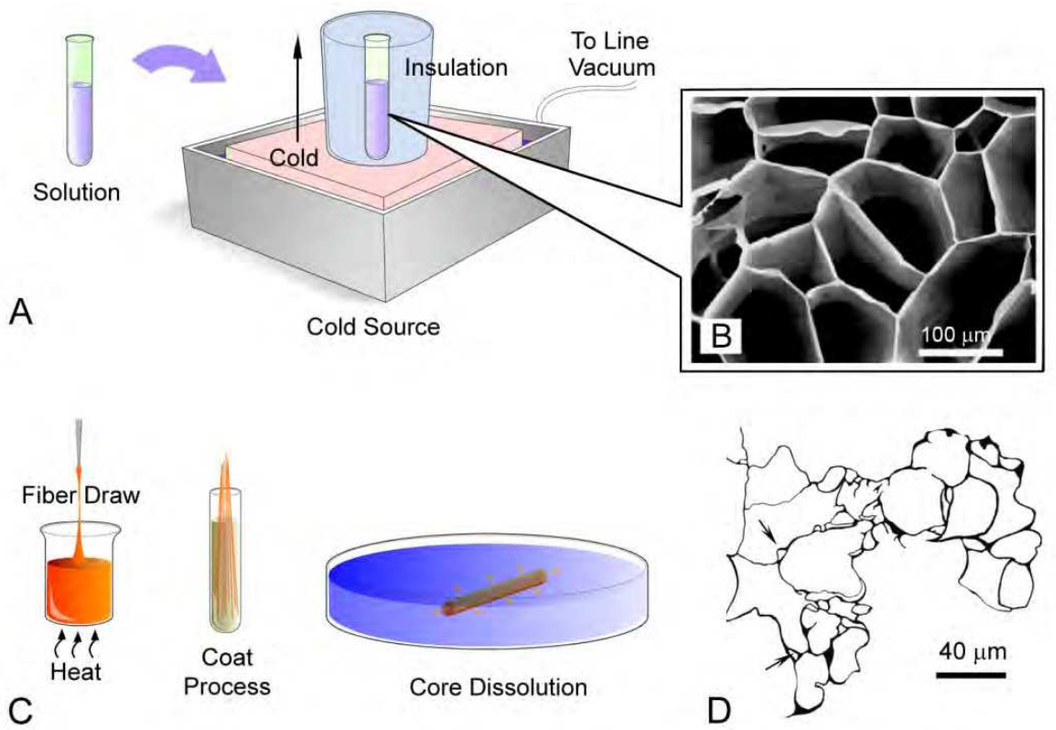

Fig. 4. (A) Diagram of thermal phase separation technique. A solution of dissolved polymer is placed on a cold source. The induced thermal gradient causes precipitation of the solute in a unixial fashion. Reprinted and adapted from (Stokols and Tuszynski 2004). (B) Corresponding microstructure of microchannels from phase separation (Stokols and Tuszynski 2004). (C) Depiction of selective dissolution for fabricating microchannels. Sucrose templates are coated with a polymer. The core is preferentially dissolved with another solvent. (D) Cross sectional images of microtubular geometries created by sacrificial templating. Reprinted with permission from (Li, Rickett et al. 2009).

One of the primary advantages of using phase separation approaches is the simplicity of the process. Phase separation generally requires minimal equipment, resources and the gamut of structures can range from nanoscale fibrils to micrometer tubes capable of sustaining

fluid flow. In addition to the basic underlying structure, additional pores or discontinuities may be imparted via particulate leaching. Thus, scaffolds with several orders of architectural complexity may be realized.

1.3.3 Self Assembly

Self assembly is a process that describes the spontaneous formation of material patterns or structures without external influence. The forces that govern the aggregation phenomenon are local molecular interactions such as electrostatic forces, hydrophobic interactions and hydrogen bonding (Tu and Tirrell 2004). Self assembly is a key concept in "bottoms up" nanofabrication, where structures are constructed from individual molecular units. Self assembly is a fundamental theme in nature and is responsible for simple or complex hierarchal shapes including lipid bilayers (micelles), DNA, proteins and viral capsids. Consequently, understanding the basic tenants of self assembly has given researchers insight into forming biocompatible supramolecular structures. The key in self-assembled structures is design of base units that have two distinguishing components: a segment for directing the aggregation process and a biologically active moiety that encodes cell-specific instruction (Tu and Tirrell 2004).

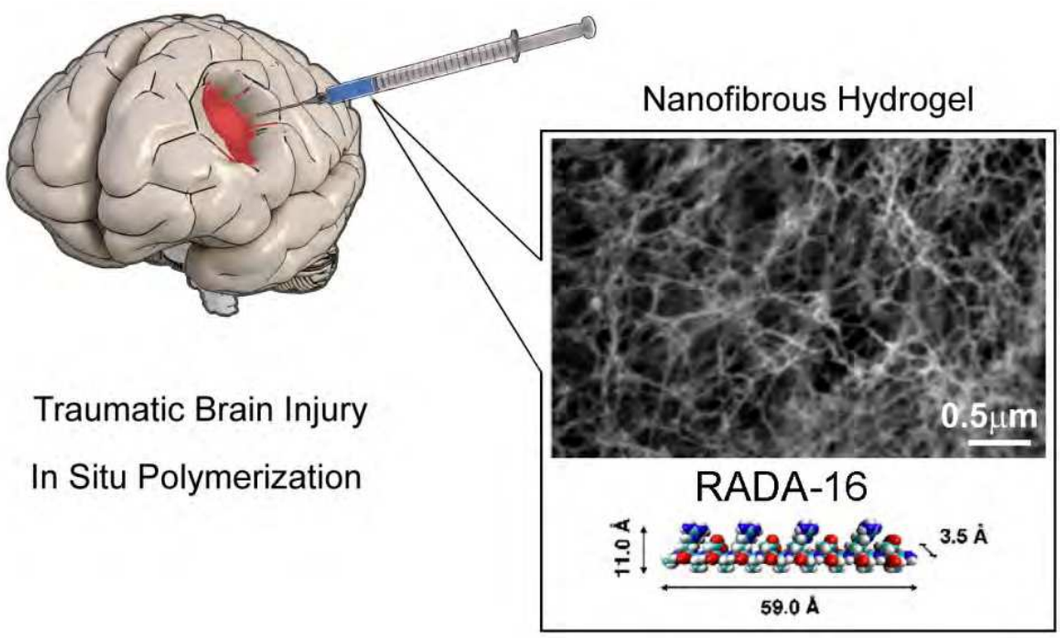

Fig. 5. *In situ* hydrogel polymerization for instances where significant soft tissue damage occurs. Inset shows the nanofibrillar arrangement of RADA-16 peptide amphiphiles. Reprinted with permission (Nagai, Unsworth et al. 2006).

Peptide amphiphiles (PA) are common building blocks for use in self assembly. This approach was first used to fabricate membranes composed of nanofibrillar meshes based on repeating amino acid ((Ala-Glu-Ala-Glu-Ala-Lys-Ala-Lys)$_2$) sequences (Zhang, Holmes et al. 1993). Recently, the same group developed an arginine, alanine, aspartate, and alanine (RADA-16) sequence that assembled into three-dimensional hydrogels composed of

nanofibrils (Figure 5). The primary base sequences were additionally functionalized with an osteogenic growth peptide and two other cell adhesion motifs via direct solid phase extension of the C-termini (Horii, Wang et al. 2007).
Similarly, Silva et al. incorporated a pentapeptide amino acid sequence (isolucinelysine-valine-alanine-valine, IKVAV onto a hydrocarbon tail (Silva, Czeisler et al. 2004). The IKVAV epitope is found in laminin protein and enhances neural cell adhesion and growth (Chen 2003). A special glutamine residue was added to give the amphiphile a net negative charge at physiologic pH. Exposure to positive charges, such as from the cell medium or body fluid would trigger the polymerization event, causing the amphiphile to form a network of nanofibers. Other attributes of individual nanorods include high aspect ratios (micrometer length vs 5-8 nanometer diameter) and high IKVAV epitope density for cell interaction. Other designer hydrogels based on peptide amphiphile self assembly have also been created. Unique characteristics of hydrogels include high water content (99%), compatibility with *in situ* application and resemblance to natural ECM.

1.4 Application of Biomimetic Architectures

1.4.1 Vasculature

Blood vessels are a major constituent of the human cardiovascular system and function as a vehicle for metabolic and gaseous transport. In arteries, three layers exist. The outmost wall consists of fibroblasts embedded within a randomly aligned collagen type I matrix for structural support. Smooth muscle cells (SMC) surrounded by elastin fibers and collagen types I and III compose the elastic medial zone. Finally, the innermost layer is an endothelial (EC) monolayer rich in collagen type IV and elastin that provides a smooth surface for blood flow.
Atherosclerosis is a common cardiovascular disease characterized by the pathological reduction in arterial elasticity and narrowing of the arteries. Decreased diameter of the interior lumen subsequently leads to loss of circulation. Once blood flow is compromised, the preferred treatment option is vascular bypass surgery. Autologous replacement of damaged arteries frequently involves the internal mammary artery or the saphenous vein. However, such treatments yield donor site morbidity and are often unable to bridge large diameter vessels (Conte 1998). Alternative options using traditional synthetic grafts, including expanded polytetrafluoroethylene (e-PTFE) and woven polyethylene terephthalate (PET) fibers are suitable for medium (6-10mm) and large (>10 mm) vascular substitutes, but are not successful as small diameter prosthetics (Ma, Kotaki et al. 2005). Development of a small-diameter vascular graft capable of withstanding the immense environmental pressures of the circulatory system presents a significant challenge. Conquering this remaining obstacle is often seen as the Holy Grail of tissue engineering (Conte 1998).

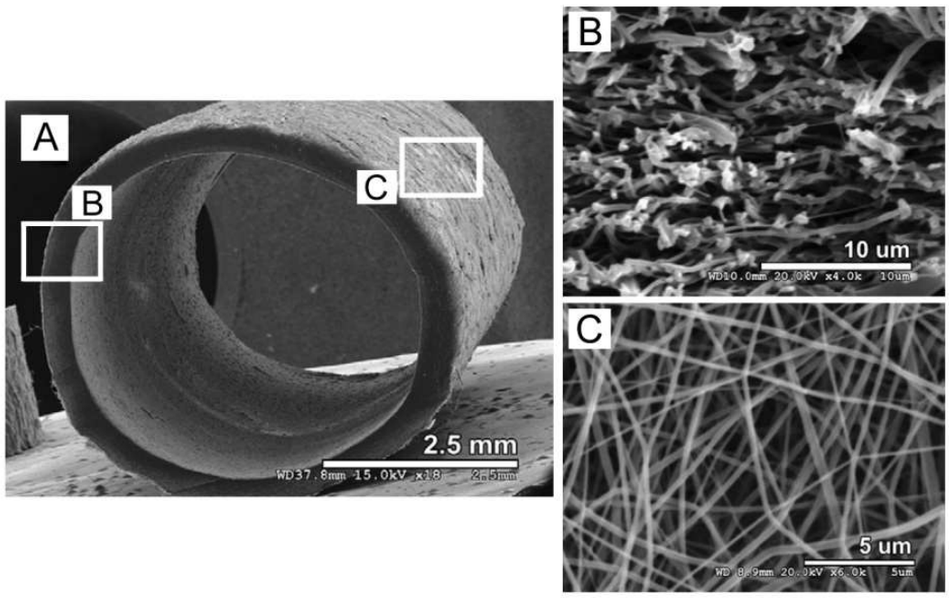

Fig. 6. Tissue engineered vascular grafts. (A) Gross overview of an electrospun composite scaffold composed of collagen type I and poly(ε-caprolactone) (PCL). (B) SEM image of the circumferential morphology (x4.0K) and (C) Exterior surface (x6.0K) (Lee, Liu et al. 2008).

New advancements in nanofiber-based scaffold production (i.e. electrospinning, phase separation and self-assembly) allow for the creation of grafts that imitate the molecular and structural properties of the vasculature ECM. For instance, Ramakrishna et al. developed a synthetic biodegradable aligned poly(L-lactide-co-ε-caprolactone) [PLLA-CL] nanofibrous scaffold(Mo, Xu et al. 2004; Xu, Inai et al. 2004; Xu, Inai et al. 2004). Aligned fibers produced a scaffold topography that mimicked the circumferential orientation of cells and fibrils found in the medial layer of arteries. When cultured with SMC and ECs, the scaffold promoted cell attachment and migration along the axis of the aligned nanofibers and demonstrated increased levels of cell proliferation when compared to an inert control. Integration of cells within the nanofibers formed a three-dimensional cellular network while maintaining phenotypic shape (Mo, Xu et al. 2004; Xu, Inai et al. 2004; Xu, Inai et al. 2004). Alternatively, Boland et al. produced a natural, three-layered vascular construct composed of collagen and elastin. Outer layers of the construct were seeded with SMC and FB with the interior lumen seeded with ECs. This cell seeded scaffold allows for natural tissue development in the injured site by promoting cell motility, proliferation and matrix deposition/remodeling (Matthews, Wnek et al. 2002). Figure 6 demonstrates a composite vascular system composed of poly(ε-caprolactone) (PCL) and collagen. The composite scaffolds exhibited adequate tensile strength (4±0.4 MPa), and elasticity (2.7±1.2MPa) for appropriate physiological performance. Composite scaffolds aided the formation of a confluent outer layer and interior lumen when seeded with smooth muscle cells and endothelial cells, respectively (Lee, Liu et al. 2008).

Similar tri-laminate vascular constructs were developed using cell self-assembly as reported by L'Heureux et. Al (L'Heureux, Paquet et al. 1998; L'Heureux, McAllister et al. 2007). Intact layers of human vascular cells were cultured past confluency to form a uniform cell sheet with a naturally produced randomly aligned ECM. The sheet was then rolled over a support

mandrel to create a tubular arrangement. An outermost layer was constructed in the same fashion by placing a sheet of fibroblasts on top of the previous SMC layer. Further maturation of the tube allowed for prolific ECM production before the interior lumen was seeded with ECs (L'Heureux, Paquet et al. 1998; L'Heureux, McAllister et al. 2007).

1.4.2 Ligament and Tendon Tissue Engineering

Ligaments are comprised of dense, parallel collagen (types I and III) bundles that serve as load bearing tissues. Fibroblasts preferentially aligned along the longitudinal axis of the crosslinked bundles regulate mechanical attributes through matrix remodeling. The high tensile strength of this configuration and elastic properties of the collagen fibrils allow for controlled movement and joint stability.

Mechanical trauma disrupting the integrity of the ligament produces significant joint dysfunction resulting in abnormal kinematics and potentially long term degenerative joint diseases (Lin, Lee et al. 1999). Conventional reconstructive surgical procedures using autografts have had limited success (Arnoczky, Tarvin et al. 1982). Major shortfalls include donor site morbidity, stress shielding and tendonitis (Lee, Shin et al. 2005).

Ligament tissue engineering is a viable alternative to surgical approaches. However, several challenges remain in the realization of scaffolds capable of withstanding the large cyclic loads. Nanofiber manipulation techniques provide promising solutions by replicating the natural orientation and spatial distribution of molecules and cells within an engineered construct.

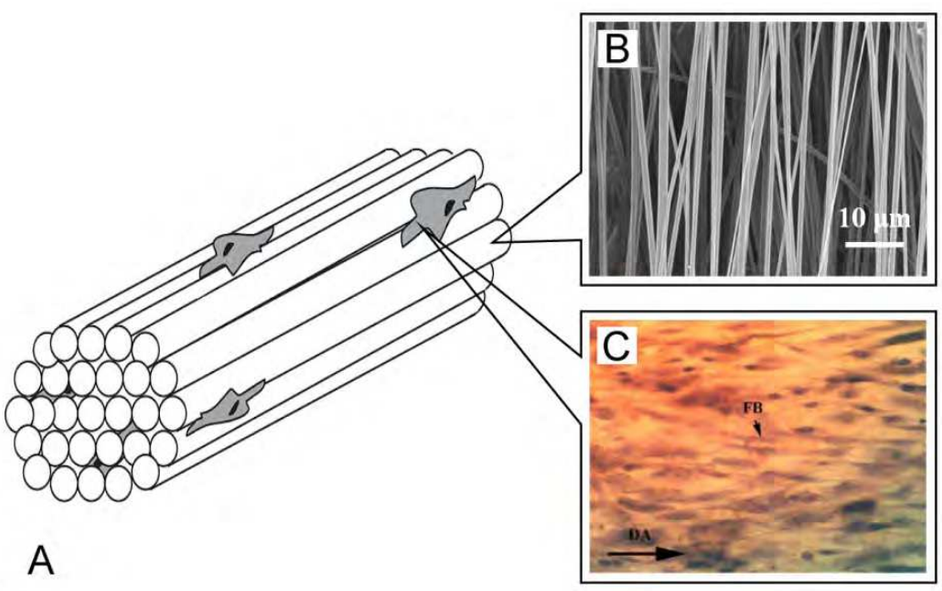

Fig. 7. Ligament Engineering (A) Ligament schema showing parallel fibers with adhered fibroblasts (Doroski, Brink et al. 2007). (B) SEM micrograph of aligned PU fibers (500 to 800 nm) (C) cultured with fibroblasts (FB) indicating the direction of alignment (DA) along aligned fibers (Lee, Shin et al. 2005).

For example, using a modified electrospinning process, Lee et al. collected polyurethane (PU) nanofibers on a rotating collecting target to produce a uniformly aligned matrix as

shown in Figure 7. Fibers were then seeded with human ligament fibroblasts (HLFs) to investigate the affects of fiber alignment on cell behavior. Results demonstrated cellular alignment with fiber orientation and increased matrix deposition under longitudinal load compared to HLFs seeded on randomly oriented fibers (Lee, Shin et al. 2005).

An alternative method for controlled collagen assembly was proposed by Akkus and collaborators. The novel electrochemical technique passed electric current through a dialyzed collagen solution placed between two parallel electrodes to promote molecular migration. This process allowed for the generation of highly oriented and densely packed elongated collagen bundles ranging between 50-400μm in diameter and 3-7cm in length. Polarized imaging was used to assess collagen orientation and fibrillar assembly as shown in Figure 8. Electrochemically oriented collagen bundles showed color patterning similar to natural tendon fibers, indicating a comparable degree of fiber orientation. Furthermore, the synthetically aligned fibers exhibited a 30-fold increase in mechanical strength compared to randomly oriented collagen fibers. Mechanically robust and highly oriented bundles formed using this method highlight a promising tissue engineering approach for tendon and ligament repair (Cheng, Gurkan et al. 2008).

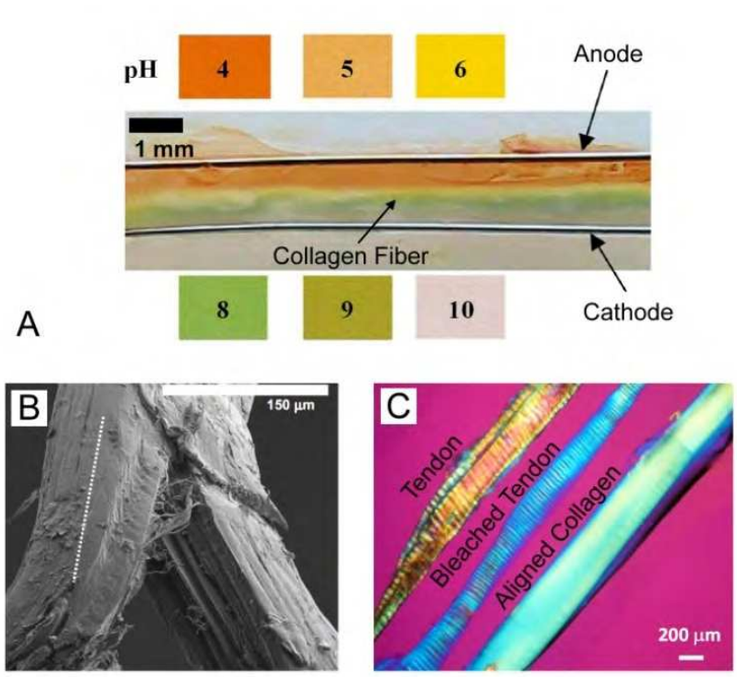

Fig. 8. Synthetic alignment of collagen fibers using an electrochemical technique. (A) Electrochemical cell with collagen solution between two parallel electrodes and color coded pH gradient required for rotational alignment. (B) SEM image of an intentionally split collagen bundle, indicating uniform orientation and alignment along the longitudinal axis (C) Polarized image of bleached native tendon and electrochemically aligned collagen construct. Both tissues depict the blue retardation coloring, indicating comparable fiber orientation (Cheng, Gurkan et al. 2008).

1.4.3 Skin

Skin is the largest organ in the body and consists of two main layers: dermis and epidermis. The keratinized stratified epidermis is the primary cell layer while the dermal zone provides structural support and nutrient renewal. Skin engineering has a history spanning 100 years yet autografts remain the gold standard for treating full thickness skin injuries. A design goal for therapeutic skin substitutes resides in emulating the skin's stratified composition. Since the dermal layer is a dense network of collagen and elastin, porous fibrillar constructs make excellent candidates as a substrate for skin reconstruction. Indeed, this approach has been used since the 1970's as the basis for artificial skin (Yannas and Burke 1980). The first skin constructs used a scaffold composed of a collagen base with added glycosaminoglycans to promote wound healing (Schulz, Tompkins et al. 2000). An additional silicone membrane ("pseudo-epidermis") was overlaid on the collagen layer to function as a heat and moisture barrier. Variations in this bilayered theme are presently employed in skin graft products (Falanga and Sabolinski 1999).

However, a significant drawback in artificial skin is the lack of vasculature, pigmentation and anti-bacterial properties. To circumvent these deficiencies, scientists are using either stem cells or genetically modified cells in combination with biomatrices. The goal is to develop artificial skin fragments that are vascularised and have the ability to ward off infection, sweat and protect against UV radiation. In one report, researchers transfected keratinocytes to secrete an antimicrobial protein (Smiley, Gardner et al. 2007). Bacterial growth was suppressed on these skin cultures, which suggests genetic manipulation to be a viable method for reducing post-operative infection. Alternatively, Katti et al. employed electrospinning to produce nanofibrous poly(lactide-coglycolide) membranes incorporating a broad spectrum antibiotic (Katti, Robinson et al. 2004).

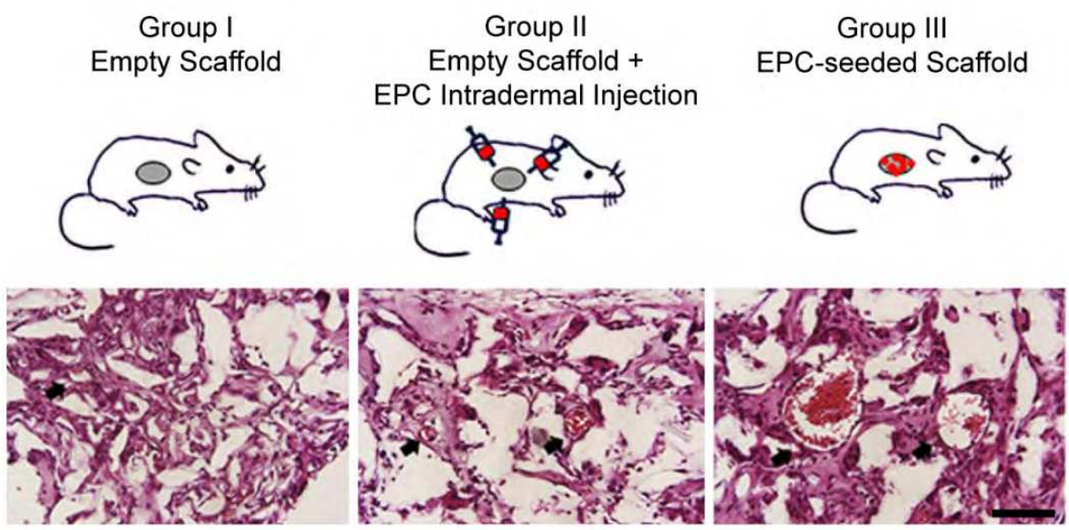

Fig. 9. Schematic showing the presence of endothelial progenitor cells in a mouse model of skin trauma (Kim, Han et al. 2009). Empty (cell free) scaffolds showed some endogenous remodeling and blood vessel formation. Injection of EPCs improved the degree of vascularization while pre-seeded scaffolds provided the best results. Reprinted with permission.

To recreate other salient features of skin, research groups have suggested using bulb stem cells residing in hair follicles to reproduce epidermis (Larouche, Tong et al. 2008). Similarly, Kim et al harvested endothelial progenitor cells (EPCs) for use in a mouse skin excision injury model (Figure 9). Experimental data showed EPC seeded scaffolds promoted angiogenesis and microvasculature at the wound site (Kim, Han et al. 2009). Newer developments in skin technology have added combinations of fibroblasts, keratinocytes or epithelial cells into biodegradable scaffolds (Priya, Jungvid et al. 2008; Schulz, Tompkins et al. 2000). After a designated culture time, the cells populate and remodel the matrix, creating a rudimentary living skin. Other studies on substrate architecture shed light on optimum fibril diameters and packing density for enhancing collagen expression (Kumbar, Nukavarapu et al. 2008) microbial infiltration (Powell and Boyce 2008).
The collective cellular and scaffolding data suggests a promising future for patients with severe skin damage. The combination of appropriately modified physical substrates coupled with cell seeding may lead to skin products that provide necessary protection while simultaneously reducing the appearance of scar (Metcalfe and Ferguson 2007).

1.4.4 Nervous system

Neurons are highly ordered cells specializing in a variety of integrative and signal transmission functions. When damaged, neurons have the intrinsic ability to regenerate, although the degree of regeneration is heavily dependent upon the local microenvironment. In the central nervous system (CNS) damaged neurons undergo a form of abortive regeneration. Multiple avenues are being pursued to coax adult neurons to recapitulate developmental processes (Rossignol, Schwab et al. 2007; Ruff, McKerracher et al. 2008). In contrast, peripheral nervous system (PNS) axons readily sprout and extend after injury. However, the quality of regeneration is lacking due to insufficient guidance cues (Lundborg 2000). It has been postulated that in addition to biochemical signals, physical guidance may further aid and direct the regeneration process.
For treating brain and spinal cord injuries hydrogels have been proposed. Hydrogels form a loose network of fibers representative of the endogenous CNS matrix. For example, Silva et al. (Silva, Czeisler et al. 2004), used a pentapeptide IKVAV sequence mounted onto a hydrophobic tail to create self assembling peptide amphiphiles. These amphiphiles directed differentiation of neural progenitor cells while inhibiting astrocyte differentiation *in vitro*. Additional investigation using a spinal cord compression model showed IKVAV nanofibers reduced glial scar formation, cell death and encouraged migration of oligodendrocytes into the implant region (Tysseling-Mattiace, Sahni et al. 2008).
In another study, peptide amphiphiles consisting of RADA16 were used to successfully restore vision in an optic nerve guinea pig animal model (Ellis-Behnke, Liang et al. 2006). In this case, the peptide hydrogel created a permissive environment that promoted neural tissue bridging of severed tracts. Other studies with hydrogels have shown promising outcomes. Stokols et al. reported a unique agarose guidance scaffold consisting of long linear channels (Stokols and Tuszynski 2004). These constructs had pore diameters (channels) of 119±26μm and were capable of sustained growth factor release. Follow-up assessment of the scaffolds in a spinal cord injury paradigm showed organized axonal regeneration through the agarose channels (Stokols and Tuszynski 2006).
In contrast to the brain, nerves within the PNS have a unique global structural arrangement. Peripheral nerve axons are longitudinally oriented, with individual axons ensheathed in

collagen based tubes (endoneurial tube). Following axotomy, these continuous tubes facilitate the regeneration process by providing a physical template that directs axon extension. Imitating this structural feature is therefore vital for encouraging outgrowth and reducing fiber misdirection (Sumner 1990).

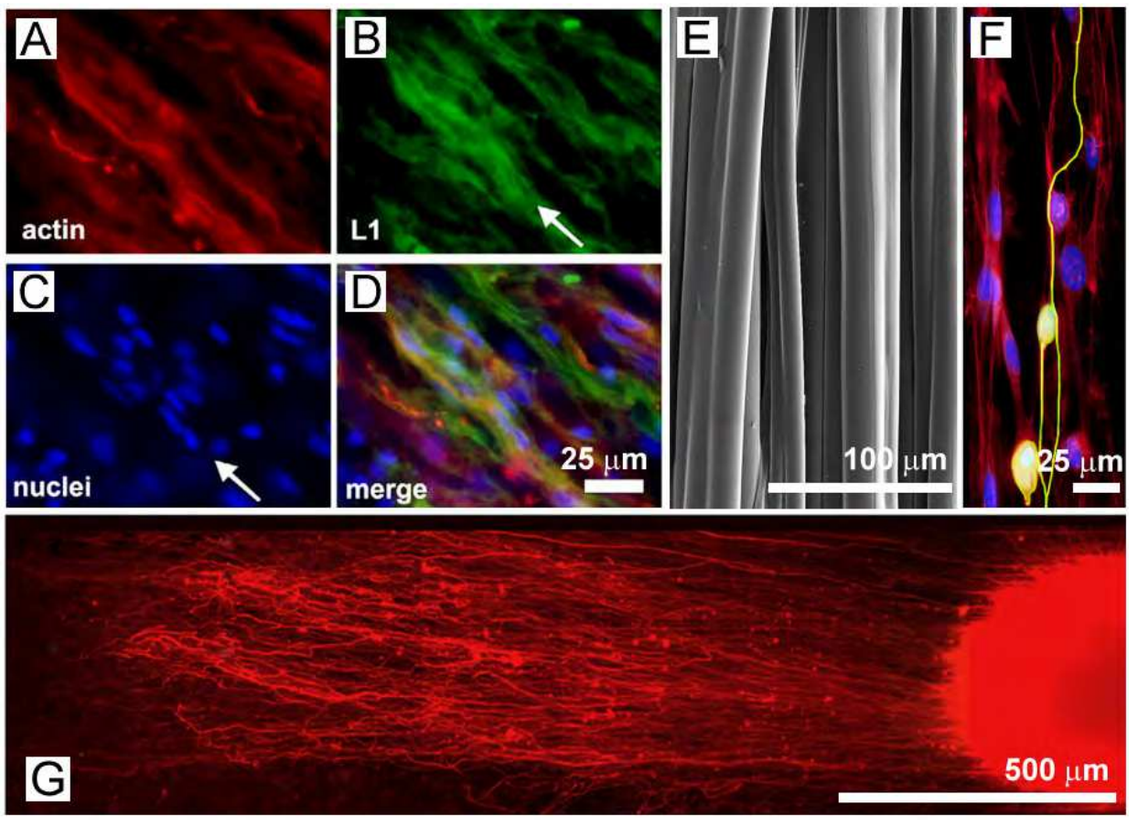

Fig. 10. (A)-(D) Fluorescent longitudinal sections of rat sciatic nerve and the structural arrangement. Reprinted from (Ribeiro-Resende, Koenig et al. 2009). (E) Microtubular polymeric arrays which mimic the oriented nature of peripheral nerves. (F) Dorsal root ganglia neurons and Schwann cells co-cultures align on tubules depicted in (E). Reprinted with permission from (Li, Rickett et al. 2009). (G) Dorsal root ganglia neurons extend along electrospun nanofibers (Kim, Haftel et al. 2008).

Conduits containing aligned electrospun fibers have been used to mimic the function of endoneurial tubes (Figure 10). For instance, Kim et al. created a 17mm gap defect in rat sciatic nerve and used an artificial conduit to bridge the damage site (Kim, Haftel et al. 2008). Acrylonitrile-co-methylacrylate (PAN-MA) polymer was spun into aligned or unaligned configurations. Both types of conduits were implanted and after 16 weeks, animals were sacrificed for analysis. Histological and functional data showed superior axonal regeneration in animals receiving the aligned fiber conduits. This study showed that for the PNS, aligned fiber morphology can be used to facilitate the regeneration process. Moreover, Li et al. used microtubular arrays to emphasize oriented Schwann cell and neurite alignment *in vitro* (Li, Rickett et al. 2009). The microtubes supported cell growth on the exterior surface and within the lumen. Other investigations have demonstrated

improved regeneration on polymer filaments in long gap injuries with rats (Cai, Peng et al. 2005).

1.5 Future Directions and Conclusion

Bioengineering has evolved from the simple notion of biocompatibility to an age which values intelligent materials for providing cell-specific instructional cues. One of these signaling mechanisms is physical and is intimately tied to cell mechanotransduction. Indeed, the local spatial arrangement of the extracellular environment plays a pivotal role in governing morphogenesis, migration and differentiation. Thus, understanding how nano and microscale architectures influence cell response and the subsequent macroscopic properties is fundamental in developing restorative constructs.

Recent manufacturing innovations have yielded tissue analogs approximating the natural ECM at multiple length scales. However, significant challenges remain. In terms of the three-dimensional aspect, most artificial scaffolds are relatively homogeneous and do not represent the stratified or graded physiochemical properties found *in vivo*. Therefore, a design challenge for future 3-D structures lies in implementation of zonal transitions. Such scaffolds would encourage heterotypic cell interactions essential to the constructive remodeling process. Additionally, we now recognize that the ECM is more than a framework for cell residence. Natural ECM contains multiple support proteins and biochemical factors that maintain local dynamic homeostasis, reinforce mechanical properties and direct wound healing (Badylak 2007). Incorporating such compounds synergistically within the material architecture is critical for optimum success.

Finally, stem cells and biomolecular delivery are inevitably going to play a larger role in regenerative medicine. Current investigations with stem cells have already demonstrated promise in various experimental injury models. New insights into controlling stem cell fate are necessary in realizing safe and effective therapies. Nonetheless, the confluence of cellular methods with biomimetic architectures bodes well for functional restoration of damaged or diseased tissues.

Acknowledgments

We would like to thank Michel Schweinsberg for his contributions in artwork.

2. References

Arnoczky, S. P., Tarvin, G. B. & Marshall, J. L. (1982). Anterior Cruciate Ligament Replacement Using Patellar Tendon - an Evaluation of Graft Revascularization in the Dog. *Journal of Bone and Joint Surgery-American Volume* **64**(2): 217-224.

Badylak, S. F. (2007). The extracellular matrix as a biologic scaffold material. *Biomaterials* **28**(25): 3587-93.

Bellan, L. M., Singh, S. P., Henderson, P. W., Porri, T. J., Craighead, H. G. & Spector, J. A. (2009). Fabrication of an artificial 3-dimensional vascular network using sacrificial sugar structures. *Soft Matter* **5**: 1354-1357.

Cai, J., Peng, X., Nelson, K. D., Eberhart, R. & Smith, G. M. (2005). Permeable guidance channels containing microfilament scaffolds enhance axon growth and maturation. *J Biomed Mater Res A* **75**(2): 374-86.

Chen, R. N., Ho, H. O., Tsai, Y. T. & Sheu, M. T. (2004). Process development of an acellular dermal matrix (ADM) for biomedical applications. *Biomaterials* **25**(13): 2679-86.

Chen, Z., Strickland, S. (2003). Laminin gamma1 is critical for Schwann cell differentiation, axon myelination, and regeneration in the peripheral nerve. *J Cell Biol* **163**(4): 889-899.

Cheng, X. G., Gurkan, U. A., Dehen, C. J., Tate, M. P., Hillhouse, H. W., Simpson, G. J. & Akkus, O. (2008). An electrochemical fabrication process for the assembly of anisotropically oriented collagen bundles. *Biomaterials* **29**(22): 3278-3288.

Conte, M. S. (1998). The ideal small arterial substitute: a search for the Holy Grail? *Faseb Journal* **12**(1): 43-45.

Doroski, D. M., Brink, K. S. & Temenoff, J. S. (2007). Techniques for biological characterization of tissue-engineered tendon and ligament. *Biomaterials* **28**(2): 187-202.

Doshi, J. & Reneker, D. H. (1995). Electrospinning process and applications of electrospun fibers. *J. Electrost.* **35**: 151.

Ellis-Behnke, R. G., Liang, Y. X., You, S. W., Tay, D. K., Zhang, S., So, K. F. & Schneider, G. E. (2006). Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision. *Proc Natl Acad Sci U S A* **103**(13): 5054-9.

Falanga, V. & Sabolinski, M. (1999). A bilayered living skin construct (APLIGRAF) accelerates complete closure of hard-to-heal venous ulcers. *Wound Repair Regen* **7**(4): 201-7.

Frank, C. B. (2004). Ligament structure, physiology and function. *J Musculoskelet Neuronal Interact* **4**(2): 199-201.

Horii, A., Wang, X., Gelain, F. & Zhang, S. (2007). Biological designer self-assembling Peptide nanofiber scaffolds significantly enhance osteoblast proliferation, differentiation and 3-D migration. *PLoS One* **2**(2): e190.

Ingber, D. E. (2008). Tensegrity-based mechanosensing from macro to micro. *Prog Biophys Mol Biol* **97**(2-3): 163-79.

Jayasinghe, S. N., Irvine, S. & McEwan, J. R. (2007). Cell electrospinning highly concentrated cellular suspensions containing primary living organisms into cell-bearing threads and scaffolds. *Nanomed* **2**(4): 555-67.

Jiang, H., Hu, Y., Li, Y., Zhao, P., Zhu, K. & Chen, W. (2005). A facile technique to prepare biodegradable coaxial electrospun nanofibers for controlled release of bioactive agents. *J Control Release* **108**(2-3): 237-43.

Katti, D. S., Robinson, K. W., Ko, F. K. & Laurencin, C. T. (2004). Bioresorbable nanofiber-based systems for wound healing and drug delivery: optimization of fabrication parameters. *J Biomed Mater Res B Appl Biomater* **70**(2): 286-96.

Kim, K. L., Han, D. K., Park, K., Song, S. H., Kim, J. Y., Kim, J. M., Ki, H. Y., Yie, S. W., Roh, C. R., Jeon, E. S., Kim, D. K. & Suh, W. (2009). Enhanced dermal wound neovascularization by targeted delivery of endothelial progenitor cells using an RGD-g-PLLA scaffold. *Biomaterials* **30**(22): 3742-8.

Kim, Y. T., Haftel, V. K., Kumar, S. & Bellamkonda, R. V. (2008). The role of aligned polymer fiber-based constructs in the bridging of long peripheral nerve gaps. *Biomaterials* **29**(21): 3117-27.

Kumbar, S. G., Nukavarapu, S. P., James, R., Nair, L. S. & Laurencin, C. T. (2008). Electrospun poly(lactic acid-co-glycolic acid) scaffolds for skin tissue engineering. *Biomaterials* **29**(30): 4100-7.

L'Heureux, N., McAllister, T. N. & de la Fuente, L. M. (2007). Tissue-engineered blood vessel for adult arterial revascularization. *New England Journal of Medicine* **357**(14): 1451-1453.

L'Heureux, N., Paquet, S., Labbe, R., Germain, L. & Auger, F. A. (1998). A completely biological tissue-engineered human blood vessel. *Faseb Journal* **12**(1): 47-56.

Larouche, D., Tong, X., Fradette, J., Coulombe, P. A. & Germain, L. (2008). Vibrissa hair bulge houses two populations of skin epithelial stem cells distinct by their keratin profile. *FASEB J* **22**(5): 1404-15.

Lee, C. H., Shin, H. J., Cho, I. H., Kang, Y. M., Kim, I. A., Park, K. D. & Shin, J. W. (2005). Nanofiber alignment and direction of mechanical strain affect the ECM production of human ACL fibroblast. *Biomaterials* **26**(11): 1261-1270.

Lee, J. Y., Bashur, C. A., Goldstein, A. S. & Schmidt, C. E. (2009). Polypyrrole-coated electrospun PLGA nanofibers for neural tissue applications. *Biomaterials* **30**(26): 4325-35.

Lee, S. J., Liu, J., Oh, S. H., Soker, S., Atala, A. & Yoo, J. J. (2008). Development of a composite vascular scaffolding system that withstands physiological vascular conditions. *Biomaterials* **29**(19): 2891-2898.

Li, D., Wang, Y. & Xia, Y. (2004). Electrospinning Nanofibers as Uniaxially Aligned Arrays and Layer-by-Layer Stacked Films. *Adv. Mater.* **16**: 361-366.

Li, J., Rickett, T. A. & Shi, R. (2009). Biomimetic nerve scaffolds with aligned intraluminal microchannels: a "sweet" approach to tissue engineering. *Langmuir* **25**(3): 1813-7.

Lin, V. S., Lee, M. C., O'Neal, S., McKean, J. & Sung, K. L. P. (1999). Ligament tissue engineering using synthetic biodegradable fiber scaffolds. *Tissue Engineering* **5**(5): 443-451.

Lundborg, G. (2000). A 25-year perspective of peripheral nerve surgery: evolving neuroscientific concepts and clinical significance. *J Hand Surg Am* **25**(3): 391-414.

Ma, P. X. & Zhang, R. (1999). Synthetic nano-scale fibrous extracellular matrix. *J Biomed Mater Res* **46**(1): 60-72.

Ma, Z. W., Kotaki, M., Yong, T., He, W. & Ramakrishna, S. (2005). Surface engineering of electrospun polyethylene terephthalate (PET) nanofibers towards development of a new material for blood vessel engineering. *Biomaterials* **26**(15): 2527-2536.

Matthews, J. A., Wnek, G. E., Simpson, D. G. & Bowlin, G. L. (2002). Electrospinning of collagen nanofibers. *Biomacromolecules* **3**(2): 232-238.

McCann, J. T., Marquez, M. & Xia, Y. (2006). Melt coaxial electrospinning: a versatile method for the encapsulation of solid materials and fabrication of phase change nanofibers. *Nano Lett* **6**(12): 2868-72.

Metcalfe, A. D. & Ferguson, M. W. (2007). Tissue engineering of replacement skin: the crossroads of biomaterials, wound healing, embryonic development, stem cells and regeneration. *J R Soc Interface* **4**(14): 413-37.

Mo, X. M., Xu, C. Y., Kotaki, M. & Ramakrishna, S. (2004). Electrospun P(LLA-CL) nanofiber: a biomimetic extracellular matrix for smooth muscle cell and endothelial cell proliferation. *Biomaterials* **25**(10): 1883-1890.

Nagai, Y., Unsworth, L. D., Koutsopoulos, S. & Zhang, S. (2006). Slow release of molecules in self-assembling peptide nanofiber scaffold. *J Control Release* **115**(1): 18-25.

Ortiz, G. (2009). Nanocontacts: The importance of being entangled. *Nat Mater* **8**(7): 541-2.

Pham, Q. P., Sharma, U. & Mikos, A. G. (2006). Electrospinning of polymeric nanofibers for tissue engineering applications: a review. *Tissue Eng* **12**(5): 1197-211.

Powell, H. M. & Boyce, S. T. (2008). Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal-epidermal skin substitutes. *J Biomed Mater Res A* **84**(4): 1078-86.

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

Priya, S. G., Jungvid, H. & Kumar, A. (2008). Skin tissue engineering for tissue repair and regeneration. *Tissue Eng Part B Rev* **14**(1): 105-18.

Ribeiro-Resende, V. T., Koenig, B., Nichterwitz, S., Oberhoffner, S. & Schlosshauer, B. (2009). Strategies for inducing the formation of bands of Bungner in peripheral nerve regeneration. *Biomaterials* **30**(29): 5251-9.

Rossignol, S., Schwab, M., Schwartz, M. & Fehlings, M. G. (2007). Spinal cord injury: time to move? *J Neurosci* **27**(44): 11782-92.

Ruff, R. L., McKerracher, L. & Selzer, M. E. (2008). Repair and neurorehabilitation strategies for spinal cord injury. *Ann N Y Acad Sci* **1142**: 1-20.

Rutledge, G. C. & Fridrikh, S. V. (2007). Formation of fibers by electrospinning. *Adv Drug Deliv Rev* **59**(14): 1384-91.

Sands, R. W. & Mooney, D. J. (2007). Polymers to direct cell fate by controlling the microenvironment. *Curr Opin Biotechnol* **18**(5): 448-53.

Schulz, J. T., 3rd, Tompkins, R. G. & Burke, J. F. (2000). Artificial skin. *Annu Rev Med* **51**: 231-44.

Silva, G. A., Czeisler, C., Niece, K. L., Beniash, E., Harrington, D. A., Kessler, J. A. & Stupp, S. I. (2004). Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* **303**(5662): 1352-5.

Singelyn, J. M., DeQuach, J. A., Seif-Naraghi, S. B., Littlefield, R. B., Schup-Magoffin, P. J. & Christman, K. L. (2009). Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering. *Biomaterials* **30**(29): 5409-16.

Smiley, A. K., Gardner, J., Klingenberg, J. M., Neely, A. N. & Supp, D. M. (2007). Expression of human beta defensin 4 in genetically modified keratinocytes enhances antimicrobial activity. *J Burn Care Res* **28**(1): 127-32.

Stephens, J. S., Fahnestock, S. R., Farmer, R. S., Kiick, K. L., Chase, D. B. & Rabolt, J. F. (2005). Effects of electrospinning and solution casting protocols on the secondary structure of a genetically engineered dragline spider silk analogue investigated via Fourier transform Raman spectroscopy. *Biomacromolecules* **6**(3): 1405-13.

Stevens, M. M. & George, J. H. (2005). Exploring and engineering the cell surface interface. *Science* **310**(5751): 1135-8.

Stokols, S. & Tuszynski, M. H. (2004). The fabrication and characterization of linearly oriented nerve guidance scaffolds for spinal cord injury. *Biomaterials* **25**(27): 5839-46.

Stokols, S. & Tuszynski, M. H. (2006). Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. *Biomaterials* **27**(3): 443-51.

Sumner, A. J. (1990). Aberrant reinnervation. *Muscle Nerve* **13**(9): 801-3.

Theron, A., Zussman, E. & Yarin, A. L. (2001). Electrostatic field-assisted alignment of electospun nanofibers. *Nanotechnology* **12**(384): 2001.

Tu, R. S. & Tirrell, M. (2004). Bottom-up design of biomimetic assemblies. *Adv Drug Deliv Rev* **56**(11): 1537-63.

Tysseling-Mattiace, V. M., Sahni, V., Niece, K. L., Birch, D., Czeisler, C., Fehlings, M. G., Stupp, S. I. & Kessler, J. A. (2008). Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. *J Neurosci* **28**(14): 3814-23.

Wang, T., Pan, T. W., Xing, Z. W. & Glowinski, R. (2009). Numerical simulation of rheology of red blood cell rouleaux in microchannels. *Phys Rev E Stat Nonlin Soft Matter Phys* **79**(4 Pt 1): 041916.

Wang, X., Gao, W., Peng, W., Xie, J. & Li, Y. (2009). Biorheological properties of reconstructed erythrocytes and its function of carrying-releasing oxygen. *Artif Cells Blood Substit Immobil Biotechnol* **37**(1): 41-4.

Xie, J., Macewan, M. R., Li, X., Sakiyama-Elbert, S. E. & Xia, Y. (2009). Neurite Outgrowth on Nanofiber Scaffolds with Different Orders, Structures, and Surface Properties. *ACS Nano*.

Xu, C. Y., Inai, R., Kotaki, M. & Ramakrishna, S. (2004). Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering. *Biomaterials* **25**(5): 877-886.

Xu, C. Y., Inai, R., Kotaki, M. & Ramakrishna, S. (2004). Electrospun nanofiber fabrication as synthetic extracellular matrix and its potential for vascular tissue engineering. *Tissue Engineering* **10**(7-8): 1160-1168.

Yannas, I. V. & Burke, J. F. (1980). Design of an artificial skin. I. Basic design principles. *J Biomed Mater Res* **14**(1): 65-81.

Zhang, S., Holmes, T., Lockshin, C. & Rich, A. (1993). Spontaneous assembly of a self-complementary oligopeptide to form a stable macroscopic membrane. *Proc Natl Acad Sci U S A* **90**(8): 3334-8.

IntechOpen

Biomimetics Learning from Nature

Edited by Amitava Mukherjee

ISBN 978-953-307-025-4
Hard cover, 534 pages
Publisher InTech
Published online 01, March, 2010
Published in print edition March, 2010

Nature's evolution has led to the introduction of highly efficient biological mechanisms. Imitating these mechanisms offers an enormous potential for the improvement of our day to day life. Ideally, by bio-inspiration we can get a better view of nature's capability while studying its models and adapting it for our benefit. This book takes us into the interesting world of biomimetics and describes various arenas where the technology is applied. The 25 chapters covered in this book disclose recent advances and new ideas in promoting the mechanism and applications of biomimetics.

How to reference

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

Jianming Li, Sean Connell and Riyi Shi (2010). Biomimetic Architectures for Tissue Engineering, Biomimetics Learning from Nature, Amitava Mukherjee (Ed.), ISBN: 978-953-307-025-4, InTech, Available from: http://www.intechopen.com/books/biomimetics-learning-from-nature/biomimetic-architectures-for-tissue-engineering

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen