Nanofibers and their applications in tissue engineering

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Abstract: Developing scaffolds that mimic the architecture of tissue at the nanoscale is one of the major challenges in the field of tissue engineering. The development of nanofibers has greatly enhanced the scope for fabricating scaffolds that can potentially meet this challenge. Currently, there are three techniques available for the synthesis of nanofibers: electrospinning, self-assembly, and phase separation. Of these techniques, electrospinning is the most widely studied technique and has also demonstrated the most promising results in terms of tissue engineering applications. The availability of a wide range of natural and synthetic biomaterials has broadened the scope for development of nanofibrous scaffolds, especially using the electrospinning technique. The three dimensional synthetic biodegradable scaffolds designed using nanofibers serve as an excellent framework for cell adhesion, proliferation, and differentiation. Therefore, nanofibers, irrespective of their method of synthesis, have been used as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, vascular tissue engineering, neural tissue engineering, and as carriers for the controlled delivery of drugs, proteins, and DNA. This review summarizes the currently available techniques for nanofiber synthesis and discusses the use of nanofibers in tissue engineering and drug delivery applications.

Keywords: electrospinning, phase separation, self-assembly, nanofiber, biomaterial, tissue engineering, scaffold, drug delivery

Introduction

Tissue repair by autologous cell/tissue transplantation is one of the most promising techniques for tissue regeneration. However, autografts are associated with limitations such as donor site morbidity and limited availability. An alternative to autografts is allografts (ie, tissue taken from another subject of the same species). Allografts are not limited in supply; however, they have the potential to cause an immune response and also carry the risk of disease transfer. Tissue engineering has emerged as an excellent approach for the repair/regeneration of damaged tissue, with the potential to circumvent all the limitations of autologous and allogenic tissue repair.

Tissue engineering represents an emerging interdisciplinary field that applies the principles of biological, chemical, and engineering sciences towards the goal of tissue regeneration (Skalak and Fox 1988; Langer and Vacanti 1993; Hoerstrup and Vacanti 2004). Tissue engineering approaches make use of biomaterials, cells, and factors either alone or in combination to restore, maintain, or improve tissue function. The tissue engineering strategy generally involves the isolation of healthy cells from a patient, followed by their expansion in vitro. These expanded cells are then seeded onto a three dimensional (3D) biodegradable scaffold that provides structural support and can also act as a reservoir for bioactive molecules such as growth factors. The scaffold gradually degrades with time to be replaced by newly grown tissue from the seeded cells (Langer and Vacanti 1993).

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Biomaterials play a crucial role in tissue engineering by serving as 3D synthetic frameworks (commonly referred to as scaffolds, matrices, or constructs) for cellular attachment, proliferation, and in growth ultimately leading to new tissue formation. A number of novel approaches have been developed for the fabrication of biomaterial-based 3D scaffolds (Atala and Lanza 2002). More recently, nanofiber-based scaffolding systems are being explored as scaffolds for tissue engineering (Ma and Zhang 1999; Kisiday et al 2002; Li et al 2002).

The development of nanofibers has enhanced the scope for fabricating scaffolds that can potentially mimic the architecture of natural human tissue at the nanometer scale. The high surface area to volume ratio of the nanofibers combined with their microporous structure favors cell adhesion, proliferation, migration, and differentiation, all of which are highly desired properties for tissue engineering applications (Bhattari et al 2004; Ma et al 2005a). Therefore, current research in this area is driven towards the fabrication, characterization, and applications of nanofibrous systems as scaffolds for tissue engineering. Due to their potential, the nanofiber-based systems are also being pursued for a variety of other biological and non-biological applications (Li et al 2002; Wang et al 2002a, 2002b; Nair et al 2004).

This review summarizes the currently available approaches for the fabrication of nanofibers and discusses their application in the engineering of a variety of tissue types.

Methods for nanofiber synthesis

Currently, there are three techniques available for the synthesis of nanofibers: electrospinning, self-assembly, and phase separation. Of these, electrospinning is the most widely studied technique and also seems to exhibit the most promising results for tissue engineering applications. Nanofibers synthesized by self-assembly and phase separation have had relatively limited studies that explored their application as scaffolds for tissue engineering.

Although there are a number of techniques for the synthesis of carbon nanofibers, such as chemical vapor deposition using a template method (Che et al 1998), catalytic synthesis (catalytic deposition, floating catalyst method) (Teo et al 2003), synthesis using radiofrequency-supported microwave plasmas (Cui et al 2000), the description of each of these techniques is beyond the scope of this review. Therefore, for carbon and alumina nanofibers, the discussion is restricted to their applications in tissue engineering.

Electrospinning

Electrospinning represents an attractive technique for the processing of polymeric biomaterials into nanofibers. This technique also offers the opportunity for control over thickness and composition of the nanofibers along with porosity of the nanofiber meshes using a relatively simple experimental setup (Doshi and Reneker 1995; Reneker and Chun 1996; Dzenis 2004; Jayaraman et al 2004).

Although the concept of electrospinning or electrospraying has been known for more than a century, polymeric nanofibers produced by electrospinning have become a topic of great interest only in the past decade (Rayleigh 1882; Doshi and Reneker 1995). The high surface area and high porosity of electrospun nanofibers allow favorable cell interactions and hence make them potential candidates for tissue engineering applications (Li et al 2002; Smith and Ma 2004; Khil et al 2005; Ma et al 2005a).

In the electrospinning process, fibers ranging from 50 nm to 1000 nm or greater (Reneker and Chun 1996; Shin et al 2001a; Fridrikh et al 2003) can be produced by applying an electric potential to a polymeric solution (Hohman et al 2001a, 2001b) (see Figure 1a). The solution is held at the tip of a capillary tube by virtue of its surface tension. The electrical potential applied provides a charge to the polymer solution. Mutual charge repulsion in the polymer solution induces a force that is directly opposite to the surface tension of the polymer solution. An increase in the electrical potential initially leads to the elongation of the hemispherical surface of the solution at the tip of the capillary tube to form a conical shape known as the Taylor cone (Doshi and Reneker 1995; Yarin et al 2001). A further increase causes the electric potential to reach a critical value, at which it overcomes the surface tension forces to cause the formation of a jet that is ejected from the tip of the Taylor cone. The charged jet undergoes instabilities and gradually thins in air primarily due to elongation and solvent evaporation (Zeleny 1914; Reneker et al 2000; Shin et al 2001a, 2001b; Frenot et al 2003). The charged jet eventually forms randomly oriented nanofibers that can be collected on a stationary or rotating grounded metallic collector (Doshi and Reneker 1995; Kameoka and Craighead 2003) (see Figure 1b).

Electrospinning has originated from electrospraying, where an electric charge is provided to a conducting liquid and produces a jet which splits into fine particles that resemble a spray, hence the name electrospraying (Rayleigh 1882; Zeleny 1914). However, when a polymer is used in place of a low-molecular-weight substance for the

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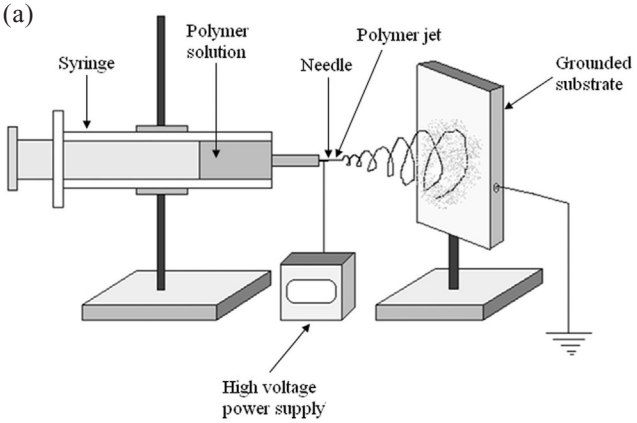

(b)

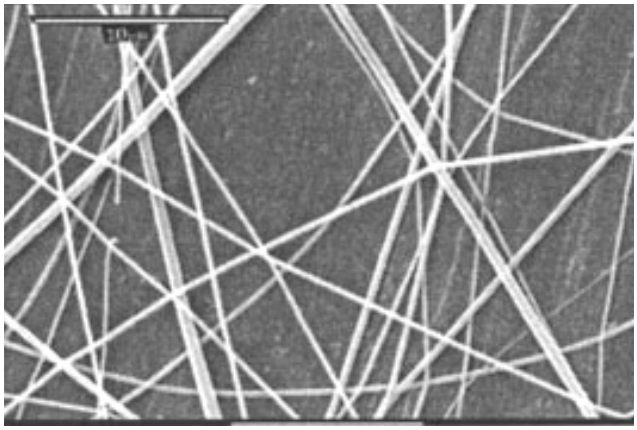

Figure 1 (a) Schematic of the electrospinning process. (b) Scanning electron micrograph of poly(lactic-co-glycolic acid) (PLGA) nanofibers synthesized using the electrospinning technique (scale bar = 10 μm). Source for 1b: Katti DS, Robinson KW, Ko FK, et al. 2004. Bioresorbable nanofiber based systems for wound healing and drug delivery: optimization of fabrication parameters. *J Biomed Mater Res*, 70B:286–96. Copyright © 2004 J Wiley. Reprinted with permission of John Wiley & Sons Inc.

electrospraying process, the long-chain nature of polymers does not allow the splitting of the jet into particles. Instead, the jet undergoes instabilities and thins to form nanofibers. Therefore, one has to use polymers (natural or synthetic) to form nanofibers using the electrospinning/electrospraying technique.

A wide range of polymers has been used to electrospin nanofibers. Natural polymers such as collagen (Huang et al 2001; Matthews et al 2002; Gersbach et al 2004; Shields et al 2004), gelatin (Zhang et al 2005), chitosan (Bhattarai et al 2005; Geng et al 2005), hyaluronic acid (Um et al 2004), and silk fibrion (Jin et al 2002, 2004) have been used to produce nanofibers that can form potential scaffolds for tissue engineering applications. More recently, nanofibers of protein (Li et al 2005; Woerdeman et al 2005) have been demonstrated to have promising use in tissue engineering.

Among the synthetic polymers explored for the fabrication of nanofibers, poly(lactic acid) (PLA) (Yang et al 2004, 2005), polyurethane (PU) (Verreck et al 2003b; Riboldi et al 2005), poly(ε-caprolactone) (PCL) (Reneker et al 2002; Li et al 2003; Li et al 2005c), poly(lactic-co-glycolic acid) (PLGA) (Luu et al 2003; Kim et al 2004; Uematsu et al 2005), poly(ethylene-co-vinylacetate) (PEVA) (Kenawy 2002), and poly(l-lactide-co-ε-caprolactone) (PLLA-CL) (Mo et al 2004; Mo and Weber 2004) have been well studied.

The process of electrospinning is affected by two sets of parameters: system parameters and process parameters. (1) System parameters such as polymer molecular weight and distribution determine the rate of degradation of nanofibers. System parameters such as polymer solution properties, ie, viscosity, surface tension, and conductivity, determine the nanofiber diameter and reduce the possibility of bead formation. (2) Process parameters such as orifice diameter, flow rate of polymer, and electric potential influence fiber diameter. Process parameters such as distance between capillary and metal collector determine the extent of evaporation of solvent from the nanofibers, and deposition on the collector, whereas motion of collector determines the pattern formation during fiber deposition. The systemic and process parameters vary with different polymeric systems and in most cases lend themselves to modification, thereby enabling tailoring of nanofibers for specific end uses (Shin et al 2001b; Zong et al 2002; Fridrikh et al 2003; Katti et al 2004).

During the process of electrospinning, the charge on the polymer solution makes it possible to control its trajectory using an electric field (Hohman et al 2001a, 2001b). This control enables the production of oriented nanofibers that can be useful in the designing of scaffolds for tissue engineering (Sundaray et al 2004; Li et al 2005a). Conventional electrospinning produces nanofibers that are randomly oriented. Recent studies on nanofibers explore the possibility of providing an orientation to the nanofibers. The intent for nanofiber alignment is driven by the desire to direct cell growth and achieve more defined cell growth. This would especially be useful in some tissue engineering applications such as neural tissue engineering, where directional neuronal/axonal growth is desired (Yang et al 2005). Some of the recent studies have tried to achieve nanofiber alignment by making use of a rotating disc with sharpened edges for deposition of nanofibers (Theron et al 2001; Sundaray et al 2004; Lee et al 2005; Yang et al 2005). The sharpened edge provides concentrated amounts of electrostatic force that causes the attraction of ions and deposition of the nanofibers along the edge of the rotating

disc to produce aligned nanofibers. Sundaray et al (2004) demonstrated that several other parameters can influence the alignment of nanofibers, such as reduction in inter electrode distance, higher polymer concentration, and use of single sharp pin as a collecting electrode (Sundaray et al 2004). In another recent study, Li et al (2005a) developed a method for collecting electrospun nanofibers using patterned electrodes. They demonstrated that by introducing insulating gaps on the conductive collector, uniaxially aligned nanofibers can be obtained. In this study, the authors took advantage of the fact that discrete fiber segments tend to align themselves in the direction of minimum net torque to obtain orientation. These studies indicated that alignment and assembly of nanofibers can be altered by varying the design pattern of the collecting electrode.

Along with the advantage of producing nanofiber meshes with high porosity and surface area, the electrospinning technique can be applied to a wide variety of natural and synthetic polymers, making it a very versatile technique. However, this technique is also associated with limitations such as broad range of fiber thickness, random orientation of nanofibers, and low mechanical properties of the fiber meshes. Overall, electrospinning is a relatively robust and simple technique to produce nanofibers from a wide variety of polymers.

Self-assembly

Eukaryotic cells can sense their local environment through cell receptors that recognize their corresponding extracellular tissue markers such as collagen and fibronectin. Therefore, mimicking the extracellular matrix (ECM) using biomaterials would be a logistic approach for engineering of a variety of tissue types. To mimic the human ECM, Berndt et al synthesized a peptide amphiphile (PA)-based self-assembling system with the goal of designing a simple self-assembly system that allows for the formation of thermally stable protein-like molecular architectures. The authors developed PAs that consisted of a dialkyl chain moiety (hydrophobic component/tail group) attached to an N-alpha amino group of a peptide chain (hydrophilic component/head group), resulting in a "peptide amphiphile" (Berndt et al 1995). The peptide head groups were derived from the ECM collagen ligand sequence. The synthesized PA hydrophilic head group consisted of Gly-Val-Lys-Gly-Asp-Lys-Gly-Asn-Pro-Gly-Trp-Pro-Gly-Ala-Pro [IV-H1], which is similar to the human α1 (IV) 1263-1277 collagen sequence. In another study, Yu et al replaced the dialkyl chains of the PA used in the previous study with monoalkyl chains (Berndt et al 1995; Yu et al 1996, 1998; Fields et al 1998). They demonstrated that with an increase in the length of the monoalkyl chain from C_6 to C_{16}, the thermal stability of the PA increased because of the hydrophobic interaction between alkyl chains (Yu et al 1998). Both the dialkyl and monoalkyl chain-based PAs readily self-assembled to form a stabilized triple-helical conformation in an aqueous solvent at the liquid–air interface (Yu et al 1996, 1999).

In a more recent study, Malker et al determined the bioactivity of the PA self-assemblies by incorporating bioactive sequences within the PA. Their results indicated that the formation of the triple-helix for such a PA (ie, containing a bioactive sequence) produced an ordered structure of the bioactive sequence on the exterior of the triple helix that led to a favorable cell response (ie, cell adhesion, spreading, and proliferation) because of the similarity of the self-assembled triple helix to natural ECM. The results of this study and another previous study by Fields et al indicated that these PA structures have potential to be used as surface coatings for biomaterials to improve biocompatibility (Fields et al 1998; Malkar et al 2003).

Based on prior knowledge of PA self-assembling systems (Berndt et al 1995; Stupp et al 1997; Fields et al 1998; Yu et al 1998; Malkar et al 2003), Stupp et al designed di- and tri-block PAs that self-assembled into a rod-like architecture. By engineering the peptide head group of the PA, the authors developed a new technique for the self-assembly of PAs into nanofibers using pH control (Hartgerink et al 2001).

The synthesis of the PA involved the following salient features (Hartgerink et al 2001).

1. Incorporation of phosphoserin residue to enable enhanced hydroxyapatite (HA) mineralization.
2. Incorporation of RGD (Arg-Gly-Asp) peptide to increase integrin-mediated cell adhesion.
3. Incorporation of four consecutive cystine residues, which form inter-molecular disulfide bonds that polymerize to provide improved structural stability.
4. Incorporation of a flexible linker region consisting of three glycine residues to provide flexibility to the head group.

The preparation of nanofibers involved reduction of cystine residues of the PA to free thiol groups using dithiotheritol followed by acidification below pH 4 to cause self-assembly of the PAs into cylindrical micelles/nanofibers. The resulting nanofibers had a hydrophobic core of alkyl residues and a hydrophilic exterior lined by peptide

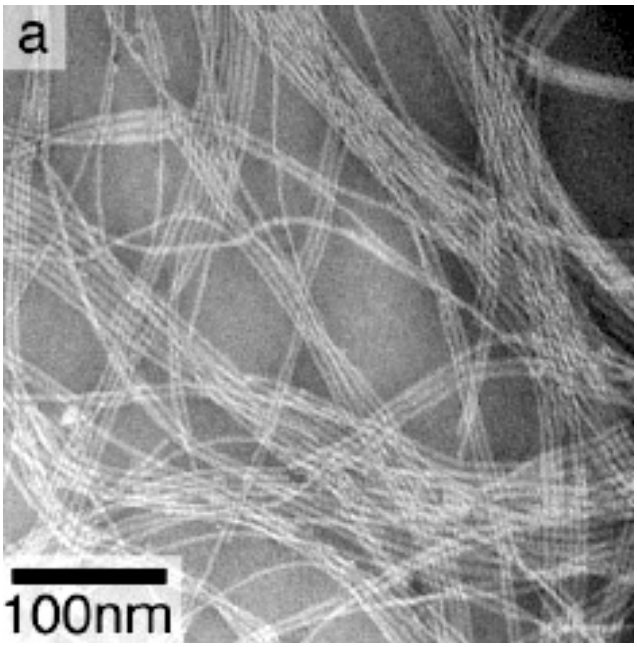

Figure 2 Transmission electron micrograph (TEM) of nanofibers formed from peptide amphiphile molecules (N terminus – $C_{10}H_{19}O$ and Peptide – CCCCGG $GS^{(PO4)}$RGD) that self-assembled by drying directly onto a TEM grid without adjusted pH (negatively stained with phosphotungstic acid). The morphology of the nanofibers was similar to that observed by pH-induced self-assembly. Source: Hartgerink JD, Beniash E, Stupp SI. 2002. Peptide-amphiphile nanofibers: a versatile scaffold for the preparation of self-assembling materials. *Proc Natl Acad Sci U S A*, 99:5133–8. Copyright © 2002 National Academy of Sciences, USA. Reprinted with permission of the National Academy of Sciences, USA.

residues. Their results indicated that the nanofibers produced by self-assembly were approximately 5–8 nm in diameter and several microns in length (see Figure 2). Hartgerink et al further investigated the mineralization potential of these nanofibers. The authors observed the formation of HA crystals that were oriented along the length of the nanofibers. This nanoscale orientation resembles the orientation of HA crystals in mineralized ECM and collagen fibers of bone tissue. Since the mineralized, self-assembled nanofibers were similar to the lowest level of the hierarchical structure of bone tissue, the authors believe that the nanofibers show potential to be used as primary building blocks for the engineering of bone or other mineralization tissue (Hartgerink et al 2001).

In another study, Hartgerink et al (2002) investigated the effect of variations in the molecular structure of the PAs on the self-assembled nanofibers. It was observed that modifications in the alkyl chain length of the PA alter the pH sensitivity of nanofibers, which affects self-assembly. Modification of the C- terminal region (ie, the region that is expressed on the surface of the nanofibers after self-assembly) led to changes in length and stiffness of the nanofibers. Replacement of cystine residues by alanine did not affect the self-assembly of the PAs into nanofibers. These results suggested that the self-assembled nanofibers show potential for development as novel biomaterials (Hartgerink et al 2002; Hwang et al 2002). This study also introduced three different methods of forming self-assembled PAs, including pH-controlled self-assembly, drying on surface-induced self-assembly, and divalent-ion-induced self-assembly. The study demonstrated that PAs can be self-assembled reversibly into nanofibers that result in the formation of gels through pH changes. These PA nanofibers can also be reversibly polymerized to improve their stability. The reversibility of these two procedures makes the self-assembly technique attractive as it enables the fabrication of remarkably versatile materials. In addition, this technique produces a good yield of nanofibers with low polydispersity.

Therefore, the self-assembly technique, by virtue of the modifications possible in the structure of the PA, enables a variety of self-assemblies including layered and lamellar structures, and by virtue of the aforementioned reversibilities lends flexibility to the system. Thus, the self-assembly technique shows good potential for further exploration with the goal of designing novel scaffolds for tissue engineering applications.

Phase separation

The motivation to mimic the 3D structure of collagen present in natural ECM, led Ma and Zhang to develop a new technique called thermally induced liquid–liquid phase separation for the formation of nanofibrous foam materials (Ma and Zhang 1999; Zhang and Ma 2002). The nanofibrous foams produced using the phase separation technique are very similar in size to the natural collagen present in the ECM of tissue in terms of their size (50–500 nm) (see Figure 3). This technique involves five basic steps (Ma and Zhang 1999; Zhang and Ma 2002).

1. Dissolution of polymer.
2. Liquid–liquid phase separation process.
3. Polymer gelation (controls the porosity of nanoscale scaffolds at low temperature).
4. Extraction of solvent from the gel with water.
5. Freezing and freeze-drying under vacuum.

Gelation was found to be the most critical step that controlled the porous morphology of the nanofibrous foams. The duration of gelation varied with polymer concentration and gelation temperature. Low gelation temperature led to the formation of the nanoscale fiber networks, whereas high gelation temperature led to the formation of a platelet-like

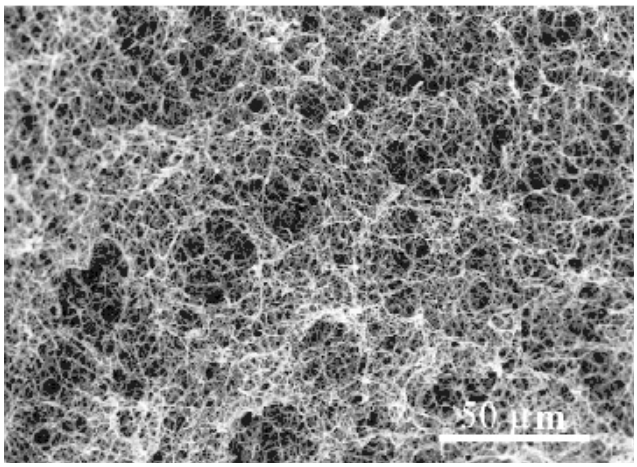

Figure 3 Scanning electron micrograph of poly(l-lactic acid) (PLLA) nanofibrous foam synthesized from 2.5% (wt/v) PLLA/tetrahydrofuran solution at a gelation temperature of 8°C using the phase separation technique (image 500 ×). Source: Ma PX, Zhang R. 1998. Synthetic nano-scale fibrous extracellular matrix. *J Biomed Mater Res*, 46:60–72. Copyright © 1998 J Wiley. Reprinted with permission of John Wiley & Sons Inc.

structure due to the nucleation of crystals and their growth. This limitation of platelet-like structure formation was overcome by increased cooling rates that produced uniform nanofibers. However, the average diameter of fibers was not significantly affected by gelation condition or polymer concentration. Process parameters such as polymer concentration were found to have a significant effect on the nanofiber properties. An increase in polymer concentration decreased porosity and increased mechanical properties (Young's modulus and tensile strength). Other process parameters, such as type of polymer, type of solvent, and thermal treatment also influenced the morphology of the nanofibrous scaffolds (Zhang and Ma 2000).

The 3D porous continuous fibrous network formed by the phase separation process showed high porosity of about 98% within blocks of the material (Ma and Zhang 1999). The authors introduced macroporosity into the scaffold by incorporating porogens such as sugar and salt in the mold along with the polymer solution during phase separation. The macroporosity was introduced with the intent of improving mass transport, cell distribution, and tissue organization. Therefore, scaffolds obtained by this method had three levels of architecture: first, macroporous (~100 μm) wherein the pore size and shape is controlled by porogen; second, interfiber distance, which is determined by polymer concentration; and third, fiber diameter (Zhang and Ma 2000, 2002). The authors then studied these nanofibrous scaffolds for their interaction with osteoblastic cells. The results demonstrated that the nanofibers increased cell adhesion and protein adsorption (fibronectin and collagen), which are properties that are necessary for cell and ECM interaction (Woo et al 2003). The authors attributed the increased cell attachment and distribution in these 3D macroporous scaffolds to their architecture.

The advantage of the phase separation process is that it is a relatively simple procedure and the requirements are very minimal in terms of equipment compared with the previously discussed techniques, electrospinning, and self-assembly. It is possible to directly fabricate the scaffold for a desired anatomical shape of a body part with a mold. Another advantage is the simultaneous presence of nano and macro architecture that can be beneficial in terms of cell response at the nanofiber level, and in terms of cell distribution and tissue architecture at the macroporosity level (Ma and Choi 2001).

Natural polymeric materials for nanofibers

Natural polymers offer the advantage of being very similar, often identical, to macromolecular substances present in the human body. Therefore, the biological environment is prepared to recognize and interact with natural polymers favorably. Some of the natural polymers used as biomaterials are collagen, hyaluronic acid, gelatin, chitosan, elastin, silk, and wheat protein (Yannas 2004).

Collagen is the most popular and well investigated natural biomaterial (Shields et al 2004). Collagen nanofibers (Matthews et al 2002) have been demonstrated to show compatibility with a number of cell types, including myoblasts and chondrocytes (Gersbach et al 2004; Shields et al 2004). In addition, the cross-linking in collagen type II scaffolds provides good mechanical properties, thereby making these scaffolds a suitable environment for cell growth (Shields et al 2004). Huang et al studied the blending of type I collagen nanofibers (produced by electrospinning) with poly(ethylene oxide) (PEO). Their results demonstrated that due to a high number of inter-molecular interactions between collagen and PEO, the mechanical strength of the nanofiber system was significantly increased (Huang et al 2001). These studies illustrated the promising role of collagen in tissue engineering.

Chitosan is another natural biomaterial that has been used to make nanofibers (Geng et al 2005). Nonwoven or aligned chitosan/PEO (90:10) nanofibers have been developed using the electrospinning technique. These nanofibers possessed structural integrity in water and their cell studies demonstrated enhanced attachment of human osteoblasts

and chondrocytes onto the nanofibers. In addition, the cells maintained their characteristic morphology and viability on these nanofibers, thereby indicating good cytocompatibility (Bhattarai et al 2005). Hence, chitosan nanofibers could be a good candidate material for scaffolds in tissue engineering.

Hyaluronic acid is a natural component of the ECM of tissue and has been used as a biomaterial. Um et al (2004) developed nanofibers of hyaluronic acid using the technique of electrospinning and electroblowing (blowing hot air during the process of electrospinning). The authors observed that electrospinning of hyaluronic acid does not allow the consistent production of high-quality nonwoven nanofibers. Therefore, they employed a new technique of electroblowing that was a combination of electrospinning and air flow. In this study, the authors successfully produced nanofibers of hyaluronic acid via electrospinning and by blowing air at 57 °C with a 70 ft/hour flow rate.

Another natural biomaterial that has been well studied is gelatin. Zhang et al (2005) developed gelatin/PCL composite fibrous scaffolds using the electrospinning technique. Their study indicated that the composite nanofibers have improved mechanical strength and wettability compared with gelatin or PCL alone. In addition, the nanofibrous scaffold of gelatin-PCL showed good cell attachment, growth, and migration of bone marrow stromal cells. Therefore, composite nanofibers of natural and synthetic materials could be a good methodology for improving mechanical properties of natural biomaterials for tissue engineering applications.

Silk fibroin is another potential natural biomaterial for nanofibrous scaffolds (Jin et al 2002). Min et al (2004) have reported the in vitro cytocompatibility of silk nanofibers with keratinocytes and fibroblasts. The cytocompatibility, fiber diameter, and high porosity together make it a suitable candidate material for scaffolding technology.

Recent studies by Li et al (2005b) explored the possible usage of electrospun protein fibers as scaffolds for tissue engineering. The authors developed human tropoelastin for electrospinning. The results of this study indicated that tropoelastin nanofibers seeded with human embryonic palatal mesenchymal cells supported cell adhesion and proliferation satisfactorily when compared with nanofibers of collagen or elastin. In another recent study, Woerdeman et al (2005) have explored the possibility of using wheat gluten, a plant protein, as a new material for electrospinning nanofibers that can be used for tissue engineering applications.

Therefore, based on these studies a wide variety of natural polymers have been explored for the synthesis of nanofibers as scaffolds for tissue engineering.

Synthetic polymeric materials for nanofibers

Synthetic polymers represent the largest class of biomaterials (Peter et al 1998; Cooper et al 2004). A wide variety of synthetic polymers has been used to form nanofibers. These include PLA (Tu et al 2003; Yang et al 2004); poly(ethylene terephthalate) (PET) (Ma et al 2005b) for blood vessel tissue engineering; PCL (Li et al 2005c) in neural and cartilage tissue engineering; and several copolymeric compounds such as PLLA-CL as a biomimetic ECM for smooth muscle and endothelial cells (Mo et al 2004; Mo and Weber 2004); PLGA (Katti et al 2004; Uematsu et al 2005), one of the most commonly used polymers to fabricate nanofibers for bone and cartilage tissue engineering and controlled drug delivery; PEVA (Kenawy et al 2002) nanofibers for controlled drug delivery; and PLGA-poly(ethylene glycol) (PLGA-PEG) block copolymeric nanofibrous scaffolds produced via electrospinning as a matrix for DNA delivery (Luu et al 2003). Recently, carbon and alumina nanofibers have been explored as biomaterials for applications in dental and orthopedic implants (Elias et al 2002; Price et al 2003a; Price et al 2003b; Webster et al 2005). Therefore, a large variety of synthetic polymers has been explored for nanofiber synthesis primarily because of the electrospinning technique that easily lends itself to synthetic polymer usage.

Applications of nanofibers in tissue engineering

A variety of methods has been reported previously for the fabrication of scaffolds to be used in tissue engineering (Atala and Lanza 2002). However, in the past decade, nanofibrous systems have been developed and explored as potential scaffolds for tissue engineering (Ma and Zhang 1999; Li et al 2002; Smith and Ma 2004; Ma et al 2005a). By virtue of their high surface area and porosity, they have the potential to provide enhanced cell adhesion and by virtue of the similarity of their 3D architecture to natural ECM, they provide an excellent micro/nano environment for cells to grow and perform their regular functions (Doshi and Reneker 1995; Stupp et al 1997; Zhang and Ma 2000). Therefore, nanofibrous systems have been strongly pursued as scaffolds for tissue engineering applications.

Nanofibers for musculoskeletal tissue engineering

Many materials (natural and synthetic) have been explored as nanofibrous scaffolding materials for bone, cartilage, ligament, and skeletal muscle tissue engineering, including HA (Ramay and Zhang 2003), chitosan (Bhattarai et al 2005), PLGA (Uematsu et al 2005), carbon (Price et al 2003b) and aluminum nanofibers (Webster et al 2005). Although nanofibers have been studied as scaffolds for multiple tissue types, musculoskeletal tissue is probably the most well studied.

Nanofibers for bone tissue engineering

The design of scaffolds for bone tissue engineering is based on the physical properties of bone tissue such as mechanical strength, pore size, porosity, hardness, and overall 3D architecture. For bone tissue engineering, scaffolds with a pore size in the range of 100–350 μm and porosity greater than 90% are preferred for better cell/tissue in-growth and hence enhanced bone regeneration (Bruder and Caplan 2000; Hutmacher 2000).

Yoshimoto et al (2003) developed nonwoven PCL scaffolds by electrospinning for the purpose of bone tissue engineering. To understand the influence of mesenchymal stem cells (MSCs) on nanofibers, MSCs derived from bone marrow of neonatal rats were seeded on the nanofibrous scaffold. The results indicated that the MSCs migrated inside the scaffold and produced abundant extracellular matrix in the scaffold. In continuation to this study, Shin et al tested the PCL nanofibers along with MSCs in vivo in a rat model. Their results demonstrated ECM formation throughout the scaffold along with mineralization and type I collagen synthesis (Shin et al 2004). These studies demonstrated that PCL-based nanofibrous scaffolds are potential candidates for bone tissue engineering.

In another study, Ramay et al used HA with β-tricalcium phosphate (β-TCP) to develop biodegradable nano-composite porous scaffolds (Ramay and Zhang 2004). β-TCP/HA scaffolds built from HA nanofibers with β-TCP as a matrix were used to fabricate porous scaffolds by a technique that integrated the gel casting technique with the polymer sponge method (Ramay and Zhang 2003). Their in vitro results demonstrated that incorporation of HA nanofibers as a second component in β-TCP significantly increased the mechanical strength of the porous composite scaffolds. This study introduced nano-composites with HA nanofibers as a promising scaffolding system for load bearing applications such as bone tissue engineering.

Nanofibers for cartilage tissue engineering

Articular cartilage tissue has a limited capacity for repair due to the reduced availability of chondrocytes and complete absence of progenitor cells in the vicinity of the wound to mediate the repair process. The chondrocytes available for repair are embedded in the dense extracellular matrix of the articular surface which restricts their mobility and hence limits their contribution to the wound healing process (McPherson and Tubo 2000). In addition, articular cartilage is an avascular tissue which further limits its capacity to self-regenerate. To provide a solution to this problem, multiple surgical techniques have been developed, but with limited success (Colwell et al 2001). Therefore, tissue engineering as a potential approach to regenerate cartilage tissue holds good promise. One of the methods of engineering cartilage tissue is through the use of 3D scaffolds combined with chondrocytes or progenitor cells (Tuli et al 2003; Li et al 2005c).

Li et al developed PCL-based nanofibrous scaffolds by electrospinning (Li et al 2003, 2005c). These scaffolds were then seeded with fetal bovine chondrocytes (FBC) and studied for their ability to maintain chondrocytes in a mature functional state. Their results demonstrated that FBCs seeded on the PCL nanofibers were able to maintain their chondrocytic phenotype by expressing cartilage-specific extracellular matrix genes like aggrecan, collagen type II and IX, and cartilage oligomeric matrix protein (Li et al 2003). Further, FBCs exhibited a spindle or round shape on the nanofibrous scaffold in contrast to a flat, well-spread morphology as seen when cultured on tissue culture polystyrene. Another interesting finding from this study was that serum-free medium produced more sulfated proteoglycan-rich cartilaginous matrix when compared with the same cultured in monolayer on tissue culture polystyrene. These results demonstrated that the bioactivity of FBCs depends on the architecture of the scaffold and the composition of the culture medium. Hence, the PCL nanofibers show potential to be further explored as scaffolds for cartilage tissue engineering.

In a more recent study, Li et al have further explored the PCL nanofibers for cartilage tissue engineering. In this study, the authors used adult bone marrow-derived MSCs along with PCL nanofibers to test if the nanofibrous scaffolds supported in vitro MSC chondrogenesis. Their results indicated that PCL nanofibers in the presence of a member of the transforming growth factor-β family caused the differentiation of MSCs to chondrocytes that was

comparable to that caused by cell aggregates or pellets (Li et al 2005c). However, since the PCL nanofibrous scaffolds possess better mechanical properties than cell pellets, they show potential to be developed as a scaffolding system for MSC delivery and hence cartilage tissue engineering.

Kisiday et al (2002) developed a self-assembling peptide hydrogel scaffold for cartilage repair. They used the peptide KDK-12 that had a sequence of (AcN-KLDLKLDLKLDL-CNH2) (where K is lysine, D is aspartic acid, and L is leucine). This peptide was seeded with bovine chondrocytes and then allowed to self assemble into a hydrogel. The chondrocyte-seeded hydrogels were then studied for their ability to support chondrocyte proliferation, ECM production, and phenotype maintenance. Their results demonstrated that the chondrocytes were able to produce cartilage-like ECM which was rich in proteoglycan and type II collagen (phenotypic markers of chondrocytes). Further, the authors observed that the mechanical properties continuously increased with time, which was indicative of the continuous deposition of glycosaminoglycan-rich matrix by the chondrocytes. In addition, the ability to design the peptide may offer advantages in controlling scaffold degradation, cell attachment, and growth factor delivery. Therefore, the self-assembling peptide hydrogel scaffold may be a suitable candidate for cartilage tissue engineering.

Nanofibers for ligament tissue engineering

Ligaments are bands of dense connective tissue responsible for joint movement and stability. Ligament ruptures result in abnormal joint kinematics and often irreversible damage of the surrounding tissue leading to tissue degenerative diseases, which do not heal naturally and cannot be completely repaired by conventional clinical methods (Lin et al 1999; Goulet et al 2000). Recently, tissue engineering methods involving nanofibers have been successfully employed to meet this challenge (Lin et al 1999). In particular, aligned nanofibers enhanced cell response and hence were explored as scaffolds for ligament tissue engineering.

Lee et al studied the effects of PU nanofiber alignment and direction of mechanical stimuli on the extracellular matrix generation of human ligament (anterior cruciate) fibroblasts (HLF) (Lee et al 2005). Conventional electrospinning produces randomly oriented nanofibers; however, the authors made use of a rotating target to achieve electrospun fibers that were aligned. The fibers were then seeded with HLFs to study the influence of alignment on HLF behavior. Their results demonstrated that HLFs were spindle shaped, oriented in the direction of nanofibers, and showed enhancement in the synthesis of ECM proteins (collagen) on aligned nanofibers when compared with randomly oriented nanofibers. In addition, the authors also studied the effect of direction of mechanical stimuli on the ECM produced by HLFs. For this study the authors seeded HLFs on parallel aligned, vertically aligned to the strain direction, and randomly oriented PU nanofibers. The results demonstrated that HLFs were more sensitive to strain in the longitudinal direction. Therefore, this study concluded that aligned nanofibrous scaffolds showed promise for use in ligament tissue engineering (Lee et al 2005).

Nanofibers for skeletal muscle tissue engineering

Skeletal muscles are responsible for voluntary movement of the body and once damaged (by disease or trauma) are difficult to regenerate in adults (DiEdwardo et al 1999). Therefore, tissue engineering of skeletal muscle, although challenging, is an exciting alternative to surgical techniques for skeletal muscle regeneration. Riboldi et al (2005) have explored the use of electrospun microfibers made from degradable polyester urethane (PEU) as scaffolds for skeletal muscle tissue engineering. Based on their preliminary studies using primary human satellite cells (biopsy from a 38-year-old female), C2C12 (murin myoblast cell line), and L6 (rat myoblast cell line), their results indicated that the electrospun microfibers of PEU showed satisfactory mechanical properties and encouraging cellular response in terms of adhesion and differentiation. Based on these studies, the electrospun microfibers of PEU show potential to be further explored as a scaffolding system for skeletal muscle tissue engineering.

Nanofibers for skin tissue engineering

Skin wounds normally heal by formation of epithelialized scar tissue rather than by regeneration of full skin (Clark and Singer 2000). Of the two layers of skin, epidermis and dermis, the epidermis has less capacity to heal; however, when large areas of the epidermis need to be replaced, normal regeneration is lacking. Further, the dermis has an enormous capacity to regenerate. The scar tissue that forms in the absence of dermis lacks elasticity, flexibility, and strength of the normal dermis (Clark and Singer 2000; Parenteau et al 2000). Consequently, scar tissue limits movements, causes pain, and is cosmetically undesirable. Therefore, engineered skin tissue would be an excellent

alternative, not only to close the wound but also to stimulate the regeneration of the dermis. Along with collagen, several other natural and synthetic polymers have been explored for skin tissue engineering (Matthews et al 2002); however, the use of these biomaterials as nanofibers has been very limited. Min et al (2004) developed nonwoven silk fibroin nanofibers by electrospinning for skin tissue engineering. Due to their high porosity and high surface area to volume ratio, fibroin nanofibers coated with type I collagen were found to promote keratinocytes/fibroblast adhesion and spreading. Therefore, the silk fibroin nanofibers show potential to be developed as a scaffold for skin tissue engineering.

Khil et al (2003) studied PU electrospun nanofiber membranes for the purpose of wound dressings. The PU nanofiber membranes provided excellent oxygen permeability and controlled water evaporation. By virtue of these properties, the nanofiber membranes allowed fluid from the wound to exude out while preventing dehydration of the wound. Further, the ultra-fine porosity of the PU nanofiber membranes disallowed invasion by exogenous microorganisms. These results indicated that the PU nanofiber membranes showed potential to be developed as wound dressing materials.

Nanofibers for blood vessel tissue engineering

From the days of research on developing vascular grafts using materials that produce minimal interaction with the inflowing blood and adjacent tissues, researchers have come a long way to develop constructs at the nanoscale that interact with cells and cause blood vessel formation.

Conventional electrospinning produces randomly oriented nanofibers; however, Mo et al developed an aligned biodegradable PLLA-CL (75:25) nanofibrous scaffold using a rotating collector disc for collection of aligned electrospun nanofibers (Mo and Weber et al 2004). These aligned nanofibers were explored to fabricate tubular scaffolds that could be used for engineering blood vessels. Their results demonstrated that the nano-sized fibers mimic the dimensions of natural ECM, provide mechanical properties comparable to human coronary artery, and form a well defined architecture for smooth muscle cell adhesion and proliferation (Mo and Weber 2004; Mo et al 2004; Xu et al 2004b).

Aligned fibers not only give structural integrity but also maintain vasoactivity as they provide necessary mechanical strength needed to sustain high pressure of the human circulatory system (Xu et al 2004a). Xu et al (2004a) studied the response of endothelial cells along with smooth muscle cells (SMCs) on the aligned nanofibers of PLLA-CL, and their results demonstrated that both the cell types showed enhanced adhesion and proliferation rates on the nanofibrous scaffold. In addition, it was observed that the SMCs cytoskeleton organization was along the direction of the nanofibers. These results suggested that aligned nanofibers may provide for a good scaffolding system for vascular tissue engineering.

It is now established that there is a significant effect of nanoscale-textured surface roughness on cell response in terms of cell adhesion and proliferation (Webster et al 2001). It is also known that cells attach and organize very well around fibers with diameters smaller than them (Xu et al 2004a). Therefore, Ma et al processed a conventional polymer, PET, into a nonwoven nanofibrous mat by electrospinning, and modified its surface by grafting gelatin (Ma et al 2005b). Their study demonstrated enhanced spreading and proliferation of endothelial cells on the modified PET nanofiber mats, while preserving their phenotype. Based on this study, gelatin-modified PET nanofibers could be potential candidates for the engineering of vascular grafts.

Boland et al (2004) developed electrospun micro and nanofibrous scaffolds from natural polymers such as collagen and elastin with the goal of developing constructs for vascular tissue engineering. Their results demonstrated that electrospun collagen and elastin nanofibers were able to mimic the complex architecture required of vascular constructs and were able to provide good mechanical properties that are desired in the environment of the blood stream. Their study indicated that micro and nanofibrous scaffolds synthesized from natural polymers such as collagen and elastin could be useful in the engineering of artificial blood vessels.

Nanofibers for neural tissue engineering

In the nervous system, degeneration of neurons or glial cells or any unfavorable change in the extracellular matrix of neural tissue can lead to a wide variety of clinical disorders. Neural tissue repair is a daunting challenge because almost all neural injuries lead to an irreversible loss of function (Fine et al 2000). Neural tissue engineering aims to repair neural tissue by employing biological tools such as normal or genetically engineered cells and ECM equivalents along

with potent synthetic tools such as biomaterials for scaffold design and/or drug delivery systems.

Yang et al have studied the potential of poly(l-lactic acid) (PLLA)-based electrospun nanofibrous scaffolds for the purpose of neural tissue engineering. Their study involved understanding the influence of the nanofibrous scaffolds on neural stem cells (NSCs). Their results indicated that randomly oriented nanofibers (150–350 nm) not only supported neural stem cell adhesion but also promoted NSC differentiation (Yang et al 2004, 2005). The authors attributed the aforementioned findings to higher surface area and roughness of the nanofibers. Yang et al have recently reported another study wherein they tried to understand the role of aligned nanofibers in neural tissue engineering. They obtained aligned nanofibers by collecting nanofibers on the edge of a rotating disc. The 3D scaffolds were fabricated to desired thickness by adjusting the collecting time; however, after approximately 30 minutes, residual charges on the collecting fibers led to random collection of the fibers on top of the scaffold. The scaffolds with oriented nanofibers were then studied with NSCs to determine the influence of nanofiber orientation on NSCs. The results demonstrated that NSCs elongated and their neurites outgrew along the direction of the fiber orientation of the aligned nanofibers. Further, it was observed that the NSCs show increased rate of differentiation on aligned nanofibers compared with microfibers. Therefore, the aligned PLLA nanofibrous scaffolds show potential to be developed for neural tissue engineering (Yang et al 2005).

Semino et al (2004) developed a self-assembling peptide scaffold with a goal of studying 3D culture and cell entrapment. Hippocampal slice and neuroprogenitor cells from the dentate gyrus region were cultured on top of the self-assembled nanofibrous scaffold. At the interaction layer between the hippocampal slice and the nanofibrous scaffold, migrating cell populations were readily enriched and entrapped. After 1 week of culture, glial cells and neurons increasingly migrated into the peptide scaffold to an approximate depth of 400–500 μm from the edge of the tissue slice. Entrapped cells collected from the migration zone were used to initiate new culture. A noteworthy observation from these experiments was that the mitotic activity of neural cells was maintained for 3 days after migration and the authors attribute this to the presence of the nanofibrous scaffold environment that is similar to the ECM of the cells in their native environment. The understanding gained in this study takes us one step closer to the development of a technology for neural progenitor cell isolation and enrichment in vitro. This technology once developed could greatly enhance the ability of scientists to isolate neural progenitor cells and hence engineer neural tissue.

Nanofibers for controlled drug delivery

Controlled delivery systems are used to improve the therapeutic efficacy and safety of drugs by delivering them to the site of action at a rate dictated by the need of the physiological environment. A wide variety of polymeric materials have been used as delivery matrices, and the choice of the delivery vehicle polymer is determined by the requirements of the specific application (Heller and Hoffman 2004). Polymeric nanofibers have recently been explored for their ability to encapsulate and deliver bioactive molecules for therapeutic applications.

Kenawy et al (2002) studied PEVA, PLA, and their 50:50 blend-based electrospun nanofibrous scaffolds for the delivery of tetracycline hydrochloride in periodontal applications. Their results demonstrated that the initial rate of release of tetracycline hydrochloride from the scaffold is high during the first 10–12 hours, most likely due to release of drug sequestered on the nanofiber surface. Electrospun PEVA showed a higher release rate when compared with the electrospun nanofiber meshes of pure PLA or 50/50 PLA/PEVA. Electrospun PEVA released 65% of its drug content within 120 hours, whereas a 50/50 blend of PLA and PEVA gave about 50% release of tetracycline hydrochloride for the same duration. These results indicated that the rate of release of tetracycline hydrochloride can be controlled by modulating the ratio of PLA and PEVA in the polymeric blend. Therefore, the nanofibrous delivery system showed potential to be employed in the treatment of periodontal diseases.

In another study, Verreck et al (2003b) have demonstrated the use of (nonbiodegradable polymer scaffolds) PU nanofibrous scaffolds produced by electrospinning for the delivery of water-insoluble drugs such as intraconazole and ketanserin. In their study, the authors obtained an amorphous nanodispersion of the water-insoluble drug on the nanofibrous scaffold. The large surface area of the nanofibrous scaffold allowed fast and efficient solvent evaporation that gave limited time for crystallization of incorporated drug, and favored the formation of an amorphous dispersion (Verreck et al 2003a). These studies

demonstrated that the release of poorly water-soluble drugs from water-insoluble polymers is possible and that the rate of release can be tailored by altering the concentration of the polymer. Another study by Kim et al (2003, 2004) demonstrated the potential use of PLGA nanofibrous scaffolds for controlled release of hydrophilic antibiotics. The authors incorporated Mefoxin® (cefotoxin sodium) into PLGA nanofibers during the process of electrospinning. Inhibited growth of *Staphylococcus aureus* indicated that the bioactivity and structure of the antibiotic drug is not affected by the process of electrospinning.

The above studies elucidate that nanofibrous scaffolds are suitable carriers for both hydrophilic and hydrophobic drugs, and that the drug release rate can be tailored by modulation of the nanofibrous scaffold's morphology, porosity, and composition.

Nanofibers for DNA, protein, and enzyme delivery

With the availability of multiple gene delivery systems, the selection of the most appropriate gene delivery vehicle to meet the needs of a particular therapeutic application can be challenging. Viral- and plasmid-based delivery vehicles are currently used for the production of therapeutic proteins to elicit a desired biological response (Fradkin et al 2000). This would especially be useful in the field of tissue engineering, wherein it would be possible to cause the production of a desired protein (growth factor) that can enhance the process of tissue regeneration. Therefore, gene delivery systems have been explored for applications in the engineering of a variety of tissues. The most commonly used carrier-based systems for gene delivery are cationic liposomes and condensing agents such as poly(ethylenimine), and poly (l-lysine). More recently, biomaterial-based gene delivery systems have been explored and are proving to be a promising approach. Various biomaterials such as poly(ethylene glycol) (PEG), PLGA, and PLA-PEG copolymers are currently being investigated for gene and protein delivery (Funhoff et al 2005).

Scaffolds for gene delivery need to provide structural stability and site specific delivery of genes along with protection of genes from the biological system until it is released. Further, the released DNA needs to retain its structural integrity until it is taken up by the desired cells.

Luu et al studied PLGA and PLA-PEG block copolymer based nanofibrous scaffolds for plasmid DNA delivery (Fang and Reneker 1997; Luu et al 2003). Their results indicated that the electrospun nanofibrous scaffolds delivered the gene in a controlled manner at the targeted site and consequently caused cell transfection and desired bioactivity. This approach showed higher transfection efficiency when compared with naked DNA added directly to the culture medium. Increasing the amount of DNA during scaffold fabrication increased transfection efficiency of the nanofiber DNA system. The architecture and material properties of the nanofibrous scaffold affect the rate at which the DNA is released. Therefore, the release profile of the DNA can be controlled by tailoring scaffold parameters like nanofiber diameter, pore size of scaffold, and degradation rate of the polymer. Through these modifications it is possible to sustain the delivery of DNA over a longer duration. Overall, this system seems ideally suited for the sustained/controlled delivery of intact DNA over a period of several months.

Jia et al (2002) used alpha-chymotripsin attached to electrospun polystyrene nanofibers (120 nm) as a catalytic system and examined its catalytic efficiency in biotransformations. Their results indicated that the nanofibrous enzyme system had a higher hydrolytic activity (65%) than immobilized enzyme, and three times more nonaqueous activity than immobilized alpha-chymotrypsin in organic solvents. The authors proposed that covalent binding of the enzyme to the nanofiber increased enzyme stability or decreased structural denaturation. They believe that this increased stability may be the reason for enhanced activity of the enzymes attached to the nanofibers. Therefore, the nanofibers show potential to be developed as catalytic systems to be used in biotransformations.

In a recent study, Zeng et al (2005) developed poly(vinyl alcohol) (PVA) electrospun nanofibers for protein delivery. PVA nanofibrous scaffolds were loaded with bovine serum albumin or luciferase proteins. The PVA nanofibers were then coated with poly(p-xylylene) (PPX) using chemical vapor deposition. The coated and uncoated PVA nanofibrous scaffolds were then studied for release kinetics and bioactivity of the released proteins under physiological conditions. The results demonstrated that intact protein/enzyme was continuously released from both the nanofiber types and their bioactivity was preserved after release from the nanofibrous scaffolds. However, the PPX-coated nanofibers exhibited significantly retarded release rates compared with the uncoated PVA nanofibers. Therefore, this study showed that the nanofibrous scaffold could be a good candidate material for controlled enzyme/protein delivery.

All the above studies demonstrated the potential of nanofibers as controlled delivery systems, and hence demand exploration at greater depth to enable this technology to benefit the patient.

Application of carbon nanofibers in tissue engineering

Apart from having nanoscale fiber dimensions similar to HA and collagen fibers present in bone, carbon nanofibers have exceptional mechanical properties (three times that of bone tissue), thereby giving a strong rationale to investigate them for application in orthopedic or dental tissue engineering (Elias et al 2002). Further, carbon nanofibers have also been shown to exhibit excellent conductivity, which might make them potential candidates for neural tissue engineering applications. The carbon-nanofiber-based implants can surpass in some ways the conventional metal alloy implants used in orthopedics, as they have excellent cytocompatibility properties (Price et al 2003b) and complications associated with leachables in the form of metal ions released from implants do not arise. In terms of mechanical properties, carbon nanofibers possess a Young's modulus of 2 TPa, which is significantly higher than that of bone, whereas the tensile strength of carbon nanofibers almost equals that of bone (Elias et al 2002). Therefore, Price et al explored the possibility of using carbon nanofibers for bone tissue engineering. They compared osteoblast adhesion on carbon nanofibers with that of conventional carbon fibers (Elias et al 2002; Price et al 2004) and showed greater osteoblast adhesion on carbon nanofibers. To determine the properties that caused enhanced adhesion on carbon nanofibers, the authors studied osteoblast adhesion on PLGA-coated carbon nanofibers. Their results indicated that PLGA-coated carbon nanofibers also showed enhanced osteoblast adhesion compared with conventional carbon fibers. The authors attributed this behavior to increased surface energy (due to high surface area of the nanofibers), nanometer topography, and surface chemistry of the fibers (Price et al 2004) (see Figure 4).

Due to their electrical conductivity, carbon nanofibers were initially explored as electrically conducting fibers, in nanoelectronic devices, field emitters, and also in reinforcement (Wal et al 2002; Hammel et al 2004). More recently, due to their conductivity, carbon nanofibers are being explored as potential candidates for neural tissue engineering. To determine the cytocompatibility of carbon nanofibers as neural implants, McKenzie et al (2004) studied the interaction of astrocytes (glial scar tissue-forming cells) and carbon nanofibers in terms of adhesion and proliferation. Their studies demonstrated that functions of astrocytes were minimized on nanoscale fibers and led to reduced scar tissue formation. Based on these observations, the authors concluded that minimized glial scar tissue formation and positive interaction with neurons are properties that would strongly support the success of a neural implant. Therefore, carbon nanofibers need to be investigated further to establish their potential use in neural tissue engineering.

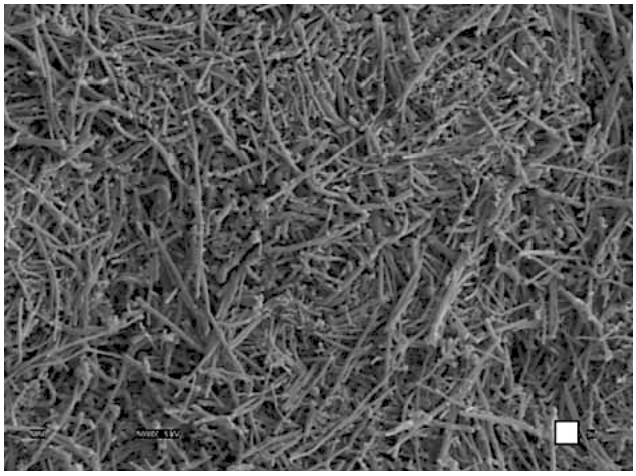

Figure 4 High magnification scanning electron micrograph of carbon nanofiber compacts. The carbon nanofibers were prepared using a chemical vapor deposition technique with a pyrolitic aromatic hydrocarbon outer layer (PR-I AG [nanophase]). The resulting carbon nanofibers were compacted serially in a steel-tool die via a uniaxial pressing cycle (0.2–0.4 GPa over a 5-minute period) at room temperature and the resulting carbon fiber compacts were used in the cell experiments (scale bar = 1 μm). Source: Elias KL, Price RL, Webster TJ. 2002. Enhanced function of osteoblasts on nanometer diameter carbon fibers. *Biomaterials*, 23:3279–87. Copyright © 2002. Reprinted with permission of Elsevier.

Applications of alumina nanofibers in tissue engineering

Osteointegration is a major requirement for bone and dental implantation. It has been demonstrated that a decrease in surface feature size can enhance osteointegration (Webster 2001).

Alumina, titania, HA, and their composites are the most well studied materials for both dental and orthopedic applications. Due to similarity between physical geometry of HA and aluminum nanofibers, Price et al hypothesized that alumina nanofibers may enhance osteointegration (Price et al 2003a). They studied the influence of alumina nanofibers on the behavior of osteoblast cells. Their results demonstrated that the alumina nanofibers enhanced cell adhesion and synthesis of osteoblastic phenotypic markers such as alkaline phosphates and calcium (Webster et al 2005).

The above studies elucidate that carbon and aluminum nanofibers can be promising materials for orthopedic/dental tissue engineering applications.

Conclusion

Mimicking the architecture of ECM is one of the major challenges of tissue engineering. Amongst all the approaches used to prepare ECM synthetically, the approach using

nanofibers has shown the most promising results. Nanofibers can be formed using either one of the three prevailing techniques: electrospinning, self-assembly, or phase separation. Electrospinning is the most widely studied technique and has also shown the most promising results. The availability of a large range of natural and synthetic biomaterials has fueled the area of nanofiber synthesis, especially using the electrospinning technique.

Nanofibers, irrespective of their method of synthesis, have provided for scaffolds with high surface area and enhanced porosity. These properties have been demonstrated to have a significant effect on cell adhesion, proliferation, and differentiation. Hence nanofibrous matrices are currently being explored as scaffolds for musculoskeletal tissue engineering (including bone, cartilage, ligament, and skeletal muscle), skin tissue engineering, neural tissue engineering, vascular tissue engineering, and controlled delivery of drugs, proteins, and DNA. The results of all these studies clearly indicate that nanofiber-based scaffolds show excellent potential to be developed for a variety of tissue engineering applications.

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