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Nanotechnology in Biomaterials: Nanofibers in Tissue Engineering

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10.1 Biomaterials

Devices made of synthetic or natural materials have been used in the medical area and introduced into the human body to improve human health since ancient times. Romans, Chinese, and Aztecs used gold wires in dentistry more than 2,000 years ago. Ancient Egyptians and Greeks sutured wounds with plant fibers and animal-derived materials and used wood for prosthetic limbs. Since then, scientists have continued their research to find bioactive agents to cure illnesses and to improve the quality of human life by using artificial prostheses.

There are various definitions of biomaterials expressed in different ways given in the literature, but more or less they have the same meaning. One of the very early definitions came from the Clemson University Advisory Board for Biomaterials (1976), which described a biomaterial as “a systemically and pharmacologically inert substance designed for implantation within or incorporation with living systems (Park, 1981).” Another definition of biomaterials is “materials of synthetic as well as of natural origin in contact with tissue, blood, and biological fluids, and intended for use for prosthetic, diagnostic, therapeutic, and storage applications without adversely affecting the living organism and its components” (Bruck, 1980). Biomaterials can be defined as materials to be used to
substitute a tissue or organ of a living system that is not fully functioning, or to support the biological system in its function while in intimate contact with the tissues in a safe, reliable, economic, and physiologically acceptable manner (Hench and Erthridge, 1982). Black (1992) defined biomaterial as “a nonviable material used in a medical device, intended to interact with biological systems.” In addition, during the Second Consensus Conference on Definitions in Biomaterials Science, a biomaterial was defined as “a material intended to interface with biological systems to evaluate, treat, augment, or replace any tissue, organ or function of the body” (Williams, 1992). The same definition is found in the Williams Dictionary of Biomaterials (Liverpool University Press, 1999).

Biomaterials are used in many application areas, such as orthopedic (total joint replacements, bone plates and screws, etc.), dental (tooth fillings, crowns, implants, etc.), cardiovascular (cardiac pacemaker, artificial heart valve, blood vessels, etc.), ophthalmic (contact and intraocular lenses), wound healing (sutures, wound dressing, tissue adhesive, ligating clips, staples, etc.), and drug delivery (oral, inhalation injectable delivery systems, microand nanodevices, sheets, fibers, and sponges, etc.) systems. Biomaterials can be found in different forms, such as tubes (artificial veins, artificial trachea), fibers (sutures), films and membranes (wound dressings, drug delivery systems, artificial kidney), gels (burn covers, topical drug delivery systems), and porous scaffolds (tissue engineering), depending on the site and type of use. Metals (titanium, stainless steel, cobalt-chromium alloys, gold, silver, platinum), polymers (nylon, silicones, Teflon, dacron, polylactides), ceramics (aluminum oxide, carbon, hydroxyapatite), natural materials (collagen, hyaluronic acid, reconstituted tissue such as porcine heart valves), and composites (carbon or ceramic wires, fiber-reinforced polymers) have been used as biomaterials in the design and production of biomedical devices. Material to be used as a biomaterial must fulfill some requirements. First, it should be compatible with the biological environment and should not cause any adverse carcinogenic or allergic tissue reaction; i.e., it should be biocompatible. Degradation products of a biomaterial must also be nontoxic and noncarcinogenic, and have the desired bioactivity toward the cells and tissue. Physical, chemical, and mechanical properties of the biomaterial should match those of the target tissue.

10.2 Tissue Engineering

Tissue engineering was once considered a subfield of biomaterials, but since it has gained too much importance in the last decades, it is now considered a field in its own right. Tissue engineering is the process of creating living three-dimensional (3D) tissues and organs by using engineering and materials methods, and specific combinations of cells and cell signals, both chemical and mechanical (Griffith, 2002). This approach aims to reproduce both the form and function of the tissue. Different from nonliving biomaterials, tissue engineering is based on the formation and regeneration of a new functional tissue by combining living components (cells, tissue fragments) and carrier scaffolds in a construct to be implanted into the body (Kneser et al., 2002; Guelcher et al., 2006).

There are three approaches in repairing tissues. The first one is the injection of tissue-specific viable cells directly into the damaged site of the tissue. The second approach is the delivery of tissue-inducing substances, such as growth and differentiation factors, to
target locations. The third one is the tissue engineering approach, in which cells are grown in 3D scaffolds. The use of cell suspensions or tissue-inducing substances is considered when the defects are small, well contained, or not suitable for implanting a 3D structure. To engineer tissues with practical size and predetermined shapes, the first two approaches are not appropriate. The main principle of the tissue engineering strategy is to seed a porous, biodegradable 3D cell carrier (scaffold, matrix) with cells, add signaling molecules like growth factors, culture the construct in a medium for growing and proliferation of cells, and then implant it into the defect area to grow a new tissue. It is expected that the cells of the natural tissue would also attach to the scaffold, proliferate, differentiate, and get organized, forming a normal, healthy tissue while the scaffold itself degrades. At the end, healthy tissue fills the defect site and no artificial matrix remains. Scaffolds, cells, and signaling biomolecules are the main components of tissue engineering devices. All of these components play very important roles during tissue regeneration. The extracellular matrix (ECM) is a microenvironment composed of parenchymal cells (functional cells), mesenchymal cells (support cells), and structural materials. Every tissue and organ in our body is formed in these microenvironments. The body serves as a bioreactor, which applies biomechanical forces and provides biochemical signals to the cells and the ECM to develop and maintain tissue and organs (Barnes et al., 2007). In the tissue engineering approach, the biological way of tissue formation in the body is mimicked by taking place in a porous matrix.

A scaffold is one of the essential components of tissue engineering since it provides 3D support for the cells, as ECM does. In vitro, cells do not grow in a way to fill a volume if there is no 3D support. Porous scaffolds provide some place for cell attachment and viability through transport of nutrients and metabolites, and allow cell expansion and tissue organization. There are some requirements for proper scaffolds. First, they should be biocompatible, and therefore should not cause any adverse responses, such as allergic and carcinogenic reactions in the body. They must have an interconnected, highly porous structure with a high surface area for cell adhesion and reorganization, for transport of nutrients and removal of metabolic waste, and for homogeneous cell distribution throughout the scaffold. They should have adequate mechanical strength to withstand the pressures and mechanical forces applied by the biological system. The degradation rate of the scaffold should be in accordance with the tissue growth; when the tissue is totally regenerated, the scaffold should be almost fully degraded. A suitable surface chemistry is extremely important for cell attachment, proliferation, and differentiation Ma, 2004a; Stevens et al., 2008. The material of the scaffold should be easily processed to achieve the desired shapes and sizes. Finally, the scaffold should have some specific properties for specific tissue engineering applications, such as osteoconductivity for bone, electrical conductivity for nerve, and contraction for myocardial tissue engineering.

Various materials have been studied as potential scaffolds. Some metals are good choices for load-bearing implants since they have superior mechanical properties (Catledge et al., 2004). Recently, porous titanium (Ti) and titanium alloys were used as scaffold biomaterials for bone tissue engineering, but they are not degradable in the body. Inorganic materials are also used in bone tissue engineering studies (Hench and Polak, 2002). Ceramics such as hydroxyapatite (HAp), Bioglass, A-W glass ceramics, and β-tricalcium phosphate (β-TCP) closely mimic bone tissues. They are osteoconductive or osteoinductive, and enhance the absorption of bone ECM proteins, resulting in improved osteoblast adhesion (Ducheyne and Qiu, 1999; Dieudonne et al., 2002; Smith et al., 2004).
However, ceramics have some drawbacks, like fragility, brittleness, low tensile strength, and being mostly not biodegradable. Biological and synthetic polymeric materials have been widely used in various tissue engineering applications due to their degradability, biocompatibility, mechanical properties suitable for many applications, and ease of processability. Some examples of natural biodegradable polymers are collagen, fibrinogen, gelatin, chitosan, alginate, starch, silk, and hyaluronic acid. The main advantages of these materials are their low immunogenicity, bioactivity, capability of interacting with the host tissue, chemical versatility, and availability from various sources, as in starch and chitosan. Of these, collagen, fibrin, hyaluronic acid, agarose, chitosan, and alginate are materials especially used in the production of scaffolds for engineering of bone and cartilage (Sabir et al., 2009). The most widely used synthetic polymers are a family of linear aliphatic polyesters, poly(α-hydroxy acid) (PHA), such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymer poly(lactic acid-co-glycolic acid) (PLGA) (Mooney et al., 1996; Seal et al., 2001). For example, PGA has been used in the engineering of cartilage, tendon, ureter, intestine, blood vessel, heart valve, and other tissues. Poly(ε-caprolactone) (PCL), poly(propylene fumarate) (PPF), poly(carbonate), poly(phosphazenes), poly(phosphoesters) and poly(anhydrides), tyrosine-derived polymers, and biodegradable urethane-based polymers are other important synthetic, biodegradable polymers used in tissue engineering applications. A problem with synthetic polymers is their degradation products, which may reduce the local pH, induce an inflammatory response, or create some adverse reactions and being nonbiocompatible.

In the past few years, polymer-ceramic composites have gained increased importance in the engineering of several types of tissues, such as bone, cartilage, tendon, and ligament (Marra et al., 1999; Zhang and Ma, 1999; Ma et al., 2001; Zhao et al., 2002; Liu and Ma, 2004). The composite is expected to have improved compressive mechanical properties compared to the polymer, and better structural integrity and flexibility than ceramics, and thus the combination of ceramic and polymer created reinforced porous scaffolds with enhanced bioactivity and controlled resorption rates (Zhang and Ma, 1999; Ma et al., 2001).

During the selection of a scaffold material, an important step is to decide on the fabrication technique. The processing technique is expected not to change certain material properties, like biocompatibility and surface chemistry. The technique should be reproducible in terms of scaffold porosity, pore size, pore distribution, and interconnectivity (Leong et al., 2003). There are many techniques that can be used to process materials into scaffolds, such as solvent casting, phase inversion, fiber bonding, melt-based technologies, high-pressure-based methods, freeze drying, and rapid prototyping (Ma and Langer, 1999; Kinikoglu et al., 2009; Sangsanoh et al., 2009; Yilgor et al., 2008; Vrana et al., 2007; Zorlutuna et al., 2007; Mooney 1996; Thomson et al., 1995; Mikos et al., 1993). Each production technique has some disadvantages and advantages over the others and may not be applicable to some polymers. Therefore, appropriate processing techniques should be chosen to produce a scaffold with the desired properties for the specific application.

To date, scaffolds have been produced in our group in various forms, like foam (Zorlutuna et al., 2007; Kinikoglou et al., 2009, Dogan et al., 2009, Ulubayram et al., 2001), film (Vrana et al., 2007; Zorlutuna et al., 2007; Kenar et al., 2008; Zorlutuna et al., 2009), and fibers (Ndreu et al., 2008; Kenar et al., 2009; Yucel et al., 2010), each having different chemistries, and a number of studies have been carried out with them either in vitro or in vivo. In the last decades, foam and film-based scaffolds were popular for 3D and 2D applications; however, in recent years there has been an increase in the use of fibrous scaffolds, especially made by electrospinning.
10.3 Importance of Nano/Microfibrous Scaffolds

Many extracellular proteins have a fibrous structure with diameters on the low or submicrometer scales. For example, collagen is the most abundant ECM protein in the human body, and it has a fibrous structure with fiber bundle diameters varying from 50 to 500 nm, depending on the site and species (Hay, 1991). In the native tissues, the structural ECM proteins are one to two orders of magnitude smaller than the cells, and therefore, the cells are in direct contact with many ECM fibers. It is now well known that many biologically active molecules, extracellular matrix components, and cells interact with each other using functional groups on the nanoscale. It is therefore a useful approach to mimic the fibrous nature of the extracellular matrix when fabricating scaffolds (Barnes et al., 2007). Nanofibers have a high surface-to-volume ratio, which enhances the number of cells that adhere to the surface (Ma and Langer, 1999). Cell migration, proliferation, and differentiation follow adhesion (Palecek et al., 1997), and it is expected that these reactions will be much higher on nanofibrous scaffolds than on foams or films. As a result, nanofibers have attracted the attention of many researchers who considered them as potential scaffolds for applications such as tissue engineered vasculature (Xu et al., 2004; Lee et al., 2007), bone (Sui et al., 2007; Cui et al., 2007), nerve (Yang et al., 2005; Schnell et al., 2007), and tendon and ligament (Lee et al., 2005; Sahoo et al., 2006).

10.4 Fabrication of Nano/Microfibrous Scaffolds

Since fibrous scaffolds with nanometric fiber diameters have been found to be satisfactory for various tissue engineering applications, extensive research toward developing processes for the fabrications of these fibrous structures is being conducted. The main methods used in the fabrication of fibrous scaffolds are self-assembly, template synthesis, drawing and extrusion, phase separation, wet spinning, and electrospinning. Each of these procedures leads to fibers with different dimensions and organizations.

In the case of self-assembly, atoms and molecules arrange themselves through weak and noncovalent interactions, and it is known as a bottom-up method since the built-up nanoscale fibers are formed starting from smaller molecules. It yields fibers with small dimensions (less than 100 nm wide and up to a few micrometers long), and it offers novel properties and functionalities that cannot be achieved by conventional organic synthesis (Hasseinkhan et al., 2006). The main disadvantage of this method is its complexity, in addition to being a long and extremely elaborate process with low productivity (Ma et al., 2005).

Another fabrication method is template synthesis. As the name implies, a nanoporous membrane is used as a mold or template in order to obtain the material of interest in the form of solid nanofibrous or hollow-shaped tubules. This method uses a polymer solution to be extruded through a porous membrane by application of pressure. Fibers are formed when the extruded polymer comes into contact with a precipitating solution (Feng et al., 2002). Fiber diameter depends on the pore size of the template used, and it varies from a few to a hundred nanometers. Many materials, like metals, polymers, and carbon, can be utilized as templates for fiber fabrication; however, this method is limited in that the length of nanofibers can only be a few micrometers.
Drawing is a method to produce single nanofibers. It requires a minimum amount of equipment and is a discontinuous process. A micropipette is dipped into a droplet near the solution-solid surface contact line via a micromanipulator, and then the micropipette is withdrawn from the liquid at a certain speed, yielding a nanofiber. These steps are repeated many times, each time with a different droplet. The solution viscosity, however, increases with solvent evaporation, and some fiber breaking occurs due to instabilities that occur during the process (Ondarcuhu and Joachim, 1998). The main limitation of this method is that it can only use solutions of viscoelastic materials, which can undergo strong deformations that are cohesive enough to withstand the stresses developed during the pull. Again, the fiber diameter is dependent on the orifice size used. It is difficult to obtain fiber diameters of less than 100 nm.

Phase separation is another process utilized in nanofiber production. The main principle of this method is that a polymer dissolved in an appropriate solvent is stirred at a certain gelation temperature for a period of time until a homogeneous solution is obtained. Then, the formed gel is immersed in water several times for solvent exchange to occur. The final gel is lyophilized. Phase separation occurs due to chemical incompatibility and results in nanofibers, but the problem is that a long time is needed to convert the polymer into a nanoporous foam. The fiber diameter ranges from 50 to 500 nm with a length of a few micrometers (Zhang et al., 2005), which is a disadvantage for some applications. Moreover, only polymers that have gelation capability can be used to obtain nanofibrous structures.

Wet spinning is a process similar to drawing, with the difference being that a polymeric solution is precipitated or coagulated by dilution, adding into a nonsolvent or a chemical reaction. Fibers produced by this method are continuous and in microscale (from 10 to 100 µm). Polymer solution concentration and the spinneret diameter are the two crucial parameters that influence the resulting fiber diameter.

The last, and one of the most preferred, methods used to prepare fibers in nano- and low microscale is the electrospinning process. High electrostatic forces are applied to draw continuous fibers from a polymeric solution through a syringe. Basically, four components are required to accomplish the process: a capillary tube ending in a needle of small diameter, a high-voltage supplier, a syringe pump, and a metallic component. One of the electrodes is connected to the needle of the syringe containing the polymer, and the other one is connected to the collector. Leakage of the polymer solution is prevented by surface tension; however, as the applied potential increases, the surface tension contribution starts to decrease as a result of charge repulsion, and the fluid shape at the needle tip becomes like a cone, which is called the Taylor cone. The voltage is increased until the repulsive electrostatic forces overcome the influence of solution surface tension. Meanwhile, the solvent evaporates and the charged jet of the solution is ejected from the needle tip in the shape of a long, continuous, thin fiber. Therefore, fibers with various diameters are collected on either a stationary or rotating collector. Rotating collectors are used to produce aligned nanofibers, which are more preferable in certain applications. A variety of polymers have been used until now to produce fibers from tens of nanometers to a few micrometers (Jin et al., 2004; Li et al., 2005; Ma et al., 2005; Ndreu et al., 2008; Erisken et al., 2008; Jeong et al., 2009; Kenar et al., 2009; Yucel et al., 2010).

Certain properties are expected from a polymer that is proven to be spinnable. The fibers should be consistent, defect-free, and have controllable diameters. The main parameters to control are process conditions such as polymer type, its concentration, solvents, applied potential, distance between the needle tip and the collector, and the collector type. These parameters are modified to control fiber thickness, porosity and pore size (or rather the spacing between the fibers or fiber density), surface chemistry, and surface topography.
(Deitzel et al., 2001; Huang et al., 2003; Katti et al., 2004; Barhate et al., 2006; Ndreu et al., 2008; Wang J et al., 2009). Therefore, optimization of the process conditions in order to get the best results is challenging with so many parameters.

Finally, it can be said that compared to the other nano/microfiber-production methods, electrospinning is more economical, simpler, yields continuous fibers, and is versatile enough to be used for the production of nanofibers from a variety of materials. Therefore, electrospinning represents an attractive technique to be used for producing nanofiber-based scaffolds for tissue engineering.

10.5 Nano/Microfibers in Tissue Engineering Applications

The use of polymeric nano/microfibers in tissue engineering applications is rapidly growing. The main areas include the engineering of skin (Venugopal and Ramakrishna, 2005; Noh et al., 2006; Yang et al., 2009; Jeong et al., 2009; Torres et al., 2009), ligament and tendon (Petrigliano et al., 2006; Teh et al., 2007, 2008; Sahoo et al., 2009; Fan et al., 2009; Yin et al., 2009), skeletal muscle (Riboldi et al., 2005; Choi et al., 2008), cartilage (Li WJ et al., 2005; Li et al., 2006; Janjanin et al., 2008; Lu et al., 2008; Hu et al., 2009), bone (Santos et al., 2008; Zhang et al., 2008; Reed et al., 2009; Wang J et al., 2011; Prabhakaran et al., 2009), cardiovascular (Kenar et al., 2009; Rockwood et al., 2008; Li WJ et al., 2006), and nerve (Yücel et al., 2010; Neal et al., 2009; Nisbet et al., 2008; Corey et al., 2007) tissues. The increase in the number of review articles (Ashammakhi et al., 2007a, 2007b, 2008; Agarwal et al., 2009; Jang et al., 2009) and publications related to the application of nano/microfibers in these fields shows the interest in electrospinning for tissue engineering. In this review we will concentrate more on the studies carried out by our research group, and also by some of the leading researchers in the fields of bone, cardiovascular, and nerve tissue engineering.

10.5.1 Bone Tissue Engineering

Prior to designing a bone substitute or a tissue-engineered bone, it is important to know well the structure and function of the natural bone so that the material type chosen is the most suitable to regenerate the function of the damaged bone. Bone is a complex, physically hard, rigid, and highly organized tissue. This tissue is composed of an intracellular matrix in a fibrous form made mainly of collagen types I and V, and some inorganic materials. Apart from the major collagen matrix, some other noncollagenous proteins, like osteocalcin and osteopontin, are also present. There are three cell types present in the bone structure: osteoblasts, osteocytes, and osteoclasts. Osteoblasts, derived from mesenchymal precursor cells in the marrow, are the bone cells responsible for formation and structural organization of the bone matrix, and also bone mineralization. When osteoblasts become trapped in the matrix, they secrete and become osteocytes. Osteocytes are the most abundant cells found in compact bone. They are networked to each other via long cytoplasmic extensions that occupy tiny canals called canaliculi, which are used for exchange of nutrients and waste. The space that an osteocyte occupies is called a lacuna. Unlike osteoblasts, these cells have reduced synthetic activity and are not capable of mitotic division. They are actively involved in the turnover of bony matrix, through various mechanosensory mechanisms. They destroy bone through a mechanism called osteocytic osteolysis. The third bone cell type, osteoclasts, are polarized cells and have a crucial role in bone resorption,
which includes an initial mineral dissolution and then an organic phase degradation (Jang et al., 2009).

As mentioned above, the main approach in the tissue engineering process is mimicking the ECM. Therefore, for bone tissue engineering it is crucial to make a design whose properties would mimic best the structure and function of bone ECM. It is important to choose a material that interacts well with bone cells in the bone formation processes. Moreover, it is favorable that materials with good mechanical properties and can withstand various external stresses applied on bone are incorporated into the material design. Since the organization of all bone compartments is in nanoscale or at the molecular level, a nanoscale-based design would be optimal to mimic the natural bone. Various strategies have been tried in designing a tissue engineered bone.

Both synthetic and natural materials have been utilized in this field. Among all polymeric materials, a group of polyesters, such as PLA, PGA, PCL, poly(3-hydroxybutyric acid) (PHB), and their copolymers, has been the most extensively studied polymers to produce the nanofibrous system for the regeneration of bone tissues (Burg et al., 2000). A summary of nano/microfibrous scaffolds used in bone tissue engineering studies is given in Table 10.1. Yoshimoto et al. (2003) used electrospun PCL nanofibers, and microscopic analysis of mesenchymal stem cell-seeded scaffolds revealed a good cell penetration in addition to a high level of mineralization and collagen type I production after 4 weeks of culture (Yoshimoto et al., 2003). Sombatmankhong et al. produced mats from PHB, poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and their 50/50 w/w blends and studied the biocompatibility of these mats by a human osteosarcoma cell line (Saos-2) and mouse fibroblasts (L929). Both cells adhered well on all types of fibrous scaffolds; however, the highest alkaline phosphatase (ALP) activity was seen on the PHB/PHBV blend (Sombatmankhong et al., 2007).

In another study, PLA and poly(ethylene glycol) (PEG)-PLA diblock polymers with controlled topographies and chemistries were prepared by electrospinning in order to study the influence of surface topography and fiber diameter on cell behavior. In the presence of osteogenic factors the cell numbers on fibrous scaffolds were reported to be equal to or higher than those on smooth surfaces. Furthermore, the authors indicated an increase in cell density with an increase in fiber diameter and no influence of surface substratum on ALP activity (Badami et al., 2006). The influence of fiber diameter on cell behavior was also studied by other researchers. Ndreu et al. (2008) studied the influence of different types of nano/microfibers (diameter range: 300 nm–1.5 µm) on Saos-2 cells. Good cell adherence, proliferation, and infiltration were observed on all types of scaffolds; however, the highest number of cell attachments was observed on the fibers with the lowest diameter (PHBV-PLLA fiber diameter: ~350 nm) when the scaffolds were examined with a scanning electron microscope (SEM) and a confocal laser scanning microscope (CLSM) (Figure 10.1).

Reed and his co-workers (2009) carried out a study on the reconstruction of a compound tissue based on the fact that healing does not occur as a single tissue, but as a compound one, such as bone-periosteum-skin. In order to perform this study, they seeded human foreskin fibroblasts, murine keratinocytes, and periosteal cells on PCL nanofibers and showed that these scaffolds supported the growth of all these cell types. Even though keratinocytes grew randomly throughout the scaffolds, they reported improved longevity of the cocultured cells and a strong degree of osteoinduction. As a consequence, construction of a composite tissue has been reported by the authors (Reed et al., 2009).

Compared to the synthetic materials, natural materials have better biocompatibility, and more importantly, they possess the main cues that are necessary for a cell to attach and grow. Natural polymers are also advantageous in that they can be degraded by naturally
### TABLE 10.1
Fibrous Scaffolds Fabricated by Electrospinning for Bone Tissue Engineering

<table>
<thead>
<tr>
<th>Polymer Type</th>
<th>Polymer Concentration/Composition/Solvent</th>
<th>Fiber Diameter</th>
<th>In Vitro Studies: Cell Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA/BG nanofiller</td>
<td>3.5%, w/v; 90:10, w:w CHL</td>
<td>Hundreds of nanometers</td>
<td>MC3T3-E1</td>
<td>Noh et al., 2010</td>
</tr>
<tr>
<td>Gelatin/siloxane</td>
<td>15–25% w/w Gelatin:GPMS:CaNO₃, formic acid</td>
<td>Nanometer scale</td>
<td>BMSCs</td>
<td>Ren et al., 2010</td>
</tr>
<tr>
<td>PVA/COL/n-HAp</td>
<td>7% w/w PVA:COL (55:45) n-HAp: 5–10% w/w of PVA deionized water, HAc</td>
<td>~320 nm</td>
<td>—</td>
<td>Asran et al., 2010</td>
</tr>
<tr>
<td>PAN/β-TCP</td>
<td>10% w/w DMF</td>
<td>~300 nm</td>
<td>MG-63</td>
<td>Liu et al., 2010</td>
</tr>
<tr>
<td>CHI</td>
<td>7% w/w TFA / DCM 70:30 v/v</td>
<td>126 ± 20 nm</td>
<td>MC3T3-E1</td>
<td>Sangsanoh et al., 2009</td>
</tr>
<tr>
<td>PCL/nanoapatite (nAp)</td>
<td>80:20% w/w 80% TFE in H₂O</td>
<td>320–430 nm</td>
<td>RBM</td>
<td>Yang F. et al., 2009</td>
</tr>
<tr>
<td>COL-HAp</td>
<td>80:20% w/w Sol concentration (0.023–0.040 g/ml) HFP</td>
<td>75–160 nm</td>
<td>MC3T3-E1</td>
<td>Song et al., 2008</td>
</tr>
<tr>
<td>CHI/PVA and CECS/PVA</td>
<td>CHI (7% w/w): PVA (10% w/w) (80:20 v/v) Aq. HAc</td>
<td>100–700 nm</td>
<td>L929</td>
<td>Yang et al., 2008</td>
</tr>
<tr>
<td>CHI-HAp</td>
<td>HAp/CHI (30:70) +10% w/w UHMWPEO HAc:DMSO (10:1, w/w)</td>
<td>214 ± 25 nm</td>
<td>hFOB</td>
<td>Zhang et al., 2008</td>
</tr>
<tr>
<td>PCL-β-TCP</td>
<td>12% w/w DCM 0–15% w/w β-TCP</td>
<td>200–2,000 nm</td>
<td>MC3T3-E1</td>
<td>Erisken et al., 2008</td>
</tr>
<tr>
<td>PLGA/COL</td>
<td>8 g/ml (50:50 (w/w)) HFP</td>
<td>382 ± 125 nm</td>
<td>BMHSCs</td>
<td>Ma et al., 2008</td>
</tr>
<tr>
<td>PLA/HAp</td>
<td>6% w/w DCM and 1,4-dioxane</td>
<td>313 nm</td>
<td>MG63</td>
<td>Sui et al., 2007</td>
</tr>
</tbody>
</table>

*(continued)*
**Fibrous Scaffolds Fabricated by Electrospinning for Bone Tissue Engineering**

<table>
<thead>
<tr>
<th>Polymer Type</th>
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<th>Fiber Diameter</th>
<th>In Vitro Studies: Cell Type</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHB, PHBV, PHB - PHBV (50:50 w/w)</td>
<td>14% w/v CHL</td>
<td>2,300–4,000 nm</td>
<td>SaOS-2 and L929</td>
<td>Sombatmankhong et al., 2007</td>
</tr>
<tr>
<td>Silk fibroin (Nang-Lai and DOAE-7 type)</td>
<td>10–40% (w/v), 85% FA</td>
<td>217–610 nm (Nang-Lai), 183–810 nm (DOAE-7)</td>
<td>MC3T3-E1</td>
<td>Meechaisue et al., 2007</td>
</tr>
<tr>
<td>PLA (L- and DL-type)/PEG</td>
<td>8.2% w/w (L-type), 26% w/w (PEG-PLLA)</td>
<td>246 ± 79 nm (L-type), 141 ± 39 nm (DL-type)</td>
<td>MC3T3-E1</td>
<td>Badami et al., 2006</td>
</tr>
<tr>
<td>PLA (L- and DL-type)/PEG</td>
<td>14% w/v HFP</td>
<td>171 ± 66 nm (PEG-PLLA), 889 ± 446 nm (PEG-PDLLA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silk/PEO/nHAp/BMP-2</td>
<td>80.2% (w/v), silk:PEO (82:18 w/w), 5 g nHAP/100 g silk, 3 mg BMP-2/mg silk fibroin</td>
<td>520 ± 55 nm</td>
<td>hMSCs</td>
<td>Li et al., 2006</td>
</tr>
<tr>
<td>Gelatin/HAp</td>
<td>80.20% (w/v), HFP</td>
<td>200–400 nm</td>
<td>MG63</td>
<td>Kim et al., 2005</td>
</tr>
<tr>
<td>PCL-CaCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>PCL-CaCO&lt;sub&gt;3&lt;/sub&gt; = 75:25 and 25:75% w/w CHL:MeOH (75:25% w/w)</td>
<td>760–900 nm</td>
<td>hFOB</td>
<td>Fujihara et al., 2005</td>
</tr>
<tr>
<td>Silk fibroin/PEO</td>
<td>7.5% w/w (80/20 w/w) in H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>700 ± 50 nm</td>
<td>Bone marrow stromal cells (BMSCs)</td>
<td>Jin et al., 2004</td>
</tr>
<tr>
<td>PCL, gelatin, PCL/gelatin</td>
<td>Gelatin: 2.5–12.5% w/v, PCL: 10% w/v, 50:50 blend: 10% w/v TFE</td>
<td>Tens of nm–1 μm</td>
<td>BMSC</td>
<td>Zhang et al., 2005</td>
</tr>
<tr>
<td>PCL</td>
<td>10% w/v CHL</td>
<td>400 ± 200 nm</td>
<td>BMSC</td>
<td>Yoshimoto et al., 2003</td>
</tr>
</tbody>
</table>

**Abbreviations:** BG, bioactive glass; BMSCs, bone marrow-derived mesenchymal stem cells; CECS, N-carboxyethyl chitosan; CHI, chitosan; CHL, chloroform; COL, collagen; DCM, dichloromethane; DMF, N,N-dimethyl formamide; DMSO, dimethyl sulfoxide; FA, formic acid; GPMS, 3-(glycidoxypropyl) trimethoxysilane; HAc, acetic acid; HAp, hydroxyapatite; hFOB, human fetal osteoblast; HFP, 1,1,1,3,3,3-hexafluor-2-propanol; hMSC, human bone marrow-derived mesenchymal stem cell; L929, mouse fibroblasts; MeOH, methanol; MC3T3-E1, mouse calvaria-derived murine preosteoblast cells; MG63, human osteoblasts cells; PAN, poly(acrylonitrile); PCL, poly(e-caprolactone); PEG, poly(ethylene glycol); PEO, poly(ethylene oxide); PHB, poly(hydroxybutyric acid); PHBV, poly(3-hydroxybutyric-co-3-hydroxyvaleric acid); PLA, poly(lactic acid); PLGA, poly(lactic acid-co-glycolic acid); RBM, rat bone marrow cells; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; β-TCP, β-tricalcium phosphate.
occurring enzymes, and therefore, they can be used as biodegradable scaffolds. The most commonly used natural polymers are proteins (collagen, gelatin, fibrinogen, silk, casein, etc.) and polysaccharides (chitosan, cellulose, hyaluronic acid, etc.). Sangsanoh et al. (2009) studied biological evaluation of electrospun chitosan nanofibers and films in vitro with four different cell types: Schwann cells, osteoblast-like cells, keratinocytes, and fibroblasts. Both substrate forms were seen to support cell attachment and especially promoted proliferation of keratinocytes. While Schwann cells attached well on nanofibrous structures, they did not adhere on films. On the other hand, even though osteoblast-like cells and fibroblasts showed good attachment on both nanofibrous and film surfaces they did not proliferate well (Sangsanoh et al., 2009). Jin et al. (2004) produced silk fibroin/poly(ethylene oxide) (PEO) nanofibrous matrices (700–750 nm), examined the behavior of human bone marrow stromal cells (BMSCs), and reported that the matrices supported BMSC attachment and proliferation during 14 days of incubation with extensive matrix coverage (Jin et al., 2004).

A blend of natural and synthetic polymers is advantageous in that they combine the better mechanical properties of synthetic polymers with the biofunctionality of natural ones. In this approach the properties of the scaffold can be changed easily and can be arranged as desired by changing the composition and the ratio of the blend. Ngiam et al. (2009) prepared nano-hydroxyapatite (n-HAp) containing electrospun PLGA and PLGA/collagen (COL) blend nanofibers. Biomineralization of n-HAp on electrospun nanofibers was achieved by using an alternate calcium and phosphate solution dipping method. n-HAp crystallite deposition was predominant on PLGA/COL nanofibers compared to on PLGA, which shows that collagen is a good template for n-HAp nucleation. It was shown that the amount of n-HAp present had a greater influence on early cell behavior, such as attachment and proliferation, than in the intermediate stages because their ALP and total protein expression results for mineralized PLGA and PLGA/COL were comparable during the culture period (Ngiam et al., 2009).

An important requirement in bone tissue engineering is that the scaffold should be highly porous so that cells can be homogeneously distributed throughout the structure. Poor cell infiltration into the structure is reported when electrospun scaffolds are used; the cells are observed more on the surface than the inside of the scaffold. Therefore, it is of great importance to find an approach to produce nanofibrous scaffolds with a higher degree of cell penetration. Ekaputra et al. (2008) used three strategies to solve this problem: selective leaching of a water-soluble fiber phase (PEO or gelatin), the use of micron-sized

FIGURE 10.1
SEM micrographs of (A) unseeded and (B) Saos-2 cell-seeded PHBV-P(L,D,L-LA) (70:30) scaffolds 14 days after incubation. (C) CLSM images of Saos-2-seeded PHBV10 scaffolds 7 days after incubation (top view showing cell-matrix interaction). (D) CLSM of cell infiltration within the scaffold as viewed in cross section (Z-axis direction). (From Ndreu et al., Nanomed, 3, 45–60, 2008.)
fibers as the scaffold, and a combination of micron-sized fibers with codeposition of a hyaluronic acid (HA)-derived hydrogel, Heprasil. In the first procedure, PCL/COL solution was electrospun in the presence of PEO, which was then removed selectively in order to increase the scaffold porosity. In the second approach, PCL/COL was electrospun in micrometer-sized PCL/COL (µmPCL/COL). The third method involved the cospraying of µmPCL/COL matrix with Heprasil. All the scaffold types supported attachment and proliferation of human fetal osteoblasts (hFOB). While selective leaching improved cellular infiltration to a low degree compared to meshes obtained by conventional electrospinning, µmPCL/COL microfibers allowed better penetration. This effect was more pronounced when Heprasil regions were present in the structure.

A fabrication method that can be used to solve the low penetration problem is the use of electrospun nano/microfibers in combination with 3D macrofiber deposition. In this design, macrofibers are expected to play a role in the structural integrity and mechanical properties of the scaffolds, whereas the nano/microfibers can act as a cell entrapment system and a cell adhesion promoter. The influence of this nano/microfibrous network on the behavior of endothelial cells (EC) seeded onto a scaffold based on a starch and PCL blend was studied by Santos et al. (2008). They have shown that the nanofibers influenced cell morphology and the maintenance of the physiological expression pattern. They also showed these EC-seeded scaffolds to be sensitive to pro-inflammatory stimulus; that is, the cells could migrate and form capillary-like structures within a 3D gel of collagen in response to some angiogenesis-stimulating factors (Santos et al., 2008).

A procedure that has been recently reported is on-site layer-by-layer (LBL) tissue regeneration, which involves electrospinning one layer of the polymeric solution, then seeding the cells onto this layer and repeating these steps to create a 3D scaffold with the cells already inside. This method is advantageous in that a highly specific 3D environment can be created by varying the composition of the fibrous layers, fiber thickness, diameter, and orientation as desired, and the neotissue formation can be unambiguously reduced (Agarwal et al., 2009).

Another way to improve cell carrier properties is surface modification of the fibers. A higher degree of spreading and growth of Saos-2 on PHBV and some of its blends has been shown by our research group by treating with oxygen plasma in order to increase hydrophilicity by adding some functional groups on the scaffold surface (Ndreu et al., 2008).

An appropriate biomechanical property is another challenge in bone tissue engineering, since the engineered tissue must be able to retain its shape and withstand the forces applied after implantation. It is well known that a significant part of the bone is constituted of calcium phosphate mineral phase. The presence of bone-inducing inorganic components within bone substitutes enhances calcium phosphate deposition after the osteogenic differentiation process. The apatite minerals and proteins/peptides, as well as drugs encapsulated within the nanofibers, are a promising strategy used for nanofibrous materials to achieve adequate mechanical properties and bone-specific bioactivity (Jang et al., 2009).

HAp plays a crucial role in the biomechanics of bone tissue, especially in increasing the compressive strength. Therefore, an increase in the number of papers reporting the addition of this material to nanofibrous scaffolds is being seen. Gelatin-HAp electrospun nanofibrous scaffolds were produced by Kim et al. (2005) to mimic the human bone matrix, and higher cellular activity was reported with these scaffolds, compared to those prepared with only gelatin. Zhang et al. (2008) have reported the use of electrospinning in combination with an in situ coprecipitation synthesis in the preparation of nanocomposite fibers of HAp/chitosan (CHI). Production of continuous nanofibers was possible after addition of a fiber-forming polymer, ultrahigh molecular weight PEO. Continuous HAp/CHI nanofibers
with a diameter of 214 ± 25 nm were produced successfully, and the HAp nanoparticles were incorporated into the electrospun nanofibers. In vitro cell culture with hFOB showed that the incorporation of HAp nanoparticles into CHI nanofibrous scaffolds led to significantly more bone formation than that with electrospun pure CHI scaffolds (Zhang et al., 2008). In another study, COL and HAp were combined in PLLA, forming three different scaffold types: PLLA, PLLA/HA, and PLLA/COL/HAp. In these scaffolds COL and HAp were expected to provide cell recognition sites and introduce osteoconductivity, an important issue in bone mineralization, respectively. Fiber diameter was found to decrease upon the addition of HAp and COL to PLLA. Human fetal osteoblasts were found to adhere and grow actively on PLLA/COL/HAp nanofibers, with mineral deposition increased by 57% in comparison to PLLA/HAp nanofibers (Prabhakaran et al., 2009).

Some other ingredients introduced to polymers to improve the biomechanical properties and material-cell interaction for bone tissue engineering applications are calcium carbonate (CaCO$_3$) and β-TCP nanoparticles. Fujihara and his co-workers (2005) used a guided bone regeneration (GBR) approach with the aim of producing a calcium-rich and mechanically stable membrane. This was achieved by the preparation of PCL nanofibers containing CaCO$_3$ nanoparticles. A PCL-based membrane was compared with that based on PCL/ CaCO$_3$, and a higher cell adherence was observed in the latter. The tensile strength of PCL nanofibrous membranes decreased when the amount of calcium carbonate nanoparticles was increased; that is, an increase in the amount of CaCO$_3$ resulted in more brittle membranes. However, it was stated that these membranes have sufficient tensile strength for guided bone regeneration (Fujihara et al., 2005).

Functionally graded, nonwoven mats of PCL with β-TCP nanoparticles were produced by using a hybrid, twin-screw extrusion/electrospinning (TSEE) process (Erisken et al., 2008). This procedure allows feeding of various liquid and solid materials, achieving melting, dispersion, pressurization, and electrospinning within a single process, with the composition changing in a time-dependent manner. The potential of these scaffolds for tissue engineering and bone-cartilage interface formation was demonstrated by using mouse calvaria-derived murine preosteoblast (MC3T3-E1) cells. The ability to control the incorporation of β-TCP resulted in a better mimicking of the compositional and structural characteristics of bone tissue (Erisken et al., 2008).

### 10.5.2 Nerve Tissue Engineering

The structure of the nervous system is important for its functionality; therefore, the architecture of the nerve tissue should be well known in order to be able to design the most suitable nerve substitute for nerve regeneration. In nervous tissue the axons are bundled into cables that provide long-distance communication between the brain and spinal cord and the rest of the body. The nerves in the peripheral nervous system (PNS) are composed of bundled motor and sensory axons in parallel arrays in connective tissue and are called the nerve trunk (Schmidt and Leach, 2003). The individual axons and their Schwann cell sheaths are surrounded by endoneurium, which is composed predominantly of oriented collagen fibers. Groups of axons are wrapped by the perineurium, which is composed of many layers of flattened cells (i.e., fibroblasts) and collagen to form fascicles. An outer sheath of loose fibrocollagenous tissue, the epineurium, assembles individual nerve fascicles into a nerve trunk. The vascularization in these nerves is provided by capillaries within the support tissue of the nerve trunk, or by vessels penetrating into the nerve from surrounding arteries and veins. Within the central nervous system (CNS), the brain and spinal cord, such bundled together axons are called nerve tracts. Typically, the neuron
consists of dendrites, an axon, and a cell body (Schmidt and Leach, 2003). A gray matter localized in the center of the spinal cord is composed of the cell bodies of excitatory neurons, glial cells, and blood vessels. The gray matter is surrounded by white matter, which provides protection and insulation of the spinal cord. White matter consists of axons and glial cells, such as oligodendrocytes, astrocytes, and microglia. Oligodendrocytes myelinate the axons in the CNS, while astrocytes are active in the blood-nerve barrier, separating the CNS from blood proteins and cells. Fascicles, axons in bundles, come out from the white matter and then exit the encasing bone of the spinal column. Following travel through the PNS-CNS transition zone, nerves enter the PNS.

Nervous system injuries may result in permanent functional disabilities, and thus have a significant impact on the patient’s quality of life. In severe damage of the peripheral nervous system, self-regeneration of the tissue is difficult, and it is almost impossible in the central nervous system (Meek et al., 2002; Schmidt and Leach, 2003). For that reason, the repair of the damaged tissue in the nervous system is complicated. Surgical interventions (end-to-end anastomosis) are used for small nerve gaps (Sanghvi et al., 2004); however, for long gaps (longer than 5 mm) the common treatment is to use autografts. The inherent drawbacks of autografts, such as limited supply, permanent loss of the nerve function at the donor site, and the requirement of a second surgery, are great challenges for researchers and compel them to investigate alternative treatments (Millesi, 1991; Terzis et al., 1997).

The rapidly developing alternative, nerve tissue engineering, aims to restore the function of damaged neural tissue through use of native cells and cell carriers. The cells commonly used in nerve tissue engineering are mature neurons, stem cells, and supportive cells, such as Schwann cells, astrocytes, and oligodendrocytes. The construct developed by nerve tissue engineering should imitate the anatomic structure of the nervous system for proper performance. When the native architecture of the nerve tissue is considered, it becomes apparent that a directional cell growth is a prerequisite for functional nerve regeneration. The desired physical properties of a cell carrier are biodegradability, high porosity, and also a cell guiding tool, such as an internal oriented matrix with appropriate chemical structure (ECM proteins, especially laminin, for nerve tissue). Topographical cues for cell migration could serve to mimic the native nerve fascicles (Hudson et al., 1999). The guidance needed for the tissue-engineered nerve construct may be achieved by the use of films with patterned surfaces, or longitudinally oriented microchannels and fibrous scaffolds.

Fibrous scaffolds to be used in nerve regeneration could be produced by electrospinning and self-assembly (Table 10.2). Since they are inherently porous and have a high surface area-to-volume ratio, these fibrous scaffolds promote attachment, survival, and growth of cells (Chiu et al., 2005). An architecture similar to ECM makes fibrous scaffolds a promising tool for nerve tissue engineering. The ideal scaffold should be biocompatible to avoid inflammatory response, be biodegradable, and have appropriate mechanical properties to provide the support required for proper healing (Hudson et al., 1999). The material of the cell carrier is a very important determinant in achieving a scaffold suitable for nerve tissue engineering. The degradation rate and mechanical properties of synthetic polymers such PLLA, PLGA, PHBV, and PCL can be controlled by chosing the appropriate compound, and as such, they are very suitable for tissue engineering. Natural materials such as PHBV, collagen, gelatin, chitosan, and especially laminin have specific physical properties and biomolecular recognition sites as in native tissue and, therefore, they have been increasingly investigated as fibrous scaffolds for nerve tissue regeneration (Cao et al., 2009). Blends of polymers have also been extensively used in nerve tissue engineering to achieve the desired scaffold properties. For instance, natural polymers lack proper
<table>
<thead>
<tr>
<th>Polymer Type</th>
<th>Polymer Concentration/Composition/Solvent</th>
<th>Fiber Diameter</th>
<th>In Vitro Studies: Cell Type</th>
<th>In Vivo Studies: Nerve Injury Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laminin-PLLA blend</td>
<td>10% w/v; 1:250, w:w HFP</td>
<td>100–500 nm (RF)</td>
<td>Rat PC12 cells</td>
<td>—</td>
<td>Koh et al., 2008</td>
</tr>
<tr>
<td>PCL</td>
<td>10% w/v CHL:MeOH 3:1, v:v</td>
<td>750 ± 100 nm (RF)</td>
<td>Brain-derived neural stem cells</td>
<td>—</td>
<td>Nisbet et al., 2008</td>
</tr>
<tr>
<td>PCL-pp*</td>
<td>16% w/w DCM:MeOH 8:2, v:v</td>
<td>2.26 ± 0.08 µm (RF)</td>
<td>Human Schwann cells</td>
<td>—</td>
<td>Chew et al., 2008</td>
</tr>
<tr>
<td></td>
<td>CHL:MeOH 1:1, v:v</td>
<td>1.03 ± 0.03 µm (AF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHL:MeOH 3:1, v:v</td>
<td>100–500 nm (RF)</td>
<td>Mouse (129 strain)</td>
<td>—</td>
<td>Thouas et al., 2008</td>
</tr>
<tr>
<td>PCL-PP</td>
<td>20% w/v DCM: DMF 80:20, v:v</td>
<td>250 nm (RF/ AF)</td>
<td>Mouse embryonic stem cells CE3 and RW4</td>
<td>—</td>
<td>Xie et al., 2009b</td>
</tr>
<tr>
<td>PCL-PP</td>
<td>10–20% w/v DCM: DMF (50 ± 3 nm shell thickness)</td>
<td>220 ± 36 nm</td>
<td>Chick embryonic (E8) dorsal root ganglia explant</td>
<td>—</td>
<td>Xie et al., 2009a</td>
</tr>
<tr>
<td>PCL/GEL</td>
<td>10% w/v; 1:1, w:w TFE</td>
<td>232 ± 194 nm (RF)</td>
<td>Rat Schwann cell line (RT4-D6P2T)</td>
<td>—</td>
<td>Gupta et al., 2009</td>
</tr>
<tr>
<td>PCL/CHI</td>
<td>6% w/w; 70:30, w:w HFP</td>
<td>189 ± 56 nm (RF/ AF)</td>
<td>Neonatal mouse cerebellum C17.2 stem cells</td>
<td>—</td>
<td>Ghasemi-Mobarakeh et al., 2008</td>
</tr>
<tr>
<td></td>
<td>PCL:CHI, 75:25, w:w PCL in HFIP, CHI in TFA/DCM</td>
<td>190 ± 26 nm (RF)</td>
<td>Rat Schwann cell line RT4-D6P2T</td>
<td>—</td>
<td>Prabhakaran et al., 2008</td>
</tr>
</tbody>
</table>

* PCL-PP: PCL coated with polypyrrole

(continued)
### TABLE 10.2 (CONTINUED)

Nano/Microfibrous Textures Used for Nerve Tissue Engineering

<table>
<thead>
<tr>
<th>Polymer Type</th>
<th>Polymer Concentration/Composition/Solvent</th>
<th>Fiber Diameter</th>
<th>In Vitro Studies: Cell Type</th>
<th>In Vivo Studies: Nerve Injury Model</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLLA</td>
<td>3 w/w% CHL</td>
<td>524 ± 305 nm (RF/AF)</td>
<td>Dorsal root ganglia explant</td>
<td>—</td>
<td>Corey et al., 2007</td>
</tr>
<tr>
<td>PLLA</td>
<td>1% w/w</td>
<td>250 nm (RF)</td>
<td>Neonatal mouse cerebellum C17.2 stem cells</td>
<td>—</td>
<td>Yang et al., 2005</td>
</tr>
<tr>
<td>PLLA</td>
<td>3% w/w</td>
<td>1.25 µm (RF)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PLLA</td>
<td>2% w/w</td>
<td>300 nm (AF)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PLLA</td>
<td>5% w/w</td>
<td>1.5 µm (AF)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PLLA</td>
<td>8% w/w CHL</td>
<td>1.2–1.6 µm (AF)</td>
<td>Embryonic (E9) chick dorsal root ganglia explants and rat Schwann cells</td>
<td>—</td>
<td>Wang et al., 2009</td>
</tr>
<tr>
<td>PLLA/PLGA</td>
<td>10% w/v THF and DMF</td>
<td>510 ± 380 nm (PLLA, RF)</td>
<td>Mouse embryonic cortical neurons</td>
<td>—</td>
<td>Nisbet et al., 2007</td>
</tr>
<tr>
<td>PLLA, 75/25 (coated with polyethylene)</td>
<td>6.5% w/w (RF)</td>
<td>520 ± 150 nm (RF)</td>
<td>PC12 cells and rat embryonic hippocampal neurons</td>
<td>—</td>
<td>Lee et al., 2009</td>
</tr>
<tr>
<td>PLLA, 75/25 (coated with polyethylene)</td>
<td>7.0% w/w (AF)</td>
<td>430 ± 180 nm (AF)</td>
<td>(85 ± 41 nm shell thickness)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(ε-caprolactone-co-ε-caprolactone co-ethyl ethylene phosphate) (PCLEEP)</td>
<td>12% w/w DCM</td>
<td>5.08 ± 0.05 µm (AF)</td>
<td>—</td>
<td>15 mm nerve lesion gap in rat sciatic nerve (cell-free)</td>
<td>Chew et al., 2007</td>
</tr>
<tr>
<td>Blend of PLGA 75:25/PCL</td>
<td>5.5% w/w PCL: 4% w/w PLGA CHL:MeOH 3:1</td>
<td>279 ± 87 nm (RF)</td>
<td>—</td>
<td>10 mm nerve gap in rat sciatic nerve (cell-free)</td>
<td>Panseri et al., 2008</td>
</tr>
<tr>
<td>Fibers Fabricated by Self-Assembly</td>
<td>RAD16-I* (Arg-Ala-Asp) and RAD16-II**</td>
<td>Cell culture media</td>
<td>10–20 nm</td>
<td>Injection of self-assembled (20 µl) in rat right leg (cell-free)</td>
<td>Holmes et al., 2000</td>
</tr>
<tr>
<td>Peptide</td>
<td>Cell culture media</td>
<td>Nanofiber Diameter (nm)</td>
<td>Cell Type</td>
<td>Injection Method</td>
<td>Depth</td>
</tr>
<tr>
<td>------------------</td>
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<td>-----------</td>
<td>------------------------------------------------------</td>
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</tr>
<tr>
<td>IKVAV (Ile–Lys–Val–Ala–Val)</td>
<td>Cell culture media</td>
<td>5–8 nm</td>
<td>Mouse (E13) neural progenitor cells</td>
<td>Spinal cord injection (T10) at a depth of 1.5 mm in rats (cell free)</td>
<td>—</td>
</tr>
<tr>
<td>RADA16-I (Arg–Ala–Asp–Ala)</td>
<td>Milli-Q water</td>
<td>10 nm</td>
<td>—</td>
<td>1.5–2 mm deep cut wound in the optic tract, superior colliculus in hamster brain (cell free)</td>
<td>—</td>
</tr>
<tr>
<td>RADA16-I peptide</td>
<td>Cell culture media</td>
<td>10 nm</td>
<td>Rat neural progenitor cells and Schwann cells</td>
<td>1 mm tissue removal by a transection in spinal cord dorsal column (between C6 and C7) in rats</td>
<td>—</td>
</tr>
</tbody>
</table>

**Abbreviations:** RF, random fiber; AF, aligned fiber; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol; PCL, poly(ε-caprolactone); CHL, chloroform; MeOH, methanol; DCM, dichloromethane; DMF, dimethylformamide; GEL, gelatin; TFE, 2,2,2-trifluoroethanol; CHI, chitosan; HFIP, hexafluoro-2-propanol; TFA, trifluoroacetic acid; PLLA, poly(L-lactic acid); PLGA, poly(lactic acid-co-glycolic acid).
mechanical properties and also lose mechanical strength very quickly due to degradation, while synthetic polymers alone are generally too hydrophobic and lack binding sites for cell adhesion (Gupta et al., 2009). Consequently, a blend or a graft of natural and synthetic polymers is an effective way to obtain scaffolds with desirable properties. A summary of nano/microfibrous scaffolds used in vitro and in vivo in nerve regeneration studies is given in Table 10.2.

Electrospinning is a straightforward technique to obtain random and aligned nano/microfibers. Even though the randomly oriented fibers are easier to obtain and are more frequently used in nerve tissue engineering, the use of the aligned fibers is a challenging but rewarding approach in mimicking the unidirectional orientation to maintain function of the nerve tissue (Table 10.2). Fibers aligned longitudinally (parallel to nerve axis) may provide a suitable environment that encourages contact guidance and the directional neuronal/axonal growth desired in nerve tissue engineering. Yang et al. (2005) observed the positive effect of contact guidance by aligned fibrous scaffolds (diameters of aligned micro- and nanofibers: 1.5 µm and 300 nm, respectively) on neural stem cells that had been induced to elongate neurites parallel to the fiber axis. The cell elongation and the neurite outgrowth were random on the randomly oriented mats (diameters of random micro- and nanofibers: 1.25 µm and 250 nm, respectively), proving the significance of guidance. This guidance was observed on aligned nano/microfibers regardless of fiber diameter. The effect of fiber arrangement was also observed in our studies carried out with neural stem cells seeded on fibrous mats. Electrospun PHBV-PLGA fibers were produced under optimized conditions (PHBV-PLGA solution: 1:1 w/w, 15% w/v, in chloroform: dimethylformamide (CHL:DMF)) with a diameter of ca. 1.5 µm (Yucel et al., 2010). The stem cells seeded on the aligned fibers were well oriented along the axis of the fibers, while the cells on the random fibers appeared to be in clusters distributed in all directions (Figure 10.2). Similar results were also obtained with dorsal root ganglion (DRG) explants cultured on PLLA nanofibers (Patel et al., 2007). Significant extension of neurites was observed on aligned fibers, while there was no neurite outgrowth on randomly oriented fibers. Like other cells, human Schwann cells responded to the fiber orientation with elongation along the fibers.

**FIGURE 10.2**
CLSM images of neural stem cells on fibronectin-coated PHBV-PLGA scaffolds. (A) Randomly oriented and (B) aligned fibers (100x; scale bar, 250 µm; two-sided arrow, direction of fiber axis; Acridine orange stain). Insets show the SEM images of the fibrous mats.
In addition, aligned electrospun fibers appeared to enhance Schwann cell maturation more than the randomly oriented fibers did, as was shown by the upregulation of the myelin-specific gene. In vitro results were confirmed by the in vivo studies in which anatomical and functional measurements showed that constructs with aligned polymer fibers significantly enhanced nerve regeneration in 17 mm peripheral nerve gaps in rats over those of randomly oriented fibers (Kim et al., 2008).

Micro- and nanoscale topography was shown to play an important role in neural stem cell differentiation. Yang et al. (2005) observed that the mouse neural stem cell differentiation rate might depend on the fiber dimension. The differentiation rate on nanofibers 300 nm in diameter was twice as high as on microfibers with a 1.5 µm diameter. However, these results on the effect of fiber diameter on neural cell differentiation are controversial. Christopherson and his colleagues (2009) found that under differentiation conditions, rat neural stem cells stretched multidirectionally and preferentially differentiated into oligodendrocytes on fibers with a diameter of 283 nm, but they elongated along a single fiber axis and preferentially differentiated into neuronal lineage on fibers 749 nm and 1,452 nm thick. Despite these conflicting results, topographical and biological cues seem to be important in the control of stem cell behavior.

Electrospinning approaches involving blending, coaxial extrusion, and emulsion spinning are used in the incorporation of bioactive agents like proteins and growth factors to enhance cell attachment, growth, differentiation, and survival of cells in nerve tissue engineering applications. Incorporation of laminin, a neurite-promoting basement membrane protein, to a synthetic polymer by blending was reported as a more efficient method to produce biomimetic scaffolds than covalent immobilization and physical adsorption of the bioactive agents (Koh et al., 2008). In a recent study, nerve growth factor (NGF), a common neurotrophic factor involved in peripheral nerve regeneration, was incorporated in poly(L-lactide-co-ε-caprolactone) fibers by emulsion electrospinning (Li et al., 2010). Pheochromocytoma 12 (PC12) cells, which differentiate into a neuronal phenotype in the presence of NGF, were used in a study where NGF was released from fibrous mats for a sustained period, revealing its bioactivity. A glial cell-derived neurotrophic factor (GDNF), important in motor neuron survival, was entrapped in aligned biodegradable polymeric fibers (a copolymer of ε-caprolactone and ethyl ethylene phosphate (PCLEEP)) (Chew et al., 2007). An in vitro release profile of GDNF showed that after an initial burst release of about 30%, the remaining GDNF was released in a fairly sustained manner for almost 2 months.

Electrical stimulation is another tool that affects neurite extension. Electrical stimulation using an external electrical field and an electroconductive polyprrole (PPy) film was shown to enhance neurite extension in vitro (Schmidt et al., 1997). Xie et al. (2009a) cultured DRG explants on conductive (PPy) core-sheath nanofibers electrospun with PCL, and results showed that upon electrical stimulation, the rate of neurite extension was enhanced on both random and aligned nanofibers without any change in the direction of the neurites’ extension. In another study, PPy was deposited on electrospun PLGA fibers to investigate the response of PC12 cells (Lee et al., 2009). Upon application of 10 mV/cm on PPy-PLGA scaffolds, PC12 cells exhibited 40–50% longer neurites and 40–90% more neurite formation than unstimulated cells on the same scaffolds.

In conclusion, the topography of nano/microfibrous scaffolds closely resembles that of the natural ECM, and this is an important factor in tissue engineering. Considering the architecture of longitudinally oriented nerve tissue, uniaxially aligned fibers could create an appropriate environment for guided nerve regeneration. The use of the aforementioned tools with aligned fibrous scaffolds could be a promising approach in the design of a biomimetic substitute for nerve regeneration.
10.5.3 Cardiac Muscle Tissue Engineering

The heart is the center of the circulation system and distributes blood throughout the body. The function of the heart is vital to supply oxygen and nutrients to, and remove waste products from, the body via the blood in order to maintain the balance that is necessary to sustain life (Vander et al., 1994). Strong muscular contractions in the ventricles pump blood out of the heart and into the circulatory system. These muscular contractions are produced by the muscle tissue that makes up the walls of the ventricles. Healthy heart muscle wall is composed of three layers: a muscular sheet, the myocardium, lined on either side by two collagenous membranes (containing type I and type III collagen and elastin); the endocardium, which is populated by endothelial cells; and the epicardium, also called visceral pericardium (van de Graaff, 1998). The myocardium is the layer of functional beating muscle that consists of fibroblasts and highly oriented cardiomyocytes (muscle fibers) in a matrix of collagen. The cardiomyocytes are connected end to end in the longitudinal direction and side by side in the transverse direction (Parker and Ingber, 2007). Collagen types I and III are the predominant interstitial collagens in the myocardium that generate structural integrity for the adjoining cardiomyocytes, providing the means by which myocyte shortening is translated into overall ventricular pump function. Basement membrane components include laminin, entactin, fibronectin, collagen type IV, and fibrillin, and proteoglycans include chondroitin sulfate, dermatan sulfate, and heparan sulfate (Kassiri and Khokha, 2005). The cardiomyocytes facilitate conduction of the electrical signals needed to initiate contractile movement in order to pump blood out of the ventricles (Vander et al., 1994). The elementary myocardial functional units are not lone cardiomyocytes, but rather are multicellular assemblies of these highly oriented cells. The cardiomyocytes are connected with intercalated discs that integrate individual electrical activation and contraction into a pumping action. Gap junctions in these intercalated discs allow the action potential to travel through the membranes of the myocytes, thus facilitating signal propagation and a synchronized contractile pulse (Vander et al., 1994).

Heart failure, stemming from cardiovascular diseases, is the number one cause of death in industrialized countries. About 5 million people in the United States have heart failure, and the number is growing; heart failure contributes to or causes about 300,000 deaths each year. The inability of the heart to deliver sufficient blood to meet the body’s metabolic requirements leads to heart failure. The most common causes are coronary artery disease and hypertension (high blood pressure), which damage the myocardium. Damage to any part of the intricate structure of the heart, though, can impair cardiac performance and result in heart failure; examples include diseases of the heart valves, the electrical conduction system, or external pressure around the heart, due to constriction of the pericardial sac in which the heart is located (Jawad et al., 2007). Congenital heart disease is also a considerable problem worldwide, affecting approximately 1% of infants (Wu et al., 2006). Congenital heart defects such as atrial septal defect, ventricular septal defect, double outlet ventricles, and hypoplastic left heart syndrome are associated with aplastic, defective, or necrotic myocardial structures, where patch closure, correction of the defect, or revascularization is usually required (Kofidis et al., 2002). Although the currently available prostheses are adequate for restoring ventricular geometry and maintaining ventricular pressure, and thus may be life saving, they do not actively adapt to the physiological environment and mechanical demands, as they are nonliving materials. When implanted into the immature heart of a child, these materials do not grow with the pediatric patient. Therefore, these prostheses have limited durability and are prone to infection, immunologic reactivity, and thrombosis, which often requires repeated operations (Mirensky and...
Breuer, 2008). These constructs are also subject to obstructive tissue ingrowths and fibrotic responses with shrinkage and calcification leading to graft failure (Endo et al., 2001). At that point, tissue engineering provides a new experimental approach aimed at vital, semi-autologous replacement structures with the capacity of growth and adaptability.

The negligible ability of adult cardiomyocytes to proliferate has triggered an intense search for progenitor cells that can be used in repairing damaged myocardium. It is clear that natural cardiomyocyte regeneration, including differentiation of progenitors residing in the myocardium or the recruitment of stem cells from outside (e.g., from endothelial cells or from the bone marrow), is insufficient to overcome cardiomyocyte death in the acutely or chronically damaged heart (Nakamura and Schneider, 2003). The potential of cardiac stem cells resident in the heart, embryonic stem cells, bone marrow stem cells, and fetal stem cells (from tissues of umbilical cord) to differentiate into cardiomyogenic lineage either in vivo or in vitro in the presence of differentiation factors, conditioned media, or cocultures has been investigated, but there is still a need for elaboration and improvement in the procedures to be able to obtain high numbers of fully differentiated cardiomyocytes. Since the main concern in myocardial tissue engineering is the functionality of the final construct, the majority of in vitro studies use rat neonatal cardiomyocytes or cardiomyocytes derived from embryonic stem cells that are already able to contract.

Fibrous scaffolds whose fiber diameter ranges from several microns to submicron sizes (Table 10.3) are increasingly gaining importance over spongy scaffolds with random pores used to obtain tissue-engineered cardiac muscle. The main obstacle in overcoming the issues involved in the engineering of cardiovascular grafts is production of substitutes that can withstand the pulsation (i.e., have adequate shape recovery), the high pressure, and the flow rate of the blood (Nisbet et al., 2009). An additional challenge in cardiovascular tissue engineering is the necessity for mechanical and biological compliance. Polymers naturally found in the cardiac tissue, like collagen and elastin, are good choices as scaffold materials, since they carry attachment sites for cells, and especially elastin has elastic properties, but they have poor mechanical properties. On the other hand, synthetic polymers have higher mechanical strength, but have problems, such as being too hydrophobic and rigid, lacking cell binding functional groups, or having inappropriate degradation rates (rapidly degrading or nondegrading). Thus, as mentioned earlier, blends or copolymers of synthetic or natural polymers are employed often in the production of scaffolds with the desired properties (Zong et al., 2005, Li et al., 2006a, 2006b). Currently cell survival in 3D cardiac grafts of spongy design is a critical issue; generally the cells are concentrated in the outer regions of the grafts and are scarce in the inside due to restricted transportation of nutrients and waste in and out of the scaffold, resulting in an undesired core effect (Bursac et al., 1999). Electrospinning enables us to fabricate highly porous scaffolds with extensively interconnected pores that allow cell-to-cell contact and migration in all directions, facilitate the transportation of nutrients and metabolites, and encourage blood vessel formation. All of these combined help cell survival throughout the scaffold, including the central portion. Electrospun mats of PCL with random, unaligned fibers were seeded with cardiomyocytes by Ishii et al. (2005), and the mats were stacked to generate a 3D cardiac graft. Five layers of these mats were successfully stacked without any indication of core ischemia. The individual layers adhered intimately, morphological and electrical communication between the layers was established, and synchronized beating was also observed. This study demonstrated the feasibility of obtaining a functional 3D cardiac graft from cell sheets supported by nanofibers mimicking the native structure of cardiac ECM. Fiber density on the electrospun mats could be tailored depending on the intended use. For example, electrospun mats with tight fiber spacing may act as a sieve to prevent blood cell
<table>
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<tr>
<th>Polymer Type</th>
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<td>PGA</td>
<td>—</td>
<td>13 μm</td>
<td>Neonatal rat cardiomyocytes</td>
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<tr>
<td>Collagen type I</td>
<td>25 mg/ml  Dl water-HCl (pH 2.5-3.0)</td>
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<tr>
<td>PCL</td>
<td>10% (w/w) CHL:MeOH (1:1)</td>
<td>100 nm–5 μm (RF)</td>
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<td>PLLA</td>
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<td>Blend of PGLA (90:10)/PLLA</td>
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<td>Blend of PLGA/gelatin/α-elastin</td>
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<td>Biodegradable polyurethane</td>
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<td>Blend of PHBV/P(L-D,L)LA/PGS</td>
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<td>1.10–1.25 μm (AF)</td>
<td>Human Wharton’s jelly MSCs</td>
<td>Kenar et al., 2009</td>
</tr>
</tbody>
</table>

**Abbreviations:** PGA, poly(glycolic acid); PCL, poly(e-caprolactone); PLLA, poly(L-lactide); PGLA, poly(glycolide-co-lactide); PLGA, poly(lactide-co-glycolide); PEG, poly(ethylene glycol); PDLLA, poly(D,L-lactide); PHBV, poly(3-hydroxybutyrate-co-3-hydroxyvalerate); P(L-D,L)LA, poly(L-D,L-lactic acid); PGS, poly(glycerol sebacate); CHL, chloroform; MeOH, methanol; DMF, N,N-dimethyl formamide; HFP, 1,1,1,3,3,3-hexafluoro-2-propanol; DCM, dichloromethane; PANi, polyaniline (conductive polymer).
leakage or provide a homogeneous surface to obtain intact endothelial cell sheets, or act as a supporting outer surface in cardiovascular scaffolds (Hong et al., 2009; Soletti et al., 2010). Conversely, fiber spacing and density may also be adjusted and loosened to promote cell infiltration in the myocardial patches.

Anisotropy in the architecture of the constructs plays an important role in achieving compliance and functionality in cardiovascular tissue engineering. Parallel submicron fibers may be produced to have circumferential orientations similar to those of the cells and fibrils of the medial layer of the native artery (Xu et al., 2004). It was demonstrated that anisotropic poly(ester urethane)urea scaffolds exhibit mechanical properties closely resembling those of the native pulmonary heart valve leaflets (Courtney et al., 2006). Cardiac muscle contracts like a syncytium owing to the multicellular assembly of myofibers oriented parallel to each other, connected end to end via intercalated discs in the longitudinal direction and connected side to side in the transverse direction. It is this cellular arrangement that integrates individual contraction into a synchronous pumping action, whereas myofiber disarrays are known to cause arrhythmia. It was shown by Black et al. (2009) that cardiomyocyte alignment augments the twitch force. Prior to emergence of techniques to produce aligned nano/microfibrous scaffolds, anisotropy had been introduced to the cardiomyocytes on the polymer scaffolds in three different ways: (1) by micropatterning (e.g., using biodegradable, elastomeric polyurethane films patterned by microcontact printing of laminin (McDevitt et al., 2003)), (2) by cyclic stretching (e.g., stretching of cardiomyocyte-seeded scaffolds (Akhyari et al., 2002; Zimmermann et al., 2002, 2004; Zimmermann and Eschenhagen, 2003; Fedak et al., 2003)), and (3) by applying electrical signals designed to mimic those in the native heart to the tissue engineered constructs (e.g., rat cardiomyocytes resuspended in Matrigel were seeded onto collagen sponges (Radisic et al., 2004)). Electrospinning, on the other hand, offers a much simpler way to achieve the anisotropy and allows greater control over composition, mechanical properties, and structure of a graft, thus making it easier to match the properties of the scaffold and the native tissue. It was shown by Zong et al. (2005) that cardiomyocytes can align parallel to micron-size fibers. Their electrospun mats with oriented fibers of PLLA and PLGA were used to assess the influence of scaffolds on primary cardiomyocyte attachment, structure, and function. The primary cardiomyocytes showed a preference for relatively hydrophobic surfaces (PLLA), where they aligned along the direction of fibers and developed mature contractile machinery (sarcomers). The only drawback of these aligned fiber scaffolds was the inability of the cardiomyocytes to penetrate into the scaffold due to the inadequate space among the fibers. A very similar phenomenon was reported by Evans et al. (2003), who observed that rat embryonic cardiac myocytes were unable to penetrate the 3D aligned collagen fiber scaffold with a pore size of 1–10 microns on the outer surface of a tubular scaffold. Aligned fiber scaffolds with a fiber density suitable for cell penetration were produced by our group by using a frame collector to obtain aligned fibers (Kenar et al., 2010). At least eight or nine Wharton's jelly (WJ) MSC layers could be obtained in a single mat of average thickness of $12 \pm 3 \text{um}$. Both cytoskeletal and nuclear alignments of the cells were observed (Figure 10.3). A wrapping method was used to obtain a thicker homogeneous construct, which maintained its original anisotropic property. Another technique, microintegration, was developed by Stankus et al. (2006), where simultaneous electrospraying of cells and electrospinning of poly(ester urethane)urea (PEUU) was utilized in order to fabricate thicker constructs with more uniform cell incorporation for use in cardiovascular tissue engineering applications. Smooth muscle cell-microintegrated PEUU was strong, flexible, and anisotropic, and Trypan blue staining revealed no significant decrease in cell viability as a result of the fabrication process.
Studies carried out with aligned fiber mats with fiber diameters ranging from 0.25 to 3.00 μm show that cells grown on them align equally well along the direction of the fibers (Evans et al., 2003; Yost et al., 2004; Zong et al., 2005; Rockwood et al., 2008; Kenar et al., 2010). The single study carried out on cardiomyocyte function on electrospun random and aligned polymer fibers showed that cellular alignment enhances cardiomyocyte maturation; cells grown on aligned electrospun polyurethanes had significantly lower steady-state levels of atrial natriuretic peptide (ANP) and, as a result, released less ANP over time than cardiomyocytes on random fibers (Rockwood et al., 2008).

In conclusion, nanofibers are novel scaffold materials for tissue engineering, and the accumulating data show them to be superior to most other designs due to the versatility of the approach. They are especially suited to the highly organized nature of biological tissues. With developments in the processing systems, they will become the major scaffold type.

References


Nakamura T, Schneider MD. 2003. The way to a human’s heart is through the stomach: visceral endoderm-like cells drive human embryonic stem cells to a cardiac fate. *Circulation* 107: 2638–2639.


