

Chapter 2

Progenitor Cell Tissue Engineering

Scaffold Design and Fabrication

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Abstract A critical aspect of cell and tissue engineering is the design of non-cellular constructs that closely interact with cells to provide the necessary conditions for intended function. Properties such as surface chemistry, mechanical strength, porosity, and rates of degradation are important elements of a cellular support system and can deeply influence the fate of progenitor cells. To achieve the goal of successful implantation and proliferation of cells in a particular region, various biomaterials and fabrication processes have been explored. Lately, techniques with origins outside the field of biology or medicine have been used to create highly controlled morphologies. This chapter will review the fundamentals of scaffold design as it relates to brain-based therapies and give some examples of fabrication techniques.

Keywords Scaffold • Tissue engineering • Electrospinning • Rapid prototyping • Central nervous system

Abbreviations

3DP	Three-dimensional printing
BDNF	Brain-derived neurotrophic factor
CAD	Computer-aided design
CNS	Central nervous system
DCM	Dichloromethane
ECM	Extracellular matrix
ESC	Embryonic stem cell
FDM	Fused deposition modeling
GMP	Good manufacturing practices
HFIP	Hexafluoro-2-propanol

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MSC	Mesenchymal stromal cell
NGF	Nerve growth factor
P(LLA-CL)	Poly(L-lactic acid-co-caprolactone)
PCL	Poly(caprolactone)
PGA	Poly(glycolic acid)
PLGA	Poly(lactic-co-glycolic acid)
PLLA	Poly(L-lactic acid)
PNS	Peripheral nervous system
PVDF	Poly(vinylidene fluoride)
RGD	Arginine-glycine-aspartic acid
RP	Rapid prototyping
SLA	Stereolithography
SLS	Selective laser sintering
THF	Tetrahydrofuran
TIPS	Thermally induced phase separation

2.1 Introduction

The human body constantly undergoes regeneration of tissue in a natural cycle of remodeling. However, various insults, including disease or trauma, can cause significant amounts of damage that surpass the natural regenerative capabilities of the body. Many current medical techniques specialize in the removal of tissue but not the replacement and regrowth of it. The advent of tissue engineering has brought with it the possibility of filling this void in the field of medicine (Guillot et al. 2007; Langer and Vacanti 1993; Thomson 1995). Tissue engineering can be seen as an attempt at mimicking the natural structure, function, and arrangement of cells to achieve a desired goal (Ma 2008). Many times, the goal is to regenerate damaged or destroyed tissue. To meet this objective, one must be able to establish and control conditions on the cellular scale. Tissues (and organs to a greater extent) gain their remarkable capabilities by tightly controlling the organization of their cellular framework. The parameters involved in cellular differentiation and growth are consolidated in the term *microenvironment*. Owing to potentially divergent cellular outcomes control of the microenvironment is especially vital when working with progenitor cells. Minute differences in microenvironment properties can cause significantly different results. The crux of tissue engineering is striving to create analogues or replicates of natural microenvironments, using natural or synthetic materials. The field of tissue engineering goes beyond the historical confines of the life sciences by taking principles and knowledge from years of experience in materials science and manufacturing research and applying them to cellular constructs, creating microenvironments that promote robust proliferation of introduced cells in a living system. Tissue engineering must also integrate ethical, economical, and regulatory factors in the design process (Griffith and Naughton 2002; Laurencin and Nair 2008). Furthermore, many phenomena researched and understood for decades have been

performed in a two-dimensional experimental setting. Tissue engineering must expand the understanding of cellular function as it exists in the body, throughout three-dimensional space (Lee et al. 2008).

The characteristics of a cellular microenvironment are in large part directed by the properties of the extracellular matrix (ECM), generally consisting of fibrous proteins, proteoglycans, and polysaccharides. The composition of the ECM varies from tissue to tissue, providing the proper scaffolding needed for each particular area. Components of the ECM exhibit both chemical and mechanical signals to direct the differentiation and organization of cells (Bissell et al. 1982; Blaschke et al. 1994). Providing an appropriate analogue for native ECM is a critical objective of tissue engineering, especially when working with progenitor cells (Chai and Leong 2007; Lelievre et al. 1998).

Advancements in nanotechnology have given researchers the ability to fabricate structures with dimensions of less than 100 nm, with ever increasing levels of control and reproducibility. Furthermore, the use of biomaterials has become more prevalent in the study of nanoscale fabrication. Several fabrication techniques provide the capability of generating implantable scaffolds using biomaterials. The ability to create structures with such small dimensions presents the opportunity to build functional composite structures of different cell or tissue types at the cellular scale, described by Nishimura as a “precursor tissue analog” (Nishimura et al. 2003). However, limited progress has been made in the field of brain-based tissue engineering. This chapter will review the requirements of scaffolds used in tissue engineering and describe some of the methods used to fabricate scaffolds. It will also give examples of therapies specific to neurological applications and the unique challenges presented in applying tissue engineering to the central nervous system (CNS).

2.2 Tissue Scaffold Goals

To properly design a tissue scaffold, the objectives of the intended therapy as well as the constraints inherent within the zone of prospective therapy must be clearly defined. Some requirements are universal regardless of location in the body, whereas others are dependent on the location of implantation and specific mechanism of the therapy. The affinity of the scaffold to cells of a particular type and the creation of a suitable microenvironment are vital requirements for the success of a scaffold. Specific organizational patterns are responsible for much of the specialized functionality of tissues and organs. Clearly, the structure of a scaffold must foster development in a pattern similar to its natural counterpart to achieve a restorative outcome. For example, a spinal cord tissue scaffold should include aligned pathways along the axis of the spinal cord to facilitate parallel axonal growth. These features and properties are heavily influenced by the chemical and physical attributes of the scaffold.

For cells to proliferate and thrive *in vivo*, a scaffold must be designed with the limitations of mass transport in mind. Nutrients and waste products must be able to

transit to and from the local vascular bed via diffusion. In time, angiogenesis may provide augmented mass-transfer capability within the scaffold, but sufficient nutrient supply must be present at the time of implantation.

The goal of the therapy should also take into consideration whether or not the therapy is permanent or temporary. For particular applications, the goal of a tissue scaffold may be to limit or inhibit incorporation with surrounding native tissue. A temporary therapy could be an introduction of cells into an area where the intention is not to regrow tissue but rather to provide trophic or stabilizing effects to surrounding cells in situ, typically after trauma (Harting et al. 2008). Such scaffolds would be removed after a period of time. A permanent therapy would in most cases attempt to replace lost or non-functional tissue. In doing so, it may be advantageous to have the scaffold degrade over time as the implanted cells proliferate with subsequent ECM formation.

Other requirements may include practical considerations such as the cost and repeatability of scaffold construction, ability to implant the scaffold easily, minimizing lead time, or maximizing storage duration. Once these particular goals have been established, a tissue engineer can begin designing a scaffold to meet the specific requirements.

2.2.1 High Surface Area-to-Volume Ratio

Maximizing the surface area-to-volume ratio in a tissue scaffold has a number of benefits. A large amount of surface area provides more adhesion points for cells to attach to. This ensures that cells intended to reside on the scaffold stay adhered to the scaffold if and when they encounter forces due to flow or stress after implantation. A high surface area also encourages proliferation of cells within the scaffold. Diffusion-limited mass transfer of nutrients and waste is a major consideration when designing a scaffold. High-surface area scaffolds provide numerous pathways for the transport of essential biochemicals and waste products. However, highly tortuous pathways can extend diffusional path-lengths within a scaffold. The limits of transport via diffusion have been sidestepped by Harrison and colleagues through the use of oxygen-generating particles embedded within tissue scaffolds (Oh et al. 2009). However, this strategy does not combat the accumulation of waste products that are still subject to simple diffusion.

2.2.2 Suitable Microenvironment

Chemical and mechanical properties are critical elements to consider when designing a tissue scaffold. Both are instrumental in the differentiation of stem cells and also aid in the organization and proliferation of cells in tissue development. Several important aspects of the microenvironment include mechanisms of cellular adhesion to the scaffold, mechanical forces applied to adhered cells (McBeath et al. 2004), and chemical signaling factors present within the tissue construct. The impor-

tance of both chemical and mechanical microenvironments within natural ECM requires tissue engineers to deeply investigate and rigorously control the properties of a manufactured tissue scaffolding system.

The mechanisms of adhesion in cellular environments influence signal transduction cascades controlling cell differentiation and growth (Aplin et al. 1999). The attachment of a cell to any substrate occurs as a result of interactions between the surface of the cell and the substrate. Sources of non-specific adhesion include hydrogen bonds, or Van der Waals forces. Such interactions are common in synthetic biomaterials but are weak and subject to instability or disengagement. The degree of scaffold hydrophilicity (wettability) also influences the success of cell attachment. Techniques such as oxygen plasma surface treatment have been shown to significantly improve wettability, consequently improving the attachment and proliferation of cells on biomaterials (Khorasani 2008).

Specific interactions involve the use of functional groups that act as ligands – known as integrins – between the ECM and cell membrane proteins. An example within the CNS is tenascin-C, a glycoprotein involved in neuroregulatory functions (Meiners 2007). Integrins bond to peptide functional groups (such as tenascin-C) that reside in the ECM.

To augment cellular adhesion to a scaffold and to promote cellular proliferation throughout the scaffold, researchers have attempted to functionalize polymers by incorporating proteins. Long-chain proteins such as fibronectin or vitronectin have been used in cell culture to facilitate adhesion to plastic culture dishes (Lewandowska et al. 1992). Scaffolds can be coated with the same proteins to improve cellular adhesion. There are several disadvantages to using long-chain proteins. Random orientation of these proteins causes a sizeable percentage of active sites to be in a position where binding is impossible. Segments of the protein may trigger allergic responses. The efficacy of fibronectin can be maximized by aligning the active site of attachment to face the integrin proteins of the cell (Calonder et al. 2005). However, the means of aligning fibronectin (through the use of antibodies attached to a layer of dextran) appear to attenuate the rate of cell proliferation. Fibronectin and other proteins also are susceptible to denaturation of their structure through regular processing techniques.

A better method of supplying active sites for integrin bonding is to use shorter peptide chains composed of functional regions responsible for the specific binding interaction. The functional element of fibronectin is a three-peptide chain, arginine-glycine-aspartic acid (RGD). Direct attachment of short-chain proteins to biomaterials with RGD groups on the free end may augment cell adhesion to the artificial structure (Massia and Hubbell 1991; Neff et al. 1998; Pierschbacher and Ruoslahti 1984).

Chemical signals such as cytokines and growth factors present in the ECM can stimulate cells to reproduce, grow, or in the case of neurons, extend neurites. When dealing with progenitor cells, the proper chemical factors must be present to direct the cells down the neural lineage pathway. In many cases, the chemical factors are able to be introduced in vitro, prior to implantation, and can be introduced through the media of cell culture. However, chemical delivery may be preferred after the scaffold is implanted over an extended period. An additional feature of a scaffold

could be to serve as a vehicle of delivery for the signaling factors that aid in the tissue growth process.

The functional groups used to augment cellular attachment can be incorporated into the scaffold prior to scaffold fabrication by blending the additive to the bulk material, or the functional groups may be introduced after the scaffold has been fabricated. Koh et al. used several methods to incorporate laminin into an electrospun scaffold intended for implantation into the peripheral nervous system (PNS) and found that blending and coating process as both resulted in positive attachment of laminin to the scaffold, resulting in enhanced neurite outgrowth (Koh et al. 2008). Recknor et al. used micro-patterned polystyrene substrates coated with laminin to guide the direction of growth in astrocytes as well as the differentiation pathway of rat hippocampal progenitor cells (Recknor et al. 2004, 2006). The various methods for enhancing tissue scaffolds with additional components may be limited by the means of scaffold fabrication, but a solution can most likely be found from prior experience.

The mechanical characteristics of a scaffold are influential for all cell types in the body (Discher et al. 2005). Scaffold stiffness influences not only stem cell differentiation (Engler et al. 2006) but also the direction of proliferation through the scaffold (Willits and Skornia 2004). Georges et al. demonstrated that the stiffness of scaffolds was a determining factor in the outcome of neurite outgrowth and astrocyte proliferation. Substrate stiffness influences the outgrowth of neurites in that stiffer scaffolds appear to inhibit neurite outgrowth, as shown through studies with varying concentrations of collagen (Georges et al. 2006). Softer scaffolds also appear to attenuate the rate of astrocyte reproduction, an important factor when one considers the limited nutrient supply available in a given unit volume. “Tuning” the elasticity of a scaffold can be an important aspect of designing a scaffold intended for a particular tissue.

2.2.3 Appropriate Biomaterial

The choice of biomaterial to use as the matrix in a tissue engineering application must be made while keeping several requirements or features in mind. Criteria for choosing a material come in large part from the intended goals of the scaffold. For example, some therapies may be temporary, in which case the scaffold would be removed after a particular period of time.

A fundamental requirement is biocompatibility. The issue of biocompatibility is more than a simple binary result after determining whether or not a material is inert *in vivo*. Several factors come into play when determining an appropriate material for use. Important factors include whether the material degrades at all, the mode of degradation, rate of degradation, and its effect on the surrounding microenvironment.

Many bioinert materials have a very low rate of degradation *in vivo* or may not degrade at all. Such materials include polyamide or poly(caprolactone) (PCL), which degrade at a very slow rate. Copolymers like poly(lactic-co-glycolic acid) (PLGA)

can have customizable rates of degradation by changing the ratio of monomeric constituents within the polymer chain. In the case of PLGA, the most rapid rate of degradation occurs when a 50:50 ratio is used. An increase in either monomer results in a decrease in the rate of degradation. Some biomaterials may act as constituents for natural tissue, as hydroxyapatite can for bone scaffolds (Wang 2006).

The mechanics of scaffold degradation or erosion are dependent upon the type of biomaterial, location, and the morphology of the scaffold. Researchers have classified most hydrolytic catalysis as either bulk or surface erosion (Gopferich 1996; von Burkersroda et al. 2002). The type of erosion affects the pattern of structural decay within the scaffold and can also modify mechanical properties of the scaffold such as the elastic modulus.

Polymers may be biocompatible in their original form but may create an environment deleterious to cell proliferation as they break down into their base compounds. For example, synthetic polymers such as PLGA and poly(glycolic acid) (PGA) degrade hydrolytically into monomers of lactic or glycolic acid. These compounds can create an acidic zone that surrounds the scaffold construct.

Natural biomaterials have variable degradation rates based on intrinsic enzymatic activity. These rates can vary among subjects and species. Creating a construct that demonstrates repeatable and predictable degradation behavior is difficult across a spectrum of different subjects.

An intensive review of biomaterials used in applications with progenitor cells describes the necessary factors to consider and examples of both synthetic and natural biomaterials (Dawson et al. 2008). Reviews by Zhong and Bellamkonda (2008) and Little et al. (2008) focus on the biomaterials related to the CNS.

2.2.4 Brain-Specific Considerations

The unique setting and structure of the brain limit the effectiveness of conventional cellular therapy delivery mechanisms. Cellular therapy in the brain ideally involves introducing cells to a specific target site of the cortex. For example, the target in traumatic brain injury (TBI) therapy would be the penumbral area surrounding the lesion in an attempt to mitigate ischemic effects of the acute post-traumatic inflammatory response (Engstrom et al. 2005; Ito et al. 2006). Other diseases or locations of injury may require significantly different strategies.

The composition of ECM in the brain differs significantly from other parts of the body. Specifically, the brain ECM has a high concentration of proteoglycans, including versican, aggrecan, neurocan, and brevican. Additional components include hyaluronic acid and tenascin. Other components such as collagen found prolifically in other tissue ECM are conspicuously absent in the CNS (Ruoslahti 1996).

Neurotrophic factors can aid in the regeneration and stabilization of damaged areas of the nervous system. Bioactive compounds such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are important in neural cell survival and proliferation (Mahoney and Saltzman 1999; Philips et al. 2001;

Schmidt and Leach 2003). Progenitor cells may be modified to generate elevated levels of neurotrophic factors, as demonstrated by Kurozumi with BDNF-enhanced mesenchymal stromal cells (MSCs) (Kurozumi et al. 2004). Alternatively, a scaffold design may include such compounds along with progenitor cells to enhance the therapeutic effect without the need for genetic modification.

Delivering cells to a specific area and maintaining their location in the brain have proven to be challenging tasks. Although early research presented promising findings (Mahmood et al. 2004), intravenous administration of progenitor cells intended to treat TBI is inefficient, with a very low percentage of injected cells physically reaching the target area (Fischer et al. 2009; Harting et al. 2009a). Direct injection of cells into the brain via stereotaxis has marginally better success, with the cells eventually migrating away from the target zone (Harting et al. 2009b). The mobility of administered progenitor cells within the brain calls for a structure that can act as an anchor to maintain a suitable concentration in a target area, maximizing the intended effect. A scaffold also provides a simple yet effective means of providing a therapy that can be removed easily after the acute post-traumatic period, a feat that is impossible using either intravenous or intracranial injection. Implantation of a scaffold within the cranial cavity brings with it a host of challenges and limitations for the designer. Several studies in which progenitor cells are integrated into a scaffold intended for use in the CNS have been performed (Brannvall et al. 2007; Soria et al. 2006; Willerth et al. 2006).

The combination of biomaterial and culture medium influences the phenotype of progenitor cell differentiation. Specifically, Hung et al. demonstrated how chitosan and poly(vinylidene fluoride) (PVDF) substrates do not facilitate proliferation or differentiation of single neural stem cells (NSCs) in serum-free media (Hung et al. 2006). However, neurospheres were able to differentiate and proliferate in serum-free media. Clearly, there is a great deal of complex consequential interaction between a progenitor cell and its surroundings, including the biomaterial of other nearby cells and the composition of the media.

The microenvironment of damaged cortex has unique properties with respect to diffusion-based transport. Traumatic or ischemic insult to the cortex has been shown to increase extracellular space volume fraction and the degree of tortuosity (Nicholson 2004; Roitbak and Sykova 1999). Such deviations from normal conditions for mass transport must be understood when designing a tissue scaffold.

2.3 Fabrication of Tissue Scaffolds

To meet the criteria of tissue scaffolds listed above, a viable fabrication method must have sufficient resolution (feature size) using appropriate materials and have the ability to create geometries that are both repeatable and predictable.

Some scaffold fabrication methods yield morphologically suitable structures with randomly assorted channels and pathways such as foaming of polymers induced by phase change or other physical events designed to create porosity in a substrate (Chen 2002). Some groups have even attempted to use samples of coral

to act as a scaffold for neural cells (Lamghari et al. 1999). Creation of chaotically distributed channels may be suitable for in vivo use given extensive empirical testing and stringent adherence to good manufacturing practices (GMPs). A few of these methods are reviewed below.

2.3.1 *Thermally Induced Phase Separation*

Thermally Induced Phase Separation (TIPS) is a common technique used to create structures with nano- and micro-scale features. This method takes advantage of differing physical properties between two or more materials. Typically, one of the materials is intended for implantation, and the material or materials act as a solvent and a porogen. There are several different classes and subclasses of TIPS that have a significant effect on the outcome of the polymer scaffold. TIPS has an advantage over leaching processes in that a highly porous structure can be created without using toxic organic solvents that are commonly present in residual levels after leaching.

Phase separation as it pertains to tissue scaffold fabrication can be considered *solid–liquid* or *liquid–liquid*. Solid–liquid phase separation involves the freezing and subsequent sublimation of the solvent. This method is commonly known as freeze drying or lyophilization. The morphology of the pores created is a direct result of the crystalline structure formed as the solvent freezes (Schugens et al. 1996).

2.3.1.1 **Solid–Liquid Phase Separation**

Solid–liquid phase separation creates three-dimensional scaffolds by cooling a polymer solution below the freezing point of the solvent, using liquid nitrogen or a temperature-controlled water bath. The solidification of the solvent forces the polymer out of solution but creates a lattice structure that is subject to the forces applied by the solid solvent mass. Applying a vacuum to the volume induces sublimation of the solvent, leaving behind a porous scaffold. It is important to remove the solvent completely since many of them (including dioxane) are highly toxic.

Influential factors in solid–liquid phase separation include polymer type, polymer concentration, solvent characteristics, and the thermal kinetic profile. Specific solvents are applicable to particular biomaterial polymers such as PLGA or PGA. Since freeze drying is a common method of removing the solvent, the vapor pressure should be a value compatible with normal lab equipment. The polarity of the solvent can influence the polymeric structure through interactions via hydrogen bonding as the polymer drops out of solution. Possibly, the single most important process condition to control is the rate of cooling. Pore structure, geometry, and orientation can be directed by controlling the rate and direction of cooling.

Schugens et al. introduced a method of freeze drying solutions of poly (L-lactic acid) (PLLA) in 1,4-dioxane to create scaffolds that can exhibit directional porosity (Schugens et al. 1996). Specifically, they determined that the direction of cooling guided the direction of macropore formation. The average diameter of macropores

was 100 μm . The concentration of PLLA was found to be optimal at 1%, although it had a limited effect on scaffold properties. This paper represents the first significant work related to phase separation methods for fabricating tissue scaffolds. No cell implantation was attempted in this study.

Teng et al. used the solid–liquid phase separation method of Schugens with PLGA in their two-layer scaffold system for spinal cord repair to create an oriented pore structure with the intent to direct axonal growth along the long axis of the spinal cord (Park et al. 2002; Teng et al. 2002). Slowly lowering tubes into a cold solution of ethanol and dry ice (-78°C), then sublimating in a freeze dryer resulted in axially oriented pores that replicated the white matter of the spinal cord. The inner grey matter of the spinal cord is fabricated using a salt-leaching process, described later.

Deguchi et al. used the sol–gel method in conjunction with phase separation to generate a gelatin–siloxane hybrid foam scaffold impregnated with growth factors (Deguchi et al. 2006). The goal of the study was to observe the scaffold's effect on an injured rat brain after implanting the scaffold within the lesion. Although no cells were implanted in the scaffold, the authors were optimistic from their results that the conditions provided by the scaffold and concomitant growth factors could nurture progenitor cells and encourage proliferation.

2.3.1.2 Liquid–Liquid Phase Separation

The specific action of liquid–liquid phase separation is more complicated in that there are two different subclasses, each resulting in different polymer morphology. One subclass undergoes phase separation through a crystal growth process at specific points of nucleation. The alternative is known as spinodal decomposition, in which the separation occurs throughout the solution spontaneously and not at discrete nucleation sites. These two processes are different from the crystallization of the solvent that occurs during solid–liquid phase separation. Both subclasses of liquid–liquid phase separation require cooling the solution to within a narrow temperature range. After phase separation, the solvent is removed through successive dilution with a liquid that does not dissolve the scaffold material. Spinodal decomposition yields nanofibers that are desirable in scaffolds intended for CNS applications, namely fibers that are on the order of hundreds of nanometers in diameter, with porosities of greater than 80%. The structure of the resulting scaffold is isotropic, a condition that may or may not be desired depending on the particular therapy.

The influential process conditions of liquid–liquid phase separation are nearly identical to those of solid–liquid phase separation, with the exception of the precise temperature at which the two species separate from each other.

Liu et al. used liquid–liquid phase separation and leaching with PLLA and microspheres of gelatin to create a nanofibrous matrix with surface modifications for a bone scaffold. The gelatin microspheres were designed to create voids in the scaffold to increase porosity. The first stage of phase separation involved mixing a solution of PLLA dissolved in tetrahydrofuran (THF) at 60°C with the gelatin microspheres. The combined solution was then placed in a -76°C freezer to initiate

a phase change of the solvent, leaving PLLA in a nanofibrous structure. Cyclohexane was used to replace the THF. The gelatin was leached out of the scaffold to create interconnected pores throughout the scaffold (Liu et al. 2006).

Yang et al. also used PLLA in solution with THF to create a nanofibrous scaffold for nerve tissue engineering by using neonatal mouse cerebellum stem cells. Neurite outgrowth initiated shortly after seeding (Yang et al. 2004a).

2.3.2 *Leaching*

Leaching is another method that has been used extensively in the fabrication of tissue scaffolds. The process involves casting a polymer mixed with a porogen (a salt or other soluble compound) into a desired shape and then introducing a solvent that would dissolve the porogen but leave the polymer intact, leaving a foam-like structure. The pore size distribution is dependent upon the morphology of the salt crystals. Salt weight percentages of 80% or higher can be used to create highly porous structures, often with interconnected pores.

The shape of the porogen influences the characteristics of the scaffold. Salt, sugar, gelatin, or wax can be used as a porogen, as they are inexpensive and make particle size easy to control. The particular type of porogen is not important provided that its solubility profile suitably differs from the scaffold polymer. However, some groups have altered the surface of the porogen particles to create functional sites on the resulting scaffold.

Teng et al. used the salt-leaching process to build the grey matter section of their spinal cord scaffold using PLGA dissolved in chloroform over sodium chloride with crystal diameters ranging from 250 to 500 μm (Teng et al. 2002). Upon evaporation of the chloroform, water was introduced to dissolve the salt. This hybrid design is noteworthy because it uses two separate fabrication methods to create a composite scaffold, thereby taking advantage of the structural differences each material and method provides.

2.3.3 *Electrospinning*

2.3.3.1 *System Overview*

Electrospinning is a drawing process that elongates and stretches a material to create a fiber that can reach the scale of nanometers in diameter. Instead of using the traditional mechanical drawing technique in which the material is pulled through a die to the desired diameter, electrospinning uses an applied electric field to deform a material by means of attracting or repulsing surface charges from a high-voltage source to a grounded structure. Electrospinning is

very similar to electrospraying, with the major difference being that electrospraying creates droplets of material whereas electrospinning generates a continuous fiber.

Typically, the material being electrospun is dissolved into solution, with the solvent evaporating as the jet of material moves toward the grounded collector. Electrospinning was first discovered in the early twentieth century and maintained a very low level of interest since mechanical methods of drawing out material were more robust and appropriate for the intended final products such as metal wire and synthetic textiles. Apart from some research related to textiles and filtration membranes (Graham 2003), growth in the field of tissue engineering is responsible for renewed interest in electrospinning because of its ability to create nano-level features relatively cheaply without sophisticated equipment. In the past decade, a massive increase in knowledge has occurred in every aspect of electrospinning, from the exploration of new materials to the modeling of the ejected jet of fibers. Electrospinning's advantages have made it a target method of fabrication for many applications in medicine, including vascular repair (Boland et al. 2004; Vaz et al. 2005), hemostasis (Wnek 2003), osteogenesis (Fujihara et al. 2005; Xin et al. 2007; Yoshimoto et al. 2003), smooth muscle regeneration (Stankus et al. 2004, 2006), and peripheral nerve repair (Bini 2006; Ghasemi-Mobarakeh et al. 2008; Panseri et al. 2008). Pham and colleagues and Teo and Ramakrishna provide excellent reviews of all types of electrospinning setups and applications (Pham et al. 2006; Teo and Ramakrishna 2006).

The principles involved in the process of electrospinning include electrostatic attraction, surface tension, evaporation dynamics, and viscous flow behavior. Each of these physical events influences the properties of the resultant nanofiber scaffold. Therefore, a tissue engineer must have a high degree of control over all of these properties in order to create repeatable and desirable scaffolds.

Conventional electrospinning uses a solution of polymer mixed in a highly volatile solvent that evaporates during the extrusion. The fundamental equipment needed for an electrospinning system include the following:

- Syringe pump
- High-voltage supply (0–25 kV)
- Enclosed housing
- Grounded collection system (plate, rotating cylinder and so on)

Figure 2.1 shows a schematic of a simple electrospinning system. Typically, a syringe pump dispenses a solution of polymer dissolved in a volatile solvent at a low flow rate (approximately 1 mL/h) through a needle that is charged on the order of 10–20 kV. A drop at the end of the needle is created. Immediately after the electric field has been established, the spherical drop elongates toward the grounded structure, creating a geometry known as a “Taylor cone.” The Taylor cone acts as the launching pad for the jet of polymer that is propelled toward the collecting structure. At some threshold voltage (dependent on a number of different factors described later), the polymer solution begins to eject from the Taylor cone. The path

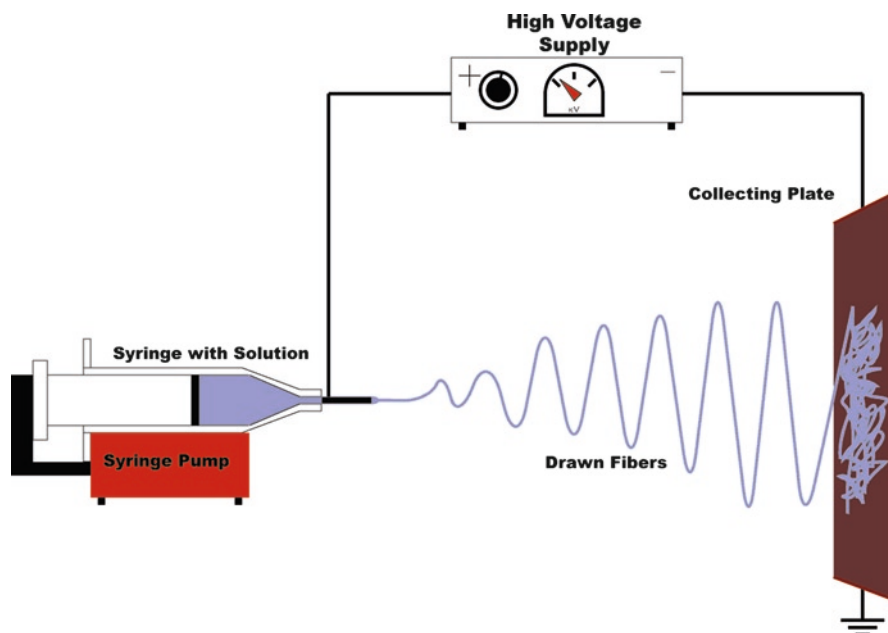


Fig. 2.1 A graphical representation of a simple electrospinning system, including a voltage supply, syringe pump loaded with syringe and solution, and a collection plate. This rudimentary setup can create fibers of less than 1 μm in diameter in a randomly oriented non-woven mat

of the ejected fiber is rather tortuous and performs a whipping motion as it progresses to the grounded area. This whipping reduces the diameter of the fiber to microns or nanometers. The volatile solvent evaporates during the transit from the Taylor cone to the grounded structure. Ideally, the solvent should be completely evaporated upon reaching the collecting structure, leaving only a thin fiber of polymer. On a planar grounded structure, the fiber lies down in a random fashion, resulting in a non-woven mat.

2.3.3.2 Process Conditions

The concept of fabrication in electrospinning may be simple, but the parameters that influence the outcome of fabrication are rather complex. If a tissue engineer is to design a scaffold to meet his or her needs, an understanding of and ability to control these parameters are needed. Researchers have devoted significant effort to gain a better understanding of the physics of electrospinning and have published reports investigating one or more of these parameters (Deitzel 2001; Nie et al. 2008; Reneker and Chun 1996; Tan 2005; Theron 2004). The next section gives a brief description of each process parameter and how changing it may influence the electrospun nanofibers.

Electric Field

The driving force of electrospinning is the presence of an electric field between a dispensing needle and the collection system. This electric field is established using a high-voltage power supply (typically direct current (DC), but alternating current (AC) has been used), with the positive lead connected to the dispensing needle and the ground lead connected to the collection system. Both the magnitude and the geometry of the applied electric field are important in electrospinning, but adjustment of the values does not translate into significant changes in the diameter of the fibers.

Field Strength

The magnitude of the electric field influences the velocity of the ejected stream of polymer. A minimum amount of electric field is required to initiate the electrospinning process and is dependent upon the viscosity of the solution and the conductivity of the solution. Since the units of the electric field are in kilovolts per centimeter, the distance between the needle and the collecting structure is not a factor. However, as described later, other factors limit the separation distance. Increasing the electric field beyond the threshold value to initiate spinning causes minimal changes to the diameter of the fibers being created. However, increasing the field strength substantially can cause the formation of multiple jets (Tan 2005) or cause discontinuities in the fiber due to the high acceleration induced by the elevated electrostatic forces. Increasing the field strength may also draw more solution from the pendant drop at the tip of the needle than can be replenished through the syringe pump.

Field Shape

In a simple setup, the electric field lines can be modeled simply as a point source for the positive end and an infinite plane for the grounded end. The field lines in the simple setup are not uniform; the outer lines follow an increasingly parabolic path. Some investigators have created setups with the intention of establishing a uniform electric field by combining the dispensing needle with a plate. Alternatively, some investigators have arranged multiple rings between the dispensing needle and the collection system, with each ring at a decreasing voltage in the attempt to localize the field Deitzel et al. (2001).

Structure

The orientation of cellular attachment and extension is heavily influenced by the mechanical properties of the nanofiber mat. Consequently, the orientation of the nanofibers is an important factor to control.

Nanofibers can be arranged in a number of different configurations by changing the geometry of the collecting structure. The conventional method of electrospinning

uses a flat plate as the collecting surface. The result is a non-woven mat of fibers that randomly distribute themselves. A mat of aligned fibers can be produced by using a rotating mandrel as the collecting surface. The result is a cylinder with fibers aligned perpendicularly to the axis of rotation. High rotational speeds are needed to ensure a high degree of alignment.

Another means of creating scaffolds with aligned fibers is through the use of two collecting plates separated by a gap that could be up to a few centimeters in width. The fibers jump back and forth perpendicularly to the direction of the gap. A cross-hatched pattern of fibers can be made by selectively grounding two different sets of electrodes, each aligned 90° apart from one another (Li 2003). Researchers have explored more exotic collection structures, including dual rings.

Solution Flow Rate

Solution must be continuously added to the pendant drop to compensate for material leaving the drop and to maintain a consistent geometry. Syringe pumps are used to provide consistent flow at flow rates ranging from 0.5 to 2 mL/h. Balancing the flow rate of the syringe pump with the rate of material being electrospun is a non-trivial matter since any imbalance results in a gradual but significant alteration of the pendant drops geometry, ultimately leading to either a cessation of fiber formation because of lack of material or the ejection of drops that may damage or destroy the integrity of the scaffold construct.

2.3.3.3 Material Solution Properties

Polymer Selection

Electrospinning can be performed with a number of different materials, including PLLA, PGA, PCL, polyamide, and collagen. Copolymers are also commonly used, with examples being PLGA and poly (L-lactide-co-caprolactone) (P(LLA-CL)). Natural materials, including collagen, sodium alginate (Nie et al. 2008), and chitosan (Ohkawa 2004), have been investigated. Additionally, composites of natural and synthetic materials have been electrospun for nerve tissue engineering (Ghasemi-Mobarakeh et al. 2008).

Molecular Weight

Biocompatible polymers are sometimes available in different molecular weights. Polymers of higher molecular weight have been shown to create better fibers at lower concentration compared with a polymer of low molecular weight (Tan 2005). Furthermore, “beading” of the fibers occurs at a higher rate with polymers of low molecular weight.

Solvent Selection

The process of electrospinning demands that the solvent evaporate fully prior to contact with the collecting surface. Additionally, appropriate solvents must be able to fully dissolve the polymer compounds intended to be electrospun. The candidate solvents for electrospinning many synthetic biocompatible polymers are highly volatile compounds such as dichloromethane (DCM), chloroform, dimethyl-formamide (DMF), and hexafluoro-2-propanol (HFIP).

Solvent electrical conductivity has also been shown to influence the quality of fibers generated from electrospinning. Since solvents such as DCM and THF are non-conductive, additives must be included to improve the conductivity of the solution. A more conductive solution results in smaller diameter fibers and a reduction in beading.

Solution Concentration

Physical properties of the solution containing the electrospinning material significantly influence the process results. Arguably, the most important parameter is the concentration of the material. Viscosity and surface tension are properties that depend upon the concentration of polymer in solution and have a large influence on nanofiber fabrication. A high polymer concentration naturally creates a viscous solution, resulting in a larger diameter extrusion and a larger fiber. Higher viscosity also requires that the applied electric field be increased. Concentrations that are too high can create poor results, causing polymer precipitate to clog the tip of the needle or discontinuous spinning. At the other end of the spectrum, low concentrations of polymer can result in a fiber that exhibits a beaded morphology. Low concentrations also can cause some solvent to reach the collecting structure, where it can dissolve and destroy the intended shape and geometry of the fibers. Typical concentrations range from 3 to 11% wt/vol for many biocompatible polymers such as PLGA and PCL. Very low concentrations are normally used in electrospraying processes, where the breakup of particles is expected.

2.3.3.4 Central Nervous System-Based Applications

The use of electrospun fibers as a cell scaffold has been investigated in many different settings both in vitro and in vivo, including the CNS. Cao et al. provide a comprehensive review of electrospinning applications for CNS tissue engineering (Cao et al. 2009). The following investigations are detailed to highlight the process conditions typically used to create scaffolds compatible with neural progenitor cell applications.

Yang et al. electrospun PLLA and investigated the effect of fiber diameter and alignment with respect to murine NSC growth (Yang et al. 2004b). A comparison was made between four groups: aligned and randomly oriented fiber mats of fibers of average diameter of 300 nm and 1.5 μm . The fiber diameter was changed by altering

the concentration of the polymer in solution (2% for smaller fibers versus 5% for the larger fibers). Alignment was controlled using the rotating mandrel method previously described. Collecting the fibers on a rotating platform caused a slight reduction in fiber diameter compared with their randomly oriented counterpart. It was surmised that a stretching force was being applied to the fibers because of the rotation of the mandrel. The NSCs were implanted in each of the four study groups. It was found that cell differentiation is based upon fiber diameter and not the degree of fiber alignment. Neurite outgrowth length was 20% greater in the aligned 300-nm diameter fiber group compared with the other three groups. The conclusion of the authors was to focus investigation on nanoscale diameter-aligned fibers for neural tissue engineering.

Nisbet et al. investigated the response of murine embryonic cortical neurons on both PLLA and PLGA electrospun scaffolds (Nisbet et al. 2007). Their electrospinning process used 10% wt solute (either PLGA or PLLA) dissolved in THF and DMF (1:1 ratio) and ejected through an 18-gauge needle (0.838 mm ID) at 0.397 mL/h. The applied voltage was 18 kV at a distance of 15 cm.

A unique feature of this experiment was the surface modification of the nanofibers using potassium hydroxide to change hydrophilicity. The authors concluded that a reduction of surface tension (that is, an increase in scaffold hydrophilicity) leads to a quicker outgrowth of neurites from the seeded cells. Another observation was that the path of neurite extension followed fibers directly if the fiber concentration was low but crossed perpendicularly to fibers when the density was greater. Highly dense regions of fibers were avoided by neurite outgrowths. This led the authors to conclude that control of the fiber density could allow the designer a limited degree of control of neurite outgrowth direction.

Carlberg et al. seeded human embryonic stem cells (hESCs) on electrospun polyurethane and promoted neuronal differentiation (Carlberg et al. 2009). Solution concentration was 11% wt, and the solvent was a mixture of THF and DMF (60:40). Eighteen kilovolts over a distance of 22 cm was applied, a non-woven mat of randomly oriented fibers was fabricated, and the mean fiber diameter was 360 nm. Neurite outgrowths were seen to extend over the network of fibers surrounding each cell, as displayed in Fig. 2.2.

Meiners et al. explored the modification of polyamide nanofibers by introducing functional units to the surface of the scaffold, hypothesizing that the functional groups would promote axonal adhesion and proliferation through the scaffold (Meiners et al. 2007). In their study three groups of rats underwent spinal column transaction. The first group received functionalized randomly oriented nanofiber mat grafts in the transected area, the second group received a graft without the functional units attached, and the third group received no scaffold and acted as the control group. The nanofiber mats in this experiment were purchased from a vendor and then modified with a peptide involved with promoting the outgrowth of neurites. The enhanced grafts were shown to enhance axonal growth in comparison with the unmodified implants.

Bini compared different structures of scaffolds made from the same biomaterial (10:90 PLGA) and observed the interactions with implanted neural stem cells (Bini 2006). Specifically, electrospun fibers were compared with a microbraided structure,

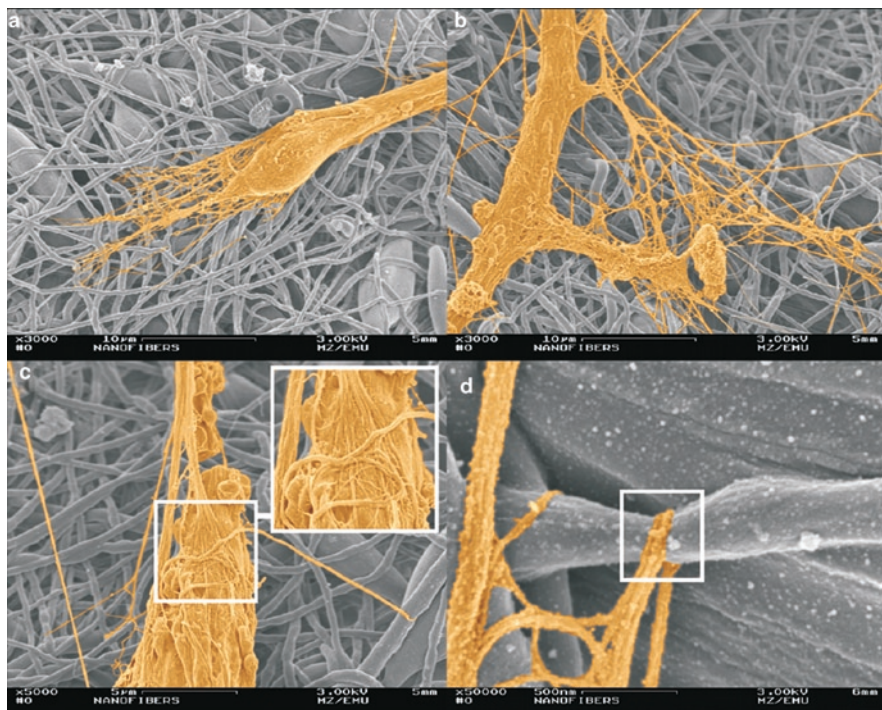


Fig. 2.2 Differentiated human embryonic stem cells seeded on a nanofiber scaffold. Note the neurite extension away from the cell body shown in frames (a) and (b) and the physical attachment of the neurites to the scaffold in frame (c) and (d). Reproduced with permission from Carlberg et al. (2009)

aligned microfibers, and a film formed by solvent casting. The electrospinning apparatus used HFIP as the solvent at a concentration of 7% wt, 12 kV as the applied voltage at a distance of 10 cm. A 0.4 mm ID needle was used to eject the solution at a flow rate of 1 mL/h. The resultant fibers were randomly oriented and their diameter appeared to be submicron, but no specific range was given. Differentiated NSC seeding demonstrated adhesion to the nanofiber scaffold and the other constructs tested. Incidentally, neurites extended along the lengths of the larger diameter microfibers of the aligned test group.

A solid foundation has been established by these and other investigators in the field of electrospinning tissue scaffolds. However, much more work needs to be done on improving the process and control of scaffold fabrication using this method.

2.3.4 *Rapid Prototyping*

The tissue fabrication methods described above have been shown to work well within various areas of the CNS. However, the methods lack the ability to control the specific shape of the scaffold other than molding it within a customized container. Techniques

developed in fields outside of biology are finding utility within the microscale world of tissue engineering. One field that has transformed the capabilities of tissue engineering is rapid prototyping (RP). RP describes a number of different manufacturing processes that allow automated fabrication using unique methods of material bonding or deposition. RP systems are capable of producing objects with geometries that are difficult, even impossible, to create using the “traditional” machining methods of milling, turning, or drilling. Furthermore, RP methods enable users to fabricate geometries directly from files generated using standard computer-aided design (CAD) software (Sun and Lal 2002). Many RP techniques are additive rather than subtractive and typically build an object one thin layer at a time. RP methods give the designer flexibility to create geometries that previously would have been impossible to achieve using any amount of labor or that are non-repeatable or both.

RP methods were not originally designed with medical applications in mind. As a result, these methods in their original form have drawbacks that limit their potential as devices for use in the CNS, apart from building a custom experimental apparatus (Tek et al. 2008). However, modifications to process conditions can yield more appropriate components. A reduction in build volume and increased resolution are often necessary for suitable scaffolds to be created. Some methods normally use cytotoxic materials that prevent concurrent deposition of cells during fabrication. The standard selective laser sintering (SLS) fabrication process occurs at temperatures far above the limit for human cells. Researchers are striving to eliminate barriers to fabrication of biomaterials (Fedorovich et al. 2007; Mironov et al. 2006; Sachlos and Czernuszka 2003; Wang et al. 2007; Wu 1996). Many problems can be bypassed by completing fabrication prior to cell seeding or by using RP methods to create negative molds wherein the biomaterial is introduced and the mold subsequently is removed. Additionally, much of the work using RP methods in cellular scaffolding has been directed toward orthopedic therapies such as osteogenesis or articular cartilage regeneration since most RP techniques produce parts that are rather stiff and strong (Schieker 2007). Such mechanically robust structures are not optimal for the brain. The topic of RP methods and their utility in cell scaffolding has been well covered in numerous review articles (Landers 2002; Peltola et al. 2008; Yang et al. 2002).

2.3.4.1 Selective Laser Sintering

SLS uses a laser to fuse powdered material into user-specified geometries in a layer-by-layer fashion. Prior to fabrication, the user would design the shape to be created in a CAD program and send it to a special program that slices the part into two-dimensional shapes that will subsequently be used by the fabrication system. An SLS machine consists of three chambers: two feed and one fabrication. The feed chambers are filled with powdered material and have a piston that moves the contents upwards. All three chambers are heated to a temperature marginally below the melting temperature of the fabrication material. A roller transfers a thin layer of powder from one feed chamber onto the fabrication chamber. A laser then raster-scans

the thin sheet of powder, melting and fusing the powder in a pattern determined by a computer program. Once the scanning is complete, the fabrication chamber is lowered slightly, and a new layer of powder is deposited for the next scan. This process is repeated until all parts have been completed. The end result is parts surrounded by un-sintered powder that acts as a structural support during the fabrication steps. The unused powder can be recycled for later use.

Tight control of temperature and laser power is required to ensure accurate fabrication of parts. Additionally, the powdered material must be homogeneous in its size distribution to generate repeatable geometries. Many machines are optimized to work with proprietary materials that are not designed for direct implantation. However, most SLS control programs allow the user to modify settings and experiment with different materials. Some materials may be problematic if they are unable to be ground to a powder-like consistency (that is, are gummy or have the tendency to clump together). The smallest feature size is dependent upon the material being used, the step size of the powder layers, and the properties of the laser (beam width, power output, and so on).

The large sizes of commercial SLS machines and the high cost of biomaterials mandate a reduction in build volume size to minimize the requisite amount of powder needed for scaffold fabrication. Zhou et al. modified their SLS system to perform research with PLLA/hydroxyapatite microspheres with the application naturally being a bone scaffold (Zhou et al. 2008). The authors also had to reduce the set temperature of the machine to match the material's properties. Their results showed that porous bone scaffolds can be made successfully using a modified SLS system. Much of the work with SLS has been limited to bone tissue engineering (Chua et al. 2004), but the technology can be applied to neurological applications.

2.3.4.2 3D Printing/Inkjet Deposition

Printing three-dimensional structures suitable for tissue scaffolds can be achieved using technology of the common inkjet printer found in most homes and offices. The principles behind depositing ink on paper can be applied to fabricate and seed a tissue scaffold.

Inkjet printing is simply the spraying of a liquid (ink in the conventional usage) through a nozzle via a driving force. The driving force could be provided by an electrostatic charge (known as electrospray) or through pressurizing the stream of liquid prior to the nozzle. Some inkjet systems use small piezoelectric pumps to provide pressure, whereas others use a small heater to generate a small bubble of steam that drives the fluid through the nozzle.

The 3D printing (3DP) rapid prototyping technique deposits a powder of the desired material one thin layer at a time. An inkjet deposition system then sprays a solvent binder (many biomaterials are soluble in chloroform or dichloromethane) that dissolves the powder into a thin puddle of solution. When the binder evaporates, the biomaterial is left behind in a solid congealed form matching the pattern of where the binder was sprayed. Three-dimensional parts are fabricated by repeating this process, creating a stack of two-dimensional slices that combine into a solid

shape. The use of 3DP has been explored in the field of drug delivery (Rowe et al. 2000) and has been investigated as a fabrication method for a number of different tissues. In this method in contrast to SLS, temperatures are not increased significantly. However, the use of cytotoxic chemicals as binders poses a significant challenge to the tissue engineer if 3DP is to be used as a means of directly fabricating a tissue scaffold. Residual solvent remains in the material even after a week in a vacuum chamber (Giordano et al. 1996). A major problem when dealing with very small features is the ability to remove unbound material from the area surrounding the part. Actions such as bead blasting or ultrasonic shaking must be performed with great care given the fragility of the small parts.

Wong et al. used a 3DP apparatus to create wax molds that were subsequently filled with a PCL solution and salt crystals to eventually create porous scaffolds (Wong et al. 2008). They compared various geometries of scaffolds built by 3DP (orthogonal channels versus unidirectional channels) with a simple porous cylindrical scaffold. The scaffolds were implanted directly into rat brains after removing a nearly identical volume of brain tissue. Native cells such as astroglia were able to migrate through both the parallel and orthogonal channels of the scaffold but not through the smaller pores. The channels also directed alignment of neuronal fibers.

A variant of the 3DP method involves direct deposition of both cells and scaffold materials using inkjet technology. Commercial off-the-shelf printers and cartridges can be used in building the apparatus. Initial research used store-bought ink cartridges that were flushed of all ink and filled with cell suspensions (Wilson and Boland 2003). Researchers have used a multi-head system to alternately deposit scaffold material and cells to create a multi-layer sandwich of cells and scaffold material. Xu et al. used this method to deposit thrombin and embryonic cortical and hippocampal cells onto a substrate containing fibrinogen. The thrombin/fibrinogen combination created a fibrin gel that encapsulated the cells. Cellular function remained normal after the deposition process (Boland et al. 2003; Xu et al. 2006). This method of concurrent direct deposition is feasible only using scaffold materials that do not require cytotoxic binders.

2.3.4.3 BioPlotting/Fused Deposition Modeling

BioPlotting is the direct deposition of cells or scaffold material or both through the use of micro-injectors. An array of syringes, each with a suspension of cells or scaffold material, is arranged on a robotic translation stage. A computer controls the movement of the stage as well as the actuation of automated pumps that deposit precise amounts of solution. Lee et al. developed a sophisticated system that can generate sophisticated composite layers of scaffold material (collagen) and cells (fibroblasts and keratinocytes) on irregular substrates, as would be seen in clinical applications (Lee et al. 2009a). The system can be used to introduce cells on an existing scaffold or to build the scaffold concurrently. Limited work has been performed with the CNS as the target area and nearly all of it is focused on spinal cord repair as opposed to brain-based therapies (Lee et al. 2009b).

Fused deposition modeling (FDM) is a technique that also has its roots in the manufacturing sector. The principle of FDM is the direct extrusion of material through the guidance of a robotic arm to generate three-dimensional structures. The material is commonly deposited in a molten form or through a dual-dispensing setup in which two liquid reagents react to form a solid or a gel (Geng 2004). FDM has advantages over 3DP and SLS in that there is no extra unused material to remove once fabrication is complete. This is useful especially when dealing with small feature sizes and pores. Kim designed an FDM system that deposited molten PCL and PLGA in interconnecting grid structures (Kim 2008). Feature sizes as small as 200 μm were created. MSCs were introduced to the scaffolds, with the PLGA scaffold having superior performance due to a greater degree of cell adhesion compared with PCL.

2.3.4.4 Stereolithography

Stereolithography uses a laser and a photosensitive liquid polymer solution to fabricate parts. The main difference between SLA and SLS is that the precursor material is a liquid rather than a solid. Fabrication occurs by a piston lowering step by step in a bath of the photocurable solution as an ultraviolet (UV) laser raster-scans over the top surface of the solution, solidifying the material. If the part being built is inherently unstable, support structures are required to keep the part upright during the build process and are removed after fabrication is complete. After the part has been fabricated, it must undergo a post-cure treatment in which it is heated in a UV oven. The feature size of a production-grade SLA system (3D Systems iPro Series) can reach as small as 0.002 in., which is the minimum layer thickness that can be generated. Industrial-grade machines are optimized for objects orders of magnitude larger than the features required on a tissue scaffold. Improvements in resolution have been investigated, both in decreasing the thickness of each successive layer and in modifying the means of selectively delivering light to the resin. Improvement in resolution is inversely proportional to the amount of time required to build a particular volume. Systems with micron-level resolution can have build rates of 1 mm/h (Bertsch 1999).

Lu et al. created a system using a digital micro-mirror array instead of a conventional raster scanning laser to build scaffolds using poly(ethylene diacrylate) as the crosslinking polymer (Lu et al. 2006). The resulting scaffold has a feature size of approximately 20 μm , as shown in Fig. 2.3. Although their goal was to create a scaffold for an osteogenic differentiation of MSCs, this method could be easily adapted for other areas of the body.

Original SLA systems required the use of toxic chemicals as the photopolymer resin. Understandably, the manufacturers of the machine did not select materials that would degrade significantly over time. Biocompatibility was not a significant material requirement. However, several biodegradable photopolymers that are suitable for in vivo use have been developed and have degradation properties similar to those of well-known biomaterials (Davis et al. 2003; Ifkovits and Burdick 2007; Lee 2007; Mizutani and Matsuda 2002). Some materials are sufficiently reactive to

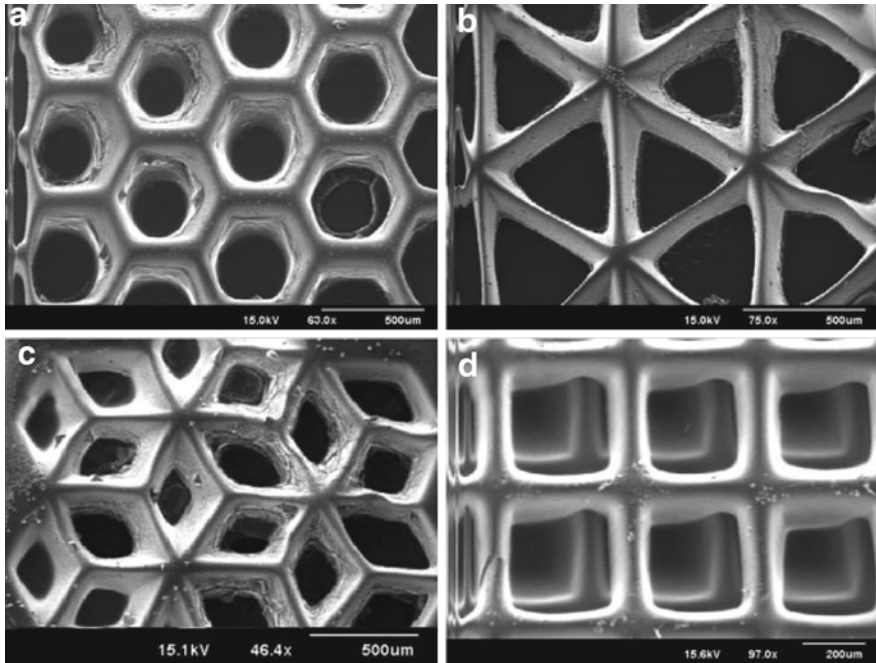


Fig. 2.3 Characteristic structures (hexagons (a), triangles (b), triangles inside hexagons (c) and squares (d)) formed through the stereolithographic rapid prototyping technique using a digital micromirror array are imaged with scanning electron microscopy. Feature sizes are approximately tens of microns thick. Reproduced with permission from Lu et al. (2006)

crosslinking, allowing cells to be deposited concurrently during photopolymerization without high levels of exposure to UV radiation.

Improvements in SLA resolution continue to be investigated using the latest in laser technology. Two-photon polymer excitation (Cumpston 1999) approaches the limits of the spatial precision of light in generating structures. Femtosecond lasers are being used not for use in SLA style applications but for ablation of monolithic biomaterials such as collagen, creating features by selectively ablating areas of the substrate (Liu et al. 2005). Further laser-related developments continue to emerge, as described by Stratakis (Stratakis 2009).

2.4 Conclusion

Tissue engineering is a young field that has made great strides not only in enhancing our understanding of the structure and function of natural tissue but also in developing techniques that allow designers to closely mimic the structure of normal tissue. Many of these techniques have been adapted from their original manifestations as rapid prototyping systems. As our understanding of the signals progenitor cells used

to differentiate and grow expands, so does our ability to create customized scaffolds that will provide the necessary inputs to guide proliferation along predetermined lineages, thereby fulfilling the promise of regenerative medicine.

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