Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery*

Stephanie M. Willerth and Shelly E. Sakiyama-Elbert[§], Department of Biomedical Engineering, Washington University in St. Louis, St. Louis, MO 63130, USA

Table of Contents

*Edited by Sangeeta Bhatia and Julia Polak. Last revised June 3, 2008. Published July 09, 2008. This chapter should be cited as: Willerth, S.M. and Sakiyama-Elbert, S.E., Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery (July 09, 2008), StemBook, ed. The Stem Cell Research Community, StemBook, doi/10.3824/stembook.1.1.1, http://www.stembook.org.

[§]To whom correspondence should be addressed: Shelly Sakiyama-Elbert, Department of Biomedical Engineering, Washington University, Campus Box 1097, One Brookings Drive, St. Louis, MO 63130, Telephone: (314) 935–7556, Fax: (314) 935–7448, sakiyama@wustl.edu.

Abstract

Combining stem cells with biomaterial scaffolds provides a promising strategy for engineering tissues and cellular delivery. This review seeks to describe the current types of scaffolds and evaluate their use in combination with stem cells for tissue engineering applications. Potential scaffold materials are classified as natural or synthetic and the advantages and drawbacks of each material are detailed. The materials are further divided into subcategories, which describe specific examples from the literature. Finally, conclusions about the current state of biomaterial scaffolds containing stem cells for tissue engineering applications are drawn and suggestions for the future direction of the field are given. Overall, this chapter seeks to give an overview of the available biomaterials for use in combination with directed stem cell differentiation as means of replacing diseased or damaged tissues.

1. Introduction

In 1987, a group of experts defined the word biomaterial as "a non-viable material used in a medical device, intended to interact with biological systems" (Europeon Society of Biomaterials Conference, 1987). This definition reflected the state of the field at the time, which was focused on developing materials and coatings to prevent the rejection of implantable medical devices. Since 1987, the field has advanced considerably with recent work resulting in the development of implantable scaffolds that consist entirely of specific biomaterials. These scaffolds demonstrate promise for tissue engineering applications and could potentially be used as replacements for diseased or damaged tissues. Such biomaterial scaffolds can be used to promote the viability and differentiation of stem cells seeded inside-based on both the intrinsic properties of the material and the incorporation of specific cues into the material. This review will focus on commonly used biomaterials that have been tested in combination with stem cells and the different applications for such scaffolds.

A wide range of biomaterials have been developed for different applications. For the purposes of this review, each biomaterial will be classified as either natural or synthetic. These categories will be broken down further into the following subtypes of materials: protein-based, polysaccharide-based, polymer-based, peptide-based, and ceramic-based biomaterials. The advantages and drawbacks of each subtype will be detailed along with an overview of the current body of work for each biomaterial as it pertains to the culture and delivery of stem cells. The different materials and applications along with the relevant literature are listed in Tables 1 and 2. This review will focus mainly on three-dimensional (3D) scaffolds as they are the most appropriate for developing engineered tissues and delivering cells for *in vivo* applications. It will also highlight studies that involve directed differentiation of stem cells into mature phenotypes as opposed to using biomaterial scaffolds for expansion of undifferentiated stem cells. Overall, this review seeks to summarize the current knowledge of using biomaterials in combination with stem cells.

2. Natural biomaterials

The different components that make up the extracellular matrix provide a starting point for developing scaffolds based on natural biomaterials. These proteins and polysaccharides perform many roles *in vivo* and thus make such materials attractive for tissue engineering applications. Additionally, their natural origin often means that these materials contain sites for cellular adhesion and tend to be biocompatible. Some of the disadvantages of these materials include potential lot to lot variability of the material, depending on the source, as well as needing to ensure the purity of the protein or polysaccharide before implantation to avoid activating an immune response. These scaffolds often have a limited range of mechanical properties and may need to be optimized for stem cell culture. The following sections will focus specifically on scaffolds made from purified proteins or polysaccharides as opposed to heterogeneous natural materials such as Matrigel or other complex protein mixtures.

2.1 Protein-based biomaterials

One of the main functions of proteins is to provide structure to tissues and this property suggests that protein-based biomaterials would be suitable for tissue engineering applications involving stem cell differentiation and transplantation. Different methods of purification exist depending on the protein desired for scaffold fabrication and the animal source. One of the most commonly used scaffold materials, collagen, can be isolated from a variety of tissues, such skin, tendon, or bone, usually of animal origin. *In vivo*, fibrin, which is derived from fibrinogen, generates the blood clots that form after injury to the vasculature. Due this role and the ability to isolate fibrinogen from blood (both human and animal), it has been used as a sealant in clinical studies and as a biomaterial scaffold. Another

Table 1. List of natural biomaterials

	Type of material	Application	Citations
Protein based biomaterials			
	Collagen	Bone, Cartilage, Heart, Ligament, Nerve, Vasculature	Baharvand et al. (2006); Battista et al. (2005); Chan et al. (2007); Chen et al. (2003); Daya et al. (2007); Gerecht-Nir et al. (2003); Ma et al. (2004); Michelini et al. (2006); Noth et al. (2005); O'Connor et al. (2000); Sumanasinghe et al. (2006); Watanabe et al. (2007)
	Fibrin	Cartilage, Nerve, Vasculature	Catelas et al. (2006); Gurevich et al. (2002); Im et al. (2005); Liu et al. (2006); Willerth et al. (2006); Willerth et al. (2007); Worster et al. (2001)
	Silk	Bone, Cartilage, Liver	Altman et al. (2002); Hofmann et al. (2007); Hofmann et al. (2006); Kim et al. (2005); Mauney et al. (2007); Meinel et al. (2005); Meinel et al. (2004a); Meinel et al. (2004b); Meinel et al. (2004c); Wang et al. (2005)
Polysaccharide based biomaterials			
	Agarose	Cartilage, Heart, Nerve	Ando et al. (2007); Awad et al. (2004); Chen et al. (2007b); Finger et al. (2007); Huang et al. (2004); Mauck et al. (2006); Moriyasu et al. (2006)
	Alginate	Cartilage, Liver, Nerve, Vasculature	Ashton et al. (2007); Awad et al. (2004); Franzesi et al. (2006); Gerecht-Nir et al. (2004); Hannouche et al. (2007); Jin et al. (2007); Maguire et al. (2006); Prang et al. (2006); Wayne et al. (2005)
	Hyaluronan	Adipose, Cartilage, Nerve, Skin, Vasculature	Angele et al. (2007); Chen et al. (2007a); Flynn et al. (2007); Flynn et al. (2008); Gerecht et al. (2007); Mehlhorn et al. (2007); Myers et al. (2007)
	Chitosan	Bone, Cartilage, Nerve, Skin	Cho et al. (2007); Franzesi et al. (2006); Gravel et al. (2006); Mrugala et al. (2007); PP et al. (2005)

protein that has been investigated for use in generating tissue engineered scaffolds is silk, which is secreted by insects and worms. Scaffolds made of silk or silk fibroin have slow degradation rates and desirable mechanical properties, providing an alternative to the collagen and fibrin. Scaffolds made from silk fibers can be fabricated into a variety of structures, such as mats, sponges, meshes and membranes, expanding the possible applications. Silk can also be chemically modified to further enhance the properties of such a scaffold. The following sections will highlight specific examples of these scaffolds being used in combination with stem cells for different tissue engineering applications.

2.1.1 Collagen

3D collagen scaffolds have been used to culture a wide variety of stem cells for different tissue engineering applications. One study demonstrated the monkey embryonic stem (ES) cells could differentiate into neural phenotypes as well as endothelial phenotypes when cultured as aggregates of cells known as embryoid bodies inside of collagen scaffolds (Chen et al., 2003; Michelini et al., 2006). When combined with appropriate cues, human ES cells can differentiate and form blood vessels when cultured inside of collagen scaffolds (Gerecht-Nir et al., 2003). A similar approach using 3D collagen scaffolds has been used to generate hepatocytes from human ES cells (Baharvand et al., 2006). Another study looked at the effect of incorporating fibronectin and laminin into collagen scaffolds on the behavior of the mouse ES cells seeded inside the scaffold (Battista et al., 2005). This study showed that fibronectin promotes differentiation into endothelial cells while adding laminin promotes differentiation into cardiomyocytes. All of these studies illustrate the suitability of collagen for use a scaffold for the culture of ES cells.

Table 2. List of synthetic biomaterials

Type of material		Application	Citations
Polymer based biomaterials			
	PLGA	Adipose, Bone, Cartilage, Muscle, Nerve	Bhang et al. (2007); Chastain et al. (2006); Choi et al. (2005, 2007); Graziano et al. (2007); Kim et al. (2003); Kim et al. (2006); Levenberg et al. (2005); Levenberg et al. (2003); Neubauer et al. (2005); Sun et al. (2007); Teng et al. (2002); Tomita et al. (2005); Uematsu et al. (2005); Xin et al. (2007); Yoon et al. (2007)
	PEG	Adipose, Bone, Cartilage, Liver, Heart, Nerve	Benoit and Anseth (2005); Benoit et al. (2007); Buxton et al. (2007); Ford et al. (2006); Hwang et al. (2006); Mahoney and Anseth (2006, 2007); Nuttelman et al. (2004); Royce Hynes et al. (2007); Salinas et al. (2007); Shin et al. (2004); Stosich et al. (2007); Underhill et al. (2007); Varghese et al. (2008)
Peptide based biomaterials		Bone, Nerve	Garreta et al. (2007); Garreta et al. (2006); Gelain et al. (2006); Hamada et al. (2008); Hosseinkhani et al. (2006); Silva et al. (2004)
Ceramic based biomaterials		Bone, Cartilage	Arinzeh et al. (2003); Arinzeh et al. (2005); Bruder et al. (1998); Dennis and Caplan (1993); Dyson et al. (2007); Gao et al. (2001); Hanada et al. (1997); Kitamura et al. (2004); Kotobuki et al. (2005); Kruyt et al. (2006); Lennon et al. (1995); Marcacci et al. (2007); Meseguer-Olmo et al. (2007); Ohgushi et al. (1996); Shimaoka et al. (2004); Toquet et al. (1999); Turhani et al. (2005); Yamada et al. (2003); Yang et al. (2006)

Additionally, other types of stem cells have been used in conjunction with collagen scaffolds to produce engineered tissues. These approaches include seeding such scaffolds with neural stem cells (Ma et al., 2004; O'Connor et al., 2000; Watanabe et al., 2007). These cells differentiate into neurons and form functional circuits inside of the scaffolds (Ma et al., 2004). Also, pre-adipocyte cells can be directed to differentiate into mature adipocytes when seeded inside of 3D collagen scaffolds (Daya et al., 2007). Other studies have cultured mesenchymal stem cells inside of such scaffolds for a variety of applications including engineering bone, ligaments, and cartilage (Chan et al., 2007; Noth et al., 2005; Sumanasinghe et al., 2006).

2.1.2 Fibrin

Although it has not been investigated as heavily as collagen, fibrin has also been studied as potential scaffold material for the culture of stem cells. Our lab has utilized fibrin scaffolds for the culture of ES cell derived neural progenitor cells and determined the necessary soluble growth factor cues needed to promote the differentiation of such cells in neurons and oligodendrocytes (Willerth et al., 2006; Willerth et al., 2007). Other groups have examined the behavior of mesenchymal stem cells seeded inside of fibrin clots and treated with growth factors for use in engineering bone (Catelas et al., 2006; Gurevich et al., 2002). Additionally, fibrin scaffolds seeded with mesenchymal stem cells have also been used for engineering cartilage (Im et al., 2005; Worster et al., 2001). Work from the Suggs laboratory has demonstrated the suitability of fibrin scaffolds for promoting vasculature formation from mouse ES cells (Liu et al., 2006). This body of work suggests that a variety of stem cell lines can be cultured inside of fibrin scaffolds for many different tissue engineering applications.

2.1.3 Silk

The properties of silk make it attractive for engineering bone and ligament tissue and extensive research has been done using 3D silk scaffolds in conjunction with mesenchymal stem cells for these applications. Specifically the Kaplan

laboratory has successfully developed such strategies (Altman et al., 2002; Hofmann et al., 2007; Hofmann et al., 2006; Kim et al., 2005; Mauney et al., 2007; Meinel et al., 2005; Meinel et al., 2004a; Meinel et al., 2004b; Meinel et al., 2004c). Work from their lab has shown that human mesenchymal stem cells combined with silk scaffolds can be used to engineer bone. One of their first studies demonstrated that the flow conditions around the scaffold as well as the properties of the scaffold influenced the rate of calcium deposition, which is an important consideration for bone tissue engineering (Meinel et al., 2004b). A companion study explored using silk scaffolds modified to contain RGD (arginine-glycine-aspartic acid) peptide sequences for the culture of human mesenchymal stem cells and showed that these scaffolds were appropriate for replacing bone due the slow scaffold degradation (Meinel et al., 2004c). Other studies have examined the role of pore size to determine its influence on the behavior of the stem cells seeded inside silk scaffolds (Kim et al., 2005; Meinel et al., 2005).

Another area of tissue engineering where this approach has been used is for the production of cartilage. Meinel and colleagues showed that silk scaffolds promoted more extensive chondrogenesis compared to collagen scaffolds when these scaffolds were seeded with human mesenchymal stem cells (Meinel et al., 2004a). A follow up study showed that macroporous silk scaffolds developed using an aqueous process could also be used for such applications (Wang et al., 2005). Similar approaches have also been used to produce tissue engineered replacements for ligaments (Altman et al., 2002). Overall, the mechanical properties of silk make it an attractive material for engineering bone, cartilage and ligament tissue from stem cells.

2.2 Polysaccharide-based biomaterials

In addition to proteins, polysaccharides, which consist of sugar monomers, also play important role in maintaining the structure of the extracellular matrix. These materials have been investigated for use as a potential scaffold material for stem cell transplantation. Polysaccharides are usually branched and can be obtained from either plant or animal sources. Depending on the source of the polysaccharide and method of isolation, an immune response may be triggered, which is one issue to consider when choosing a potential scaffold material. The structure and monomer composition contribute the properties of the specific polysaccharides. Scaffolds made from such materials can often be formulated to gel rapidly, allowing for injection into the injury site. This section will review some of the most commonly used polysaccharide-based scaffolds, including agarose, alginate, hyaluronan, and chitosan, that have been used for the culture and differentiation of stem cells.

2.2.1 Agarose

Agarose, which is isolated from red algae and seaweed, consists of a galactose-based backbone and is commonly used as a medium for cell culture in the form of agar. One of the attractive properties of agarose is that its stiffness can altered, allowing for tuning of the mechanical properties of the scaffold. Agarose scaffold have been investigated in combination with stem cells for generating a variety of applications, including cartilage, heart, and nerve. A variety of studies have demonstrated the suitability of agarose scaffolds for promoting stem cells to differentiate into chondrocytes (Awad et al., 2004; Finger et al., 2007; Huang et al., 2004; Mauck et al., 2006). The different stem cells types used in these studies included bovine mesenchymal stem cells, human mesenchymal stem cells, and adipose-derived stem cells. A different study showed that primate ES cells cultured inside of agarose scaffolds would form aggregates and differentiate into cardiomyocytes that would beat for up to one month (Chen et al., 2007b). Other studies have demonstrated that both mouse and primate ES cells can differentiate into dopaminergic neurons when encapsulated inside of agarose microcapsules (Ando et al., 2007; Moriyasu et al., 2006). This strategy could be used as a potential therapy for Parkinson's disease. Overall, agarose scaffolds provide a versatile platform for tissue engineering.

2.2.2 Alginate

Alginate, which is derived from the cell walls of brown algae, forms scaffolds through the use of ionic cross-linking, allowing for encapsulation of cells. Many studies have evaluated alginate scaffolds as a platform for generating cartilage (Awad et al., 2004; Hannouche et al., 2007; Jin et al., 2007; Wayne et al., 2005). Both adipose-derived adult stem cells as well bone marrow-derived mesenchymal stem cells have been shown to survive and differentiate in chondrocytes in these studies. Alginate has also been used for neural tissue engineering applications. One study demonstrated that adult neural progenitor cells seeded inside of alginate scaffolds survived *in vivo* for two weeks after implantation into a spinal cord injury model (Prang et al., 2006). A different study developed tunable alginate scaffolds by incorporating microspheres that released enzymes over time to degrade the scaffold. These scaffolds were successfully used to culture neural progenitor cells and increased their proliferation rate compared to when such cells were cultured in

alginate scaffolds without microspheres (Ashton et al., 2007). Alginate scaffolds have also been used in combination with ES cells to generate hepatocytes and vasculature (Gerecht-Nir et al., 2004; Maguire et al., 2006).

2.2.3 Hyaluronan

Hyaluronan, also known as hyaluronic acid, is one of the major components of the extracellular matrix. It contains sites for cell adhesion and hyaluronan expression is upregulated during embryogenesis, suggesting its suitability as a scaffold material for the culture of ES cells. A recent study from the Langer lab demonstrated that such scaffolds could be used for promoting both self renewal of human ES cells as well as vascular differentiation (Gerecht et al., 2007). Hyaluronan is also expressed in many different tissues, including cartilage and nerve, suggesting it could also be used for the culture and differentiation of adult stem cells. Some studies have used mesenchymal stem cells cultured inside of hyaluronan scaffolds as a way of repairing cartilage both *in vitro* and *in vivo* (Angele et al., 2007; Mehlhorn et al., 2007). Work from the Woodhouse group has also used such approaches to engineer adipose substitutes (Flynn et al., 2007; Flynn et al., 2008). Other approaches have combined hyaluronan scaffolds with stem cells derived from keratinocytes and adipose for engineering skin and bone respectively (Chen et al., 2007a; Myers et al., 2007).

2.2.4 Chitosan

Another polysaccharide that has been explored for tissue engineering applications is chitosan. It is derived by the deacetylation of chitin and consists of glucosamine units. Additionally, the rate of gelation of chitosan scaffolds can be controlled using pH. Chitosan has been used extensively as material for regenerating skin, bone and nerve tissue and has more recently been studied for use in combination with stem cells. One of the studies looked at the ability of such 3D scaffolds to promote osteogenic differentiation of mouse mesenchymal stem cells (Gravel et al., 2006). This study showed that the addition of corraline, another seaweed derived material, enhanced osteocalcin release over time, which is important for bone formation. A different approach for bone tissue engineering used adipose-derived mesenchymal stem cells seeded inside of chitosan particles, which were then aggregated to form scaffolds (PP et al., 2005). Chitosan scaffolds have also been demonstrated to be suitable for mouse ES cell culture as well as for the expansion of stem cells derived from human cord blood (Cho et al., 2007; Franzesi et al., 2006). For cartilage tissue engineering, an *in vivo* study looked at the effects of using chitosan scaffolds seeded with mesenchymal stem cells and transforming growth factor-β as treatment for lesions on the patella of sheep (Mrugala et al., 2007). These cells differentiated into chondrocyte-like cells, demonstrating that such strategies can be effective *in vivo*. Such studies show that these scaffolds support stem cell differentiation both *in vitro* and *in vivo*.

3. Synthetic biomaterials

Synthetic biomaterials provide an alternative to natural materials to serve as scaffolds for the culture of stem cells. These materials offer many advantages including reproducibility due to their defined chemical composition and the ability to control the mechanical properties, degradation rate, and shape independently. The mechanical properties of a scaffold can influence the resulting stem cell differentiation (Engler et al., 2006). The ability to shape a material allows for production of scaffolds that conform to specifications of the injury or transplantation site. The ability to tailor scaffolds with specific degradation rate is one advantage of synthetic scaffolds over natural biomaterials and these properties can also affect the release rate of drugs incorporated into such scaffolds. However, many of the synthetic materials lack sites for cell adhesion and may have to be chemically modified to contain such cues to allow for stem cell adhesion and culture. Other considerations include the biocompatibility of the material and its suitability for transplantation *in vivo*, as well as whether or not the material and its byproducts can trigger an immune response.

3.1 Polymer-based biomaterials

This section will discuss polymers that have been used in conjunction with stem cells for producing different kinds of tissue. These scaffolds are chemically defined and can often be formulated to have specific mechanical properties. They can also be modified to contain cues using various chemistries. There are some issues with these polymer-based scaffolds including a lack of sites for cell adhesion and the potential for toxic byproducts after degradation. This section will focus on some of the most commonly used polymer scaffolds for culture of stem cells, including poly (lactic-co-glycolic acid) and poly (ethylene glycol). Other polymers, such as poly (2-hydroxyethyl methacrylate) (pHEMA) and poly (ε-caprolactone), have been explored for stem cell culture in 2D, but these studies will not be discussed in this chapter.

3.1.1 Poly (lactic-co-glycolic acid)

Poly (lactic-co-glycolic acid) (PLGA) is a copolymer that consists of monomers of glycolic acid and lactic acid connected by ester bonds. It is an FDA approved polymer that is attractive for tissue engineering applications due to its biocompatibility and the ability to modulate the degradation rate. In the presence of cells, PLGA scaffolds degrade in the monomers, which are natural metabolites but can have negative effects due to their acidic nature. For these reasons, PLGA scaffolds have been used for engineering a wide range of tissues.

Some groups have investigated combining PLGA scaffolds with mesenchymal stem cells for the generation of adipose tissue, which can be used during reconstructive surgery. They showed that including basic fibroblast growth factor (bFGF) along with the stem cells seeded inside of PLGA scaffolds enhanced the differentiation of these cells into adipocytes (Neubauer et al., 2005). Work from the Suh lab looked at translating such an approach to a PLGA microsphere system to develop an injectable method of tissue engineering (Choi et al., 2005, 2007). Other work has investigated the use of PLGA scaffolds combined with adult adipose-derived stem cells for generating muscle tissue (Kim et al., 2006).

Neural tissue engineering represents another area where PLGA scaffolds seeded with stem cells shows promise as therapy for disorders of the nervous system. Work done by the Langer lab has shown the potential of such strategies. One study that showed that PLGA scaffolds designed to mimic the spinal cord (see Figure 1) and seeded with murine neural stem cells produced an increase in functional recovery after traumatic spinal cord injury in preclinical testing (Teng et al., 2002). An additional study demonstrated that human ES cells seeded inside of PLGA scaffolds could be directed to differentiate into neurons when treated with the appropriate cues (Levenberg et al., 2003). The same study also showed that these cells could differentiate in cartilage and liver tissue inside of such scaffolds when exposed to the appropriate cues. A follow up study further characterized the differentiation of human ES cells treated with neurotrophins when seeded inside PLGA scaffolds for engineering neural tissue (Levenberg et al., 2005). Seeding retinal progenitor cells into PLGA scaffolds provided an effective method of cell delivery *in vivo,* and the cells were able to differentiate into neurons and astrocytes (Tomita et al., 2005). PLGA has also been demonstrated to be a suitable scaffold for the culture of progenitor cells isolated from the hippocampus in terms of cell viability and differentiation (Bhang et al., 2007).

An extensive amount of research has been conducted using PLGA scaffolds for engineering bone and cartilage tissue. One study seeded human mesenchymal stem cells onto nanofiber PLGA scaffolds where they differentiated into both chondrogenic and osteogenic phenotypes (Xin et al., 2007). Another group implanted PLGA scaffolds seeded with mesenchymal stem cells into rabbits with large defects in their knees (Uematsu et al., 2005). Cartilage formation was observed 12 weeks after implantation when the scaffolds were used. Numerous studies have been conducted using PLGA scaffolds seeded with mesenchymal stem cells for bone tissue engineering (Chastain et al., 2006; Graziano et al., 2007; Kim et al., 2003; Sun et al., 2007; Yoon et al., 2007). Some of the interesting observations from these studies included determining that the addition of pits to such scaffolds enhanced osteogenic differentiation and that co-culture with endothelial cells affected differentiation (Graziano et al., 2007; Sun et al., 2007).

3.1.2 Poly (ethylene glycol)

Poly (ethylene glycol) (PEG), with high molecular weight versions being referred to as poly (ethylene oxide) (PEO), is a commonly used polymer for biomaterial applications due to its ability to resist protein absorption. Scaffolds made from PEG can be polymerized using either chemical or photoinitiators, and the amount of initiator used affects the properties of the resulting scaffolds. These scaffolds can also be chemically modified to contain bioactive molecules, including peptides and heparin. In combination with stem cells, these scaffolds have been evaluated for their suitability as potential replacements for bone, cartilage, nerve, liver and vasculature tissue.

A great deal of research has been done on using PEG scaffolds seeded with stem cells to generate bone and cartilage (Benoit and Anseth, 2005; Benoit et al., 2007; Buxton et al., 2007; Hwang et al., 2006; Nuttelman et al., 2004; Salinas et al., 2007; Shin et al., 2004; Varghese et al., 2008). For bone applications, studies explored using mesenchymal stem cells combined with a variety of cues, including RGD peptides, bone morphogenetic protein (BMP), and heparin, to promote osteogenic differentiation (Benoit and Anseth, 2005; Benoit et al., 2007; Nuttelman et al., 2004; Shin et al., 2004). Similar to the approaches for generating bone, mesenchymal stem cells were also used for producing cartilage in combination with PEG scaffolds. The cells were directed to form chondrocytes through the addition of bioactive molecules, including chondroitin sulfate, transforming growth factor-β, and BMP (Hwang et al., 2006; Varghese et al., 2008). One of the studies looked at how changing the properties of the PEG scaffold influenced

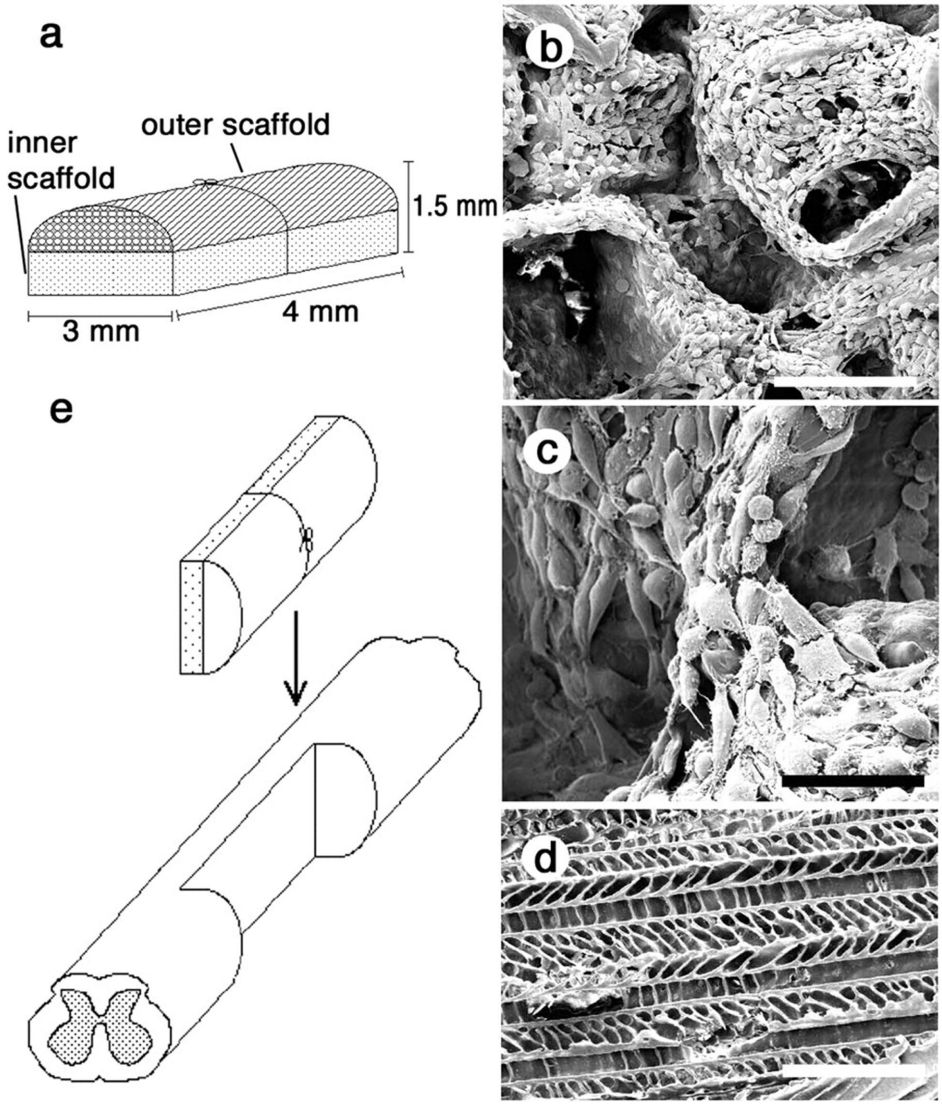

Figure 1. Designing PLGA scaffolds that mimicks the architecture of the spinal cord. A. Schematic of the scaffold design showing the inner and outer scaffolds. B. and C. Inner scaffolds seeded with NSCs. (Scale bars: 200 μm and 50 μm, respectively.) D. The outer section of the scaffold was generated by means of a solid-liquid phase separation technique that produced long, axially oriented pores for axonal guidance as well as radial pores to allow fluid transport and inhibit the ingrowth of scar tissue (scale bar, 100 μm). E. Schematic of surgical insertion of the implant into the spinal cord. **Reproduced from Teng et al. (2002), Copyright 2002, National Academy of Sciences, U.S.A.**

extracellular matrix secretion by the stem cells (Buxton et al., 2007). All of these conditions are important to consider when designing PEG scaffolds for stem cell culture.

Other examples in the literature show the suitability of PEG scaffolds for engineering nerve tissue for the treatment of central nervous system disorders, such as Parkinson's disease or spinal cord injury. Work by Mahoney and Anseth demonstrated that neural precursor cells could be cultured inside of PEG scaffolds and investigated the effects of adding bFGF and collagen to such a system (Mahoney and Anseth, 2006, 2007). The Lavik group functionalized PEG scaffolds with poly-L-lysine to add sites for cell adhesion, and the neural stem cells seeded inside these scaffolds survived and were able to differentiate into mature phenotypes (Royce Hynes et al., 2007). Another study from their lab used macroporous PEG scaffolds for the co-culture of neural progenitor cells and endothelial cells to engineer nerve tissue. The addition of the endothelial cells allowed for formation of a microvasculature inside of the nerve tissue when tested *in vivo* (Ford et al., 2006). PEG scaffolds have also been investigated in combination with human mesenchymal stem cells for adipose tissue engineering and with mouse embryonic liver cells to generate hepatocytes for liver tissue engineering, showing the versatility of such scaffolds (Stosich et al., 2007; Underhill et al., 2007).

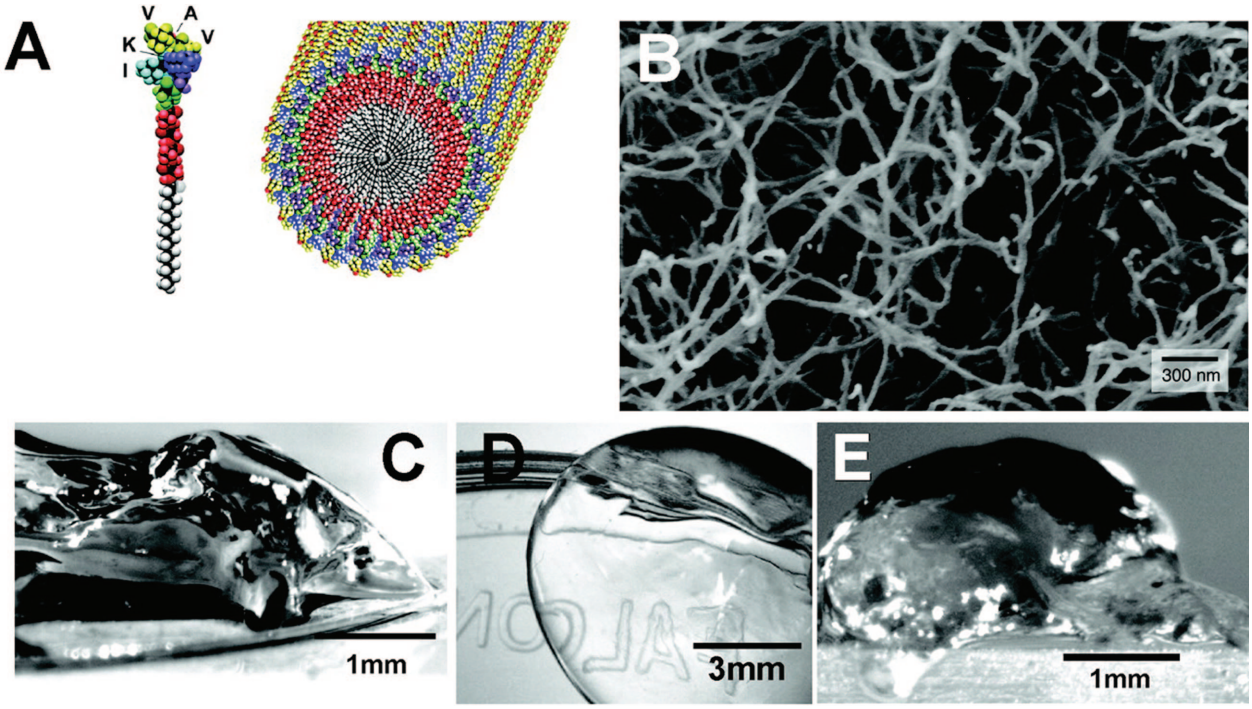

Figure 2. Self assembling peptide scaffolds for the neural tissue engineering applications. A. Molecular graphics illustration of an IKVAV-containing peptide amphiphile molecule and its self-assembly into nanofibers. B. Scanning electron micrograph of an IKVAV nanofiber network formed by adding cell media (DMEM) to a peptide amphiphile aqueous solution. The sample in the image was obtained by network dehydration and critical-point drying of samples caged in a metal grid to prevent network collapse (samples were sputtered with gold-palladium films and imaged at 10 kV). C. and D. Micrographs of the gel formed by adding to IKVAV peptide amphiphile solutions (C) cell culture media and (D) cerebral spinal fluid. E. Micrograph of an IKVAV nanofiber gel surgically extracted from an enucleated rat eye after intraocular injection of the peptide amphiphile solution. **Reproduced from Silva et al. (2004). Reprinted with permission from AAAS**. Readers may view, browse, and/or download material for temporary copying purposes only, provided these uses are for noncommercial personal purposes. Except as provided by law, this material may not be further reproduced, distributed, transmitted, modified, adapted, performed, displayed, published, or sold in whole or in part, without prior written permission from the publisher.

3.2 Peptide-based biomaterials

Peptide-based biomaterials consist of short sequences of amino acids, which can produce self assembling scaffolds. These scaffolds can potentially combine the functionality of protein-based scaffolds by using motifs derived from naturally occurring proteins with the reproducibility of synthetic scaffolds. Many of the peptide-based biomaterials can self assemble into 3D scaffolds through the use of amphiphilic peptides, which form aggregates in aqueous solutions.

The Stupp lab was one of the first groups to use such self-assembling scaffolds (shown in Figure 2) for promoting the differentiation of murine neural progenitor cells into neurons (Silva et al., 2004). These scaffolds contained the peptide sequence IKVAV (isoleucine-lysine-valine-alanine-valine) derived from laminin and this sequence had been shown previously to promote neurite outgrowth (Matsuzawa et al., 1996). This study also illustrates the importance of selecting the appropriate peptide sequence for promoting the survival and differentiation of the stem cells seeded inside of such a scaffold. A similar approach was used to develop self-assembling peptide scaffolds seeded with mesenchymal stem cells for bone tissue engineering (Hosseinkhani et al., 2006). These scaffolds incorporated an RGD sequence to allow the cells to adhere to the scaffolds.

Other groups have used scaffolds produced from the self-assembling peptide called RADA16-I combined with stem cells for tissue engineering applications. One approach to producing bone involved seeding embryoid bodies derived from mouse ES cells inside of scaffolds made from these peptides and then inducing the cells to differentiate using osteogenic medium (Garreta et al., 2006). A follow up study combined this self-assembling peptides with the ceramic hydroxyapatite to produce a scaffold that was more efficient promoting at the differentiation of mouse ES cells seeded inside (Garreta et al., 2007). More recent work by the Zhang lab looked at altering the RADA16 peptides to incorporate 18 different peptide motifs to determine the most appropriate scaffold material for mouse adult neural stem cells (Gelain et al., 2006). This study identified two motifs derived from bone marrow homing motifs that promoted

the differentiation of these stem cells into neurons and astrocytes as well as increased cell survival. This work suggests that such self-assembling peptide scaffolds can be optimized to promote the differentiation of stem cells into a desired phenotype based on the incorporated motifs.

3.3 Ceramic-based biomaterials

Ceramics are inorganic materials formed through treatment with heat and are often porous and brittle. They have crystalline structures and are used for a wide variety of applications. Common examples of such materials include bioactive glass and hydroxyapatite. Bioactive glass consists of a mixture of silicon dioxide, sodium oxide, calcium oxide, and phosphate oxide. Its chemical composition allows it to have surface reactivity and these materials were developed as means of replacing injured bone. Hydroxyapatite, which is a mineral found in bone, is a naturally occurring ceramic material. Synthetic versions of hydroxyapatite have been synthesized and investigated for use as a potential replacement for bone tissue due to its strength and biocompatibility. Other ceramic-based materials exhibit similar properties and these materials, along with hydroxyapatite, have been investigated extensively in combination with stem cells for tissue engineering of bone (Arinzeh et al., 2003; Arinzeh et al., 2005; Bruder et al., 1998; Dennis and Caplan, 1993; Dyson et al., 2007; Gao et al., 2001; Hanada et al., 1997; Kitamura et al., 2004; Kotobuki et al., 2005; Kruyt et al., 2006; Lennon et al., 1995; Marcacci et al., 2007; Meseguer-Olmo et al., 2007; Ohgushi et al., 1996; Shimaoka et al., 2004; Toquet et al., 1999; Turhani et al., 2005; Yamada et al., 2003; Yang et al., 2006). These studies include both *in vitro* and *in vivo* testing of these materials.

The Caplan lab has done a great deal of work characterizing the culture of mesenchymal stem cells on ceramic materials (Dennis and Caplan, 1993; Hanada et al., 1997; Lennon et al., 1995). These studies showed that these stem cells could differentiate into osteogenic phenotypes when seeded inside of ceramic cubes and demonstrated that bFGF and BMP could be used to enhance differentiation. Another study demonstrated the usefulness of growth/differentiation factor-5 for promoting osteogenic differentiation in such a culture system (Shimaoka et al., 2004). These scaffolds have also showed promise when tested in *in vivo* models of bone defects (Arinzeh et al., 2003; Bruder et al., 1998; Marcacci et al., 2007; Yamada et al., 2003). One of the studies demonstrated that incorporation of mesenchymal stem cells into ceramic implants results in stronger bone graft compared to implanting an acellular version of the scaffold (Bruder et al., 1998). Two other studies showed similar results with bone formation only occurring when the stem cells were seeded into the scaffold (Arinzeh et al., 2003; Yamada et al., 2003). Finally a recent study detailed the results of using such an approach in a pilot clinical study in Italy (Marcacci et al., 2007). In this study, bone marrow stromal cells were taken from the patient and seeded onto the ceramic scaffolds that were then implanted back into the patient. Long term bone regeneration consisting of fusion between the implant and the original bone was observed with no major adverse events reported. This study illustrates how such regenerative medicine strategies can be effective in a clinical setting.

More recently, these ceramic-based materials have been combined with biodegradable polymers, such as those materials mentioned earlier in this chapter, to be used for bone tissue engineering applications. Such composite materials can allow for additional drug delivery functionality and for fabrication of highly porous structures to allow for cellular infiltration when culturing stem cells inside of such scaffolds. Such composite scaffolds and drug delivery methods for bone tissue engineering have been reviewed extensively in the literature (Guarino et al., 2007; Lee and Shin, 2007; Rezwan et al., 2006).

4. Conclusions and future studies

The purpose of this review was to detail some of the most commonly used biomaterial scaffolds for transplantation of stem cells. It sought to give an idea of the types of materials available and their unique properties, allowing reader to the ability to choose a material that would best suit their specific tissue engineering application. As seen throughout the studies detailed in this chapter, the type of material and the cues that are incorporated in the scaffold play a large role in directing the fate of the stem cells seeded inside. Another important consideration when designing such scaffolds is the method of fabrication, which allows different types of patterns and architecture to be formed. These methods have been reviewed elsewhere (Tsang and Bhatia, 2004).

Many of the scaffold materials discussed in this chapter could be adapted for new tissue engineering applications and stem cell types. Although it was not discussed extensively in the chapter, different scaffold materials can be blended together to achieve additional properties and benefits. Also, many different biomaterials exist that have not yet been adapted for use with stem culture, which could be explored in future studies. Another consideration for

developing engineered scaffolds containing stem cells is how to incorporate or produce of a vascular network inside of the scaffold that would allow it to integrate with actual tissue and restore function lost after injury or disease. It can be achieved through a variety of ways including through co-culture with endothelial cells or by adding cues to promote angiogenesis into the scaffolds. Overall, this chapter has provided a starting point for further development of biomaterial scaffolds combined with stem cells for tissue engineering applications.

5. Acknowledgements

The authors would like to acknowledge the funding source for this work – NIH R01 NS051454.

6. References

Altman, G. H., Horan, R. L., Lu, H. H., Moreau, J., Martin, I., Richmond, J. C., and Kaplan, D. L. (2002). Silk matrix for tissue engineered anterior cruciate ligaments. Biomaterials *23*, 4131–4141.

Ando, T., Yamazoe, H., Moriyasu, K., Ueda, Y., and Iwata, H. (2007). Induction of dopamine-releasing cells from primate embryonic stem cells enclosed in agarose microcapsules. Tissue engineering *13*, 2539–2547.

Angele, P., Johnstone, B., Kujat, R., Zellner, J., Nerlich, M., Goldberg, V., and Yoo, J. (2007). Stem cell based tissue engineering for meniscus repair. Journal of biomedical materials research.

Arinzeh, T. L., Peter, S. J., Archambault, M. P., van den Bos, C., Gordon, S., Kraus, K., Smith, A., and Kadiyala, S. (2003). Allogeneic mesenchymal stem cells regenerate bone in a critical-sized canine segmental defect. The Journal of bone and joint surgery *85-A*, 1927–1935.

Arinzeh, T. L., Tran, T., McAlary, J., and Daculsi, G. (2005). A comparative study of biphasic calcium phosphate ceramics for human mesenchymal stem-cell-induced bone formation. Biomaterials *26*, 3631–3638.

Ashton, R. S., Banerjee, A., Punyani, S., Schaffer, D. V., and Kane, R. S. (2007). Scaffolds based on degradable alginate hydrogels and poly(lactide-co-glycolide) microspheres for stem cell culture. Biomaterials *28*, 5518–5525.

Awad, H. A., Wickham, M. Q., Leddy, H. A., Gimble, J. M., and Guilak, F. (2004). Chondrogenic differentiation of adipose-derived adult stem cells in agarose, alginate, and gelatin scaffolds. Biomaterials *25*, 3211–3222.

Baharvand, H., Hashemi, S. M., Kazemi, Ashtiani S., and Farrokhi, A. (2006). Differentiation of human embryonic stem cells into hepatocytes in 2D and 3D culture systems in vitro. The International journal of developmental biology *50*, 645–652.

Battista, S., Guarnieri, D., Borselli, C., Zeppetelli, S., Borzacchiello, A., Mayol, L., Gerbasio, D., Keene, D. R., Ambrosio, L., and Netti, P. A. (2005). The effect of matrix composition of 3D constructs on embryonic stem cell differentiation. Biomaterials *26*, 6194–6207.

Benoit, D. S., and Anseth, K. S. (2005). Heparin functionalized PEG gels that modulate protein adsorption for hMSC adhesion and differentiation. Acta biomaterialia *1*, 461–470.

Benoit, D. S., Durney, A. R., and Anseth, K. S. (2007). The effect of heparin-functionalized PEG hydrogels on three-dimensional human mesenchymal stem cell osteogenic differentiation. Biomaterials *28*, 66–77.

Bhang, S. H., Lim, J. S., Choi, C. Y., Kwon, Y. K., and Kim, B. S. (2007). The behavior of neural stem cells on biodegradable synthetic polymers. Journal of biomaterials science *18*, 223–239.

Bruder, S. P., Kurth, A. A., Shea, M., Hayes, W. C., Jaiswal, N., and Kadiyala, S. (1998). Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells. J Orthop Res *16*, 155–162.

Buxton, A. N., Zhu, J., Marchant, R., West, J. L., Yoo, J. U., and Johnstone, B. (2007). Design and characterization of poly(ethylene glycol) photopolymerizable semi-interpenetrating networks for chondrogenesis of human mesenchymal stem cells. Tissue engineering *13*, 2549–2560.

Catelas, I., Sese, N., Wu, B. M., Dunn, J. C., Helgerson, S., and Tawil, B. (2006). Human mesenchymal stem cell proliferation and osteogenic differentiation in fibrin gels in vitro. Tissue engineering *12*, 2385–2396.

Chan, B. P., Hui, T. Y., Yeung, C. W., Li, J., Mo, I., and Chan, G. C. (2007). Self-assembled collagen-human mesenchymal stem cell microspheres for regenerative medicine. Biomaterials *28*, 4652–4666.

Chastain, S. R., Kundu, A. K., Dhar, S., Calvert, J. W., and Putnam, A. J. (2006). Adhesion of mesenchymal stem cells to polymer scaffolds occurs via distinct ECM ligands and controls their osteogenic differentiation. Journal of biomedical materials research *78*, 73–85.

Chen, P. Y., Huang, L. L., and Hsieh, H. J. (2007a). Hyaluronan preserves the proliferation and differentiation potentials of long-term cultured murine adipose-derived stromal cells. Biochemical and biophysical research communications *360*, 1–6.

Chen, S. S., Fitzgerald, W., Zimmerberg, J., Kleinman, H. K., and Margolis, L. (2007b). Cell-cell and cell-extracellular matrix interactions regulate embryonic stem cell differentiation. Stem cells (Dayton, Ohio) *25*, 553–561.

Chen, S. S., Revoltella, R. P., Papini, S., Michelini, M., Fitzgerald, W., Zimmerberg, J., and Margolis, L. (2003). Multilineage differentiation of rhesus monkey embryonic stem cells in three-dimensional culture systems. Stem cells (Dayton, Ohio) *21*, 281–295.

Cho, C. H., Eliason, J. F., and Matthew, H. W. (2007). Application of porous glycosaminoglycan-based scaffolds for expansion of human cord blood stem cells in perfusion culture. Journal of biomedical materials research.

Choi, Y. S., Park, S. N., and Suh, H. (2005). Adipose tissue engineering using mesenchymal stem cells attached to injectable PLGA spheres. Biomaterials *26*, 5855–5863.

Choi, Y. S., Park, S. N., and Suh, H. (2007). The effect of PLGA sphere diameter on rabbit mesenchymal stem cells in adipose tissue engineering. Journal of materials science.

Daya, S., Loughlin, A. J., and Macqueen, H. A. (2007). Culture and differentiation of preadipocytes in two-dimensional and three-dimensional in vitro systems. Differentiation; research in biological diversity *75*, 360–370.

Definitions in Biomaterials: Proceeding of a Consensus Conference of the European Society of Biomaterials (1987). Chester, England, March 3–5 Vol 4. (Amsterdam, Elsevier).

Dennis, J. E., and Caplan, A. I. (1993). Porous ceramic vehicles for rat-marrow-derived (Rattus norvegicus) osteogenic cell delivery: effects of pre-treatment with fibronectin or laminin. The Journal of oral implantology *19*, 106–115; discussion 136–107.

Dyson, J. A., Genever, P. G., Dalgarno, K. W., and Wood, D. J. (2007). Development of custom-built bone scaffolds using mesenchymal stem cells and apatite-wollastonite glass-ceramics. Tissue engineering *13*, 2891–2901.

Engler, A. J., Sen, S., Sweeney, H. L., and Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. Cell *126*, 677–689.

Finger, A. R., Sargent, C. Y., Dulaney, K. O., Bernacki, S. H., and Loboa, E. G. (2007). Differential effects on messenger ribonucleic acid expression by bone marrow-derived human mesenchymal stem cells seeded in agarose constructs due to ramped and steady applications of cyclic hydrostatic pressure. Tissue engineering *13*, 1151–1158.

Flynn, L., Prestwich, G. D., Semple, J. L., and Woodhouse, K. A. (2007). Adipose tissue engineering with naturally derived scaffolds and adipose-derived stem cells. Biomaterials *28*, 3834–3842.

Flynn, L. E., Prestwich, G. D., Semple, J. L., and Woodhouse, K. A. (2008). Proliferation and differentiation of adipose-derived stem cells on naturally derived scaffolds. Biomaterials.

Ford, M. C., Bertram, J. P., Hynes, S. R., Michaud, M., Li, Q., Young, M., Segal, S. S., Madri, J. A., and Lavik, E. B. (2006). A macroporous hydrogel for the coculture of neural progenitor and endothelial cells to form functional

vascular networks in vivo. Proceedings of the National Academy of Sciences of the United States of America *103*, 2512–2517.

Franzesi, G. T., Ni, B., Ling, Y., and Khademhosseini, A. (2006). A controlled-release strategy for the generation of cross-linked hydrogel microstructures. Journal of the American Chemical Society *128*, 15064–15065.

Gao, J., Dennis, J. E., Solchaga, L. A., Awadallah, A. S., Goldberg, V. M., and Caplan, A. I. (2001). Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells. Tissue engineering *7*, 363–371.

Garreta, E., Gasset, D., Semino, C., and Borros, S. (2007). Fabrication of a three-dimensional nanostructured biomaterial for tissue engineering of bone. Biomolecular engineering *24*, 75–80.

Garreta, E., Genove, E., Borros, S., and Semino, C. E. (2006). Osteogenic differentiation of mouse embryonic stem cells and mouse embryonic fibroblasts in a three-dimensional self-assembling peptide scaffold. Tissue engineering *12*, 2215–2227.

Gelain, F., Bottai, D., Vescovi, A., and Zhang, S. (2006). Designer self-assembling Peptide nanofiber scaffolds for adult mouse neural stem cell 3-dimensional cultures. PLoS ONE *1*, e119.

Gerecht-Nir, S., Cohen, S., Ziskind, A., and Itskovitz-Eldor, J. (2004). Three-dimensional porous alginate scaffolds provide a conducive environment for generation of well-vascularized embryoid bodies from human embryonic stem cells. Biotechnology and bioengineering *88*, 313–320.

Gerecht-Nir, S., Ziskind, A., Cohen, S., and Itskovitz-Eldor, J. (2003). Human embryonic stem cells as an in vitro model for human vascular development and the induction of vascular differentiation. Laboratory investigation; a journal of technical methods and pathology *83*, 1811–1820.

Gerecht, S., Burdick, J. A., Ferreira, L. S., Townsend, S. A., Langer, R., and Vunjak-Novakovic, G. (2007). Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proceedings of the National Academy of Sciences of the United States of America *104*, 11298–11303.

Gravel, M., Gross, T., Vago, R., and Tabrizian, M. (2006). Responses of mesenchymal stem cell to chitosan-coralline composites microstructured using coralline as gas forming agent. Biomaterials *27*, 1899–1906.

Graziano, A., d'Aquino R., Cusella-De, Angelis, M. G., Laino, G., Piattelli, A., Pacifici, M., De Rosa, A., and Papaccio, G. (2007). Concave pit-containing scaffold surfaces improve stem cell-derived osteoblast performance and lead to significant bone tissue formation. PLoS ONE *2*, e496.

Guarino, V., Causa, F., and Ambrosio, L. (2007). Bioactive scaffolds for bone and ligament tissue. Expert review of medical devices *4*, 405–418.

Gurevich, O., Vexler, A., Marx, G., Prigozhina, T., Levdansky, L., Slavin, S., Shimeliovich, I., and Gorodetsky, R. (2002). Fibrin microbeads for isolating and growing bone marrow-derived progenitor cells capable of forming bone tissue. Tissue engineering *8*, 661–672.

Hamada, K., Hirose, M., Yamashita, T., and Ohgushi, H. (2008). Spatial distribution of mineralized bone matrix produced by marrow mesenchymal stem cells in self-assembling peptide hydrogel scaffold. Journal of biomedical materials research *84*, 128–136.

Hanada, K., Dennis, J. E., and Caplan, A. I. (1997). Stimulatory effects of basic fibroblast growth factor and bone morphogenetic protein-2 on osteogenic differentiation of rat bone marrow-derived mesenchymal stem cells. J Bone Miner Res *12*, 1606–1614.

Hannouche, D., Terai, H., Fuchs, J. R., Terada, S., Zand, S., Nasseri, B. A., Petite, H., Sedel, L., and Vacanti, J. P. (2007). Engineering of implantable cartilaginous structures from bone marrow-derived mesenchymal stem cells. Tissue engineering *13*, 87–99.

Hofmann, S., Hagenmuller, H., Koch, A. M., Muller, R., Vunjak-Novakovic, G., Kaplan, D. L., Merkle, H. P., and Meinel, L. (2007). Control of in vitro tissue-engineered bone-like structures using human mesenchymal stem cells and porous silk scaffolds. Biomaterials *28*, 1152–1162.

Hofmann, S., Knecht, S., Langer, R., Kaplan, D. L., Vunjak-Novakovic, G., Merkle, H. P., and Meinel, L. (2006). Cartilage-like tissue engineering using silk scaffolds and mesenchymal stem cells. Tissue engineering *12*, 2729–2738.

Hosseinkhani, H., Hosseinkhani, M., Tian, F., Kobayashi, H., and Tabata, Y. (2006). Osteogenic differentiation of mesenchymal stem cells in self-assembled peptide-amphiphile nanofibers. Biomaterials *27*, 4079–4086.

Huang, C. Y., Reuben, P. M., D'Ippolito, G., Schiller, P. C., and Cheung, H. S. (2004). Chondrogenesis of human bone marrow-derived mesenchymal stem cells in agarose culture. The anatomical record *278*, 428–436.

Hwang, N. S., Kim, M. S., Sampattavanich, S., Baek, J. H., Zhang, Z., and Elisseeff, J. (2006). Effects of three-dimensional culture and growth factors on the chondrogenic differentiation of murine embryonic stem cells. Stem cells (Dayton, Ohio) *24*, 284–291.

Im, G. I., Shin, Y. W., and Lee, K. B. (2005). Do adipose tissue-derived mesenchymal stem cells have the same osteogenic and chondrogenic potential as bone marrow-derived cells? Osteoarthritis and cartilage/OARS, Osteoarthritis Research Society *13*, 845–853.

Jin, X., Sun, Y., Zhang, K., Wang, J., Shi, T., Ju, X., and Lou, S. (2007). Ectopic neocartilage formation from predifferentiated human adipose derived stem cells induced by adenoviral-mediated transfer of hTGF beta2. Biomaterials *28*, 2994–3003.

Kim, H., Kim, H. W., and Suh, H. (2003). Sustained release of ascorbate-2-phosphate and dexamethasone from porous PLGA scaffolds for bone tissue engineering using mesenchymal stem cells. Biomaterials *24*, 4671–4679.

Kim, H. J., Kim, U. J., Vunjak-Novakovic, G., Min, B. H., and Kaplan, D. L. (2005). Influence of macroporous protein scaffolds on bone tissue engineering from bone marrow stem cells. Biomaterials *26*, 4442–4452.

Kim, M., Choi, Y. S., Yang, S. H., Hong, H. N., Cho, S. W., Cha, S. M., Pak, J. H., Kim, C. W., Kwon, S. W., and Park, C. J. (2006). Muscle regeneration by adipose tissue-derived adult stem cells attached to injectable PLGA spheres. Biochemical and biophysical research communications *348*, 386–392.

Kitamura, S., Ohgushi, H., Hirose, M., Funaoka, H., Takakura, Y., and Ito, H. (2004). Osteogenic differentiation of human bone marrow-derived mesenchymal cells cultured on alumina ceramics. Artificial organs *28*, 72–82.

Kotobuki, N., Ioku, K., Kawagoe, D., Fujimori, H., Goto, S., and Ohgushi, H. (2005). Observation of osteogenic differentiation cascade of living mesenchymal stem cells on transparent hydroxyapatite ceramics. Biomaterials *26*, 779–785.

Kruyt, M. C., Wilson, C. E., de Bruijn, J. D., van Blitterswijk, C. A., Oner, C. F., Verbout, A. J., and Dhert, W. J. (2006). The effect of cell-based bone tissue engineering in a goat transverse process model. Biomaterials *27*, 5099–5106.

Lee, S. H., and Shin, H. (2007). Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. Advanced drug delivery reviews *59*, 339–359.

Lennon, D. P., Haynesworth, S. E., Young, R. G., Dennis, J. E., and Caplan, A. I. (1995). A chemically defined medium supports in vitro proliferation and maintains the osteochondral potential of rat marrow-derived mesenchymal stem cells. Experimental cell research *219*, 211–222.

Levenberg, S., Burdick, J. A., Kraehenbuehl, T., and Langer, R. (2005). Neurotrophin-induced differentiation of human embryonic stem cells on three-dimensional polymeric scaffolds. Tissue engineering *11*, 506–512.

Levenberg, S., Huang, N. F., Lavik, E., Rogers, A. B., Itskovitz-Eldor, J., and Langer, R. (2003). Differentiation of human embryonic stem cells on three-dimensional polymer scaffolds. Proceedings of the National Academy of Sciences of the United States of America *100*, 12741–12746.

Liu, H., Collins, S. F., and Suggs, L. J. (2006). Three-dimensional culture for expansion and differentiation of mouse embryonic stem cells. Biomaterials *27*, 6004–6014.

Ma, W., Fitzgerald, W., Liu, Q. Y., O'Shaughnessy, T. J., Maric, D., Lin, H. J., Alkon, D. L., and Barker, J. L. (2004). CNS stem and progenitor cell differentiation into functional neuronal circuits in three-dimensional collagen gels. Experimental neurology *190*, 276–288.

Maguire, T., Novik, E., Schloss, R., and Yarmush, M. (2006). Alginate-PLL microencapsulation: effect on the differentiation of embryonic stem cells into hepatocytes. Biotechnology and bioengineering *93*, 581–591.

Mahoney, M. J., and Anseth, K. S. (2006). Three-dimensional growth and function of neural tissue in degradable polyethylene glycol hydrogels. Biomaterials *27*, 2265–2274.

Mahoney, M. J., and Anseth, K. S. (2007). Contrasting effects of collagen and bFGF-2 on neural cell function in degradable synthetic PEG hydrogels. Journal of biomedical materials research *81*, 269–278.

Marcacci, M., Kon, E., Moukhachev, V., Lavroukov, A., Kutepov, S., Quarto, R., Mastrogiacomo, M., and Cancedda, R. (2007). Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. Tissue engineering *13*, 947–955.

Matsuzawa, M., Weight, F. F., Potember, R. S., and Liesi, P. (1996). Directional neurite outgrowth and axonal differentiation of embryonic hippocampal neurons are promoted by a neurite outgrowth domain of the B2-chain of laminin. Int J Dev Neurosci *14*, 283–295.

Mauck, R. L., Yuan, X., and Tuan, R. S. (2006). Chondrogenic differentiation and functional maturation of bovine mesenchymal stem cells in long-term agarose culture. Osteoarthritis and cartilage / OARS, Osteoarthritis Research Society *14*, 179–189.

Mauney, J. R., Nguyen, T., Gillen, K., Kirker-Head, C., Gimble, J. M., and Kaplan, D. L. (2007). Engineering adipose-like tissue in vitro and in vivo utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds. Biomaterials *28*, 5280–5290.

Mehlhorn, A. T., Niemeyer, P., Kaschte, K., Muller, L., Finkenzeller, G., Hartl, D., Sudkamp, N. P., and Schmal, H. (2007). Differential effects of BMP-2 and TGF-beta1 on chondrogenic differentiation of adipose derived stem cells. Cell proliferation *40*, 809–823.

Meinel, L., Fajardo, R., Hofmann, S., Langer, R., Chen, J., Snyder, B., Vunjak-Novakovic, G., and Kaplan, D. (2005). Silk implants for the healing of critical size bone defects. Bone *37*, 688–698.

Meinel, L., Hofmann, S., Karageorgiou, V., Zichner, L., Langer, R., Kaplan, D., and Vunjak-Novakovic, G. (2004a). Engineering cartilage-like tissue using human mesenchymal stem cells and silk protein scaffolds. Biotechnology and bioengineering *88*, 379–391.

Meinel, L., Karageorgiou, V., Fajardo, R., Snyder, B., Shinde-Patil, V., Zichner, L., Kaplan, D., Langer, R., and Vunjak-Novakovic, G. (2004b). Bone tissue engineering using human mesenchymal stem cells: effects of scaffold material and medium flow. Annals of biomedical engineering *32*, 112–122.

Meinel, L., Karageorgiou, V., Hofmann, S., Fajardo, R., Snyder, B., Li, C., Zichner, L., Langer, R., Vunjak-Novakovic, G., and Kaplan, D. L. (2004c). Engineering bone-like tissue in vitro using human bone marrow stem cells and silk scaffolds. Journal of biomedical materials research *71*, 25–34.

Meseguer-Olmo, L., Bernabeu-Esclapez, A., Ros-Martinez, E., Sanchez-Salcedo, S., Padilla, S., Martin, A. I., Vallet-Regi, M., Clavel-Sainz, M., Lopez-Prats, F., and Meseguer-Ortiz, C. L. (2007). In vitro behaviour of adult mesenchymal stem cells seeded on a bioactive glass ceramic in the SiO(2)-CaO-P(2)O(5) system. Acta biomaterialia.

Michelini, M., Franceschini, V., Sihui Chen, S., Papini, S., Rosellini, A., Ciani, F., Margolis, L., and Revoltella, R. P. (2006). Primate embryonic stem cells create their own niche while differentiating in three-dimensional culture systems. Cell proliferation *39*, 217–229.

Moriyasu, K., Yamazoe, H., and Iwata, H. (2006). Induction dopamine releasing cells from mouse embryonic stem cells and their long-term culture. Journal of biomedical materials research *77*, 136–147.

Mrugala, D., Bony, C., Neves, N., Caillot, L., Fabre, S., Moukoko, D., Jorgensen, C., and Noel, D. (2007). Phenotypic and functional characterization of ovine mesenchymal stem cells: application to a cartilage defect model. Ann Rheum Dis.

Myers, S. R., Partha, V. N., Soranzo, C., Price, R. D., and Navsaria, H. A. (2007). Hyalomatrix: a temporary epidermal barrier, hyaluronan delivery, and neodermis induction system for keratinocyte stem cell therapy. Tissue engineering *13*, 2733–2741.

Neubauer, M., Hacker, M., Bauer-Kreisel, P., Weiser, B., Fischbach, C., Schulz, M. B., Goepferich, A., and Blunk, T. (2005). Adipose tissue engineering based on mesenchymal stem cells and basic fibroblast growth factor in vitro. Tissue engineering *11*, 1840–1851.

Noth, U., Schupp, K., Heymer, A., Kall, S., Jakob, F., Schutze, N., Baumann, B., Barthel, T., Eulert, J., and Hendrich, C. (2005). Anterior cruciate ligament constructs fabricated from human mesenchymal stem cells in a collagen type I hydrogel. Cytotherapy *7*, 447–455.

Nuttelman, C. R., Tripodi, M. C., and Anseth, K. S. (2004). In vitro osteogenic differentiation of human mesenchymal stem cells photoencapsulated in PEG hydrogels. Journal of biomedical materials research *68*, 773–782.

O'Connor, S. M., Stenger, D. A., Shaffer, K. M., Maric, D., Barker, J. L., and Ma, W. (2000). Primary neural precursor cell expansion, differentiation and cytosolic Ca(2+) response in three-dimensional collagen gel. Journal of neuroscience methods *102*, 187–195.

Ohgushi, H., Dohi, Y., Yoshikawa, T., Tamai, S., Tabata, S., Okunaga, K., and Shibuya, T. (1996). Osteogenic differentiation of cultured marrow stromal stem cells on the surface of bioactive glass ceramics. J Biomed Mater Res *32*, 341–348.

PP, B. M., Pedro, A. J., Peterbauer, A., Gabriel, C., Redl, H., and Reis, R. L. (2005). Chitosan particles agglomerated scaffolds for cartilage and osteochondral tissue engineering approaches with adipose tissue derived stem cells. Journal of materials science *16*, 1077–1085.

Prang, P., Muller, R., Eljaouhari, A., Heckmann, K., Kunz, W., Weber, T., Faber, C., Vroemen, M., Bogdahn, U., and Weidner, N. (2006). The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. Biomaterials *27*, 3560–3569.

Rezwan, K., Chen, Q. Z., Blaker, J. J., and Boccaccini, A. R. (2006). Biodegradable and bioactive porous polymer/. inorganic composite scaffolds for bone tissue engineering. Biomaterials *27*, 3413–3431.

Royce Hynes, S., McGregor, L. M., Ford Rauch, M., and Lavik, E. B. (2007). Photopolymerized poly(ethylene glycol)/. poly(L-lysine) hydrogels for the delivery of neural progenitor cells. Journal of biomaterials science *18*, 1017–1030.

Salinas, C. N., Cole, B. B., Kasko, A. M., and Anseth, K. S. (2007). Chondrogenic differentiation potential of human mesenchymal stem cells photoencapsulated within poly(ethylene glycol)-arginine-glycine-aspartic acid-serine thiol-methacrylate mixed-mode networks. Tissue engineering *13*, 1025–1034.

Shimaoka, H., Dohi, Y., Ohgushi, H., Ikeuchi, M., Okamoto, M., Kudo, A., Kirita, T., and Yonemasu, K. (2004). Recombinant growth/differentiation factor-5 (GDF-5) stimulates osteogenic differentiation of marrow mesenchymal stem cells in porous hydroxyapatite ceramic. Journal of biomedical materials research. *68*, 168–176.

Shin, H., Zygourakis, K., Farach-Carson, M. C., Yaszemski, M. J., and Mikos, A. G. (2004). Modulation of differentiation and mineralization of marrow stromal cells cultured on biomimetic hydrogels modified with Arg-Gly-Asp containing peptides. Journal of biomedical materials research *69*, 535–543.

Silva, G. A., Czeisler, C., Niece, K. L., Beniash, E., Harrington, D. A., Kessler, J. A., and Stupp, S. I. (2004). Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science (New York, NY *303*, 1352–1355.

Stosich, M. S., Bastian, B., Marion, N. W., Clark, P. A., Reilly, G., and Mao, J. J. (2007). Vascularized adipose tissue grafts from human mesenchymal stem cells with bioactive cues and microchannel conduits. Tissue engineering *13*, 2881–2890.

Sumanasinghe, R. D., Bernacki, S. H., and Loboa, E. G. (2006). Osteogenic differentiation of human mesenchymal stem cells in collagen matrices: effect of uniaxial cyclic tensile strain on bone morphogenetic protein (BMP-2) mRNA expression. Tissue engineering *12*, 3459–3465.

Sun, H., Qu, Z., Guo, Y., Zang, G., and Yang, B. (2007). In vitro and in vivo effects of rat kidney vascular endothelial cells on osteogenesis of rat bone marrow mesenchymal stem cells growing on polylactide-glycoli acid (PLGA) scaffolds. Biomedical engineering online *6*, 41.

Teng, Y. D., Lavik, E. B., Qu, X., Park, K. I., Ourednik, J., Zurakowski, D., Langer, R., and Snyder, E. Y. (2002). Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proceedings of the National Academy of Sciences of the United States of America *99*, 3024–3029.

Tomita, M., Lavik, E., Klassen, H., Zahir, T., Langer, R., and Young, M. J. (2005). Biodegradable polymer composite grafts promote the survival and differentiation of retinal progenitor cells. Stem cells (Dayton, Ohio) *23*, 1579–1588.

Toquet, J., Rohanizadeh, R., Guicheux, J., Couillaud, S., Passuti, N., Daculsi, G., and Heymann, D. (1999). Osteogenic potential in vitro of human bone marrow cells cultured on macroporous biphasic calcium phosphate ceramic. J Biomed Mater Res *44*, 98–108.

Tsang, V. L., and Bhatia, S. N. (2004). Three-dimensional tissue fabrication. Advanced drug delivery reviews *56*, 1635–1647.

Turhani, D., Watzinger, E., Weissenbock, M., Yerit, K., Cvikl, B., Thurnher, D., and Ewers, R. (2005). Three-dimensional composites manufactured with human mesenchymal cambial layer precursor cells as an alternative for sinus floor augmentation: an in vitro study. Clinical oral implants research *16*, 417–424.

Uematsu, K., Hattori, K., Ishimoto, Y., Yamauchi, J., Habata, T., Takakura, Y., Ohgushi, H., Fukuchi, T., and Sato, M. (2005). Cartilage regeneration using mesenchymal stem cells and a three-dimensional poly-lactic-glycolic acid (PLGA) scaffold. Biomaterials *26*, 4273–4279.

Underhill, G. H., Chen, A. A., Albrecht, D. R., and Bhatia, S. N. (2007). Assessment of hepatocellular function within PEG hydrogels. Biomaterials *28*, 256–270.

Varghese, S., Hwang, N. S., Canver, A. C., Theprungsirikul, P., Lin, D. W., and Elisseeff, J. (2008). Chondroitin sulfate based niches for chondrogenic differentiation of mesenchymal stem cells. Matrix Biol *27*, 12–21.

Wang, Y., Kim, U. J., Blasioli, D. J., Kim, H. J., and Kaplan, D. L. (2005). In vitro cartilage tissue engineering with 3D porous aqueous-derived silk scaffolds and mesenchymal stem cells. Biomaterials *26*, 7082–7094.

Watanabe, K., Nakamura, M., Okano, H., and Toyama, Y. (2007). Establishment of three-dimensional culture of neural stem/. progenitor cells in collagen Type-1 Gel. Restorative neurology and neuroscience *25*, 109–117.

Wayne, J. S., McDowell, C. L., Shields, K. J., and Tuan, R. S. (2005). In vivo response of polylactic acid-alginate scaffolds and bone marrow-derived cells for cartilage tissue engineering. Tissue engineering *11*, 953–963.

Willerth, S. M., Arendas, K. J., Gottlieb, D. I., and Sakiyama-Elbert, S. E. (2006). Optimization of fibrin scaffolds for differentiation of murine embryonic stem cells into neural lineage cells. Biomaterials *27*, 5990–6003.

Willerth, S. M., Faxel, T. E., Gottlieb, D. I., and Sakiyama-Elbert, S. E. (2007). The effects of soluble growth factors on embryonic stem cell differentiation inside of fibrin scaffolds. Stem cells (Dayton, Ohio) *25*, 2235–2244.

Worster, A. A., Brower-Toland, B. D., Fortier, L. A., Bent, S. J., Williams, J., and Nixon, A. J. (2001). Chondrocytic differentiation of mesenchymal stem cells sequentially exposed to transforming growth factor-beta1 in monolayer and insulin-like growth factor-I in a three-dimensional matrix. J Orthop Res *19*, 738–749.

Xin, X., Hussain, M., and Mao, J. J. (2007). Continuing differentiation of human mesenchymal stem cells and induced chondrogenic and osteogenic lineages in electrospun PLGA nanofiber scaffold. Biomaterials *28*, 316–325.

Yamada, Y., Boo, J. S., Ozawa, R., Nagasaka, T., Okazaki, Y., Hata, K., and Ueda, M. (2003). Bone regeneration following injection of mesenchymal stem cells and fibrin glue with a biodegradable scaffold. J Craniomaxillofac Surg *31*, 27–33.

Yang, X. B., Webb, D., Blaker, J., Boccaccini, A. R., Maquet, V., Cooper, C., and Oreffo, R. O. (2006). Evaluation of human bone marrow stromal cell growth on biodegradable polymer/bioglass composites. Biochemical and biophysical research communications *342*, 1098–1107.

Yoon, E., Dhar, S., Chun, D. E., Gharibjanian, N. A., and Evans, G. R. (2007). In vivo osteogenic potential of human adipose-derived stem cells/poly lactide-co-glycolic acid constructs for bone regeneration in a rat critical-sized calvarial defect model. Tissue engineering *13*, 619–627.