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REVIEW

Using Polymeric Materials to Control Stem Cell Behavior for Tissue Regeneration

Nianli Zhang and David H. Kohn*

Patients with organ failure often suffer from increased morbidity and decreased quality of life. Current strategies of treating organ failure have limitations, including shortage of donor organs, low efficiency of grafts, and immunological problems. Tissue engineering emerged about two decades ago as a strategy to restore organ function with a living, functional engineered substitute. However, the ability to engineer a functional organ is limited by a limited understanding of the interactions between materials and cells that are required to yield functional tissue equivalents. Polymeric materials are one of the most promising classes of materials for use in tissue engineering, due to their biodegradability, flexibility in processing and property design, and the potential to use polymer properties to control cell function. Stem cells offer potential in tissue engineering because of their unique capacity to self-renew and differentiate into neurogenic, osteogenic, chondrogenic, and myogenic lineages under appropriate stimuli from extracellular components. This review examines recent advances in stem cell–polymer interactions for tissue regeneration, specifically highlighting control of polymer properties to direct adhesion, proliferation, and differentiation of stem cells, and how biomaterials can be designed to provide some of the stimuli to cells that the natural extracellular matrix does. **Birth Defects Research (Part C) 96:63–81, 2012.** © 2012 Wiley Periodicals, Inc.

Key words: stem cells; polymer; tissue engineering; adhesion; proliferation; differentiation

INTRODUCTION

Failure of organ function due to injury, disease, or aging accounts for a significant number of clinical disorders at a tremendous social and economic cost (Freed et al., 2009). In the United States, nearly 6 million bone fractures occur each year (Zhao et al., 2010), and worldwide, 8 million persons suffer a myocardial infarction (Kraehenbuehl et al., 2008). The failure of organs also has a significant impact on quality of life. For example, patients with traumatic spinal cord injury often suffer lifetime sensory and motor deficits below the site of injury (Hsieh et al., 2010).

Current treatments for organ failure vary with the type of organ affected, but all have limitations. For cardiac functional failure, one of the current strategies is to deliver functional cells to the myocardium. However, this strategy results in low engraftment efficiency and cell viability in infracted hearts (Ye et al., 2011). Cardiac transplantation can significantly lengthen and improve quality of life. It is limited, however, due to a chronic shortage of donor hearts (Leor et al., 2000). With the replacement of diseased or damaged bone, autologous bone grafts are preferable because they contain the patient's own cells and proteins, which not only provide a framework for new bone to grow into, but also are immunogenetically compatible. Despite satisfying clinical results, autografts often lead to morbidity at the surgical site. Another strategy is to use an allogenic bone graft. However, utilization of these grafts carries the risk of immunological rejection or disease transfer (Cordonnier et al., 2011).

To overcome these limitations in organ transplantation and grafting, the field of tissue engineering emerged about two decades ago. Tissue engineering combines the disciplines of both the materials sciences and the life sciences to replace a diseased or damaged tissue or organ with a living, functional engineered substitute (Chan and Mooney, 2008; Marklein and Burdick, 2010). However, tissues such as bone, articular cartilage, and myocardium possess highly specialized structures and compositions that provide unique mechanical and transport properties (Freed et al., 2009). Therefore, to reconstruct a functional engineered tissue substitute, it is necessary to understand how these specialized structures and compositions affect cell behavior in vivo, and use this information to direct the design of substitute tissues and organs. Unfortunately, our ability to design

Nianli Zhang and **David H. Kohn** are from The Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan 48109-1078
David H. Kohn is also from The Department of Biomedical Engineering, University of Michigan, Ann Arbor, Michigan 48109-2099

*Correspondence to: David H. Kohn, Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, Michigan 48109-1078. E-mail: dhkohn@umich.edu

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a functional organ substitute is limited by an incomplete understanding of the interactions between materials and cells, and an inability to control the complex signaling pathways elicited by these interactions (Fisher et al., 2010).

The first question that needs to be answered, to optimize any tissue engineering strategy geared toward producing a functional tissue equivalent, is what cell type and substrate material are appropriate for the particular tissue engineering goal at hand. Stem cells and polymeric materials are key design choices due to their unique properties. Briefly, stem cells have the ability to self-renew and commit to specific cell lineages under appropriate stimuli. Polymeric materials are biocompatible, degradable, and flexible in processing and property design. A significant focus of tissue engineering, therefore, is to utilize polymers, or soft materials, as a means of controlling stem cell function via physical, chemical, mechanical, and/or biological cues "communicated" to from the polymer to the cells.

This review examines recent progress in stem cell–polymer interactions for tissue regeneration. Specifically, we focus on how polymer material properties affect the activity of stem cells in vitro and further tissue regeneration in vivo. The design of novel polymeric biomaterials with appropriate physical, chemical, mechanical, and biological cues to guide stem cell adhesion, proliferation, and differentiation are discussed. Finally, we discuss how the ability of a biomaterial to guide stem cell function can lead to improved outcomes for nerve, bone, cartilage, and cardiac regeneration.

Characteristics of Stem Cells

Stem cells are an important cell type for cell-based therapy and regenerative medicine, especially within the rapidly expanding field of tissue engineering, due to their two unique properties, self-renewal and differentiation. With the first unique property, these cells can be easily expanded in vitro and, therefore, a large cell number can be obtained for seeding onto three-dimensional (3D) scaffolds of clinically relevant volume and subsequent cell transplantation. Stem cells can also give rise to more committed cell types, such as osteoblasts, chondrocytes, adipocytes, and neuronal cells, when they receive the appropriate cues.

Based on their differentiation potential, stem cells used for tissue engineering can be divided into two categories, pluripotent stem cells and multipotent stem cells. Pluripotent stem cells include embryonic stem cells (ESC) and induced pluripotent stem cells (iPSC). Compared to multipotent stem cells, pluripotent cells can self-renew indefinitely. Their pluripotent nature gives them the ability to differentiate into any one of the three germ layers: endoderm, ectoderm, and mesoderm (Dawson et al., 2008). Because ESCs are isolated from the inner cell mass of the blastocyst during embryological development, their use in tissue engineering is controversial and more limited. Although iPSCs are obtained by genetically modifying somatic cells, more attention has been paid to this cell type recently. Examples of multipotent stem cells include bone marrow derived-mesenchymal stem cells (MSCs), hematopoietic stem cells, neural stem cells (NSCs), and adipose derived stem cells (ASCs). These stem cells exist in the corresponding differentiated tissues, renew themselves for the lifetime of the organism, and yield all of the specialized cell types of the tissue from which they are originated.

Factors Controlling Stem Cell Behavior

Maintaining stem cells in an undifferentiated state and subsequently directing them to differentiate in a reliable and reproducible manner into specific cell types are key issues in stem cell biology (Dawson et al., 2008) and consequently in stem cell-based tissue engineering. Cell adhesion, proliferation, and differentiation are largely dictated by signals from extracellular components, such as soluble biological and pharmacological factors in fluid, extracellular matrix (ECM), and other adjacent cells (cell–cell crosstalk; Fig. 1). It has been long recognized that not only the type, but also the dose, spatial and temporal distribution of soluble factors play an important role in mediating cell behavior (Luong et al., 2006; Beohar et al., 2010; Zhang et al., 2010b). Various properties of the ECM influence cell adhesion, proliferation, and differentiation, including physical properties (roughness, stiffness, surface patterning, and electrical conductivity), chemical properties (concentration of monomers and functional groups), as well as structural properties (cross-linking, morphology, 2D vs. 3D) (Murphy et al., 2005). Cell–cell communication is also critical to cell differentiation. For example, enhancement of gap junction intercellular communication leads to an increased magnitude and spatial distribution of differentiation markers and consequently an increased volume fraction and spatial uniformity of in vivo (Rossello et al., 2009). Individual factors, as well as combinations of factors from the extracellular environment, affect cell adhesion, viability, proliferation, and differentiation (Chan and Mooney, 2008; Dawson et al., 2008). Therefore, a key to advancing tissue engineering is the ability to control the signaling of multiple factors simultaneously.

Polymeric Materials for Tissue Repair and Regeneration

Polymeric materials for tissue regeneration are of both natural and synthetic origin (Table 1). Examples of natural polymers include collagen, fibrin, and polysaccharides, such as hyaluronic acid and alginate. Natural polymers contain a variety of biological cues, including cell adhesion sequences, and therefore, can be recognized by cells. However, natural polymers are subjected to batch-to-batch variation due to the complexity of

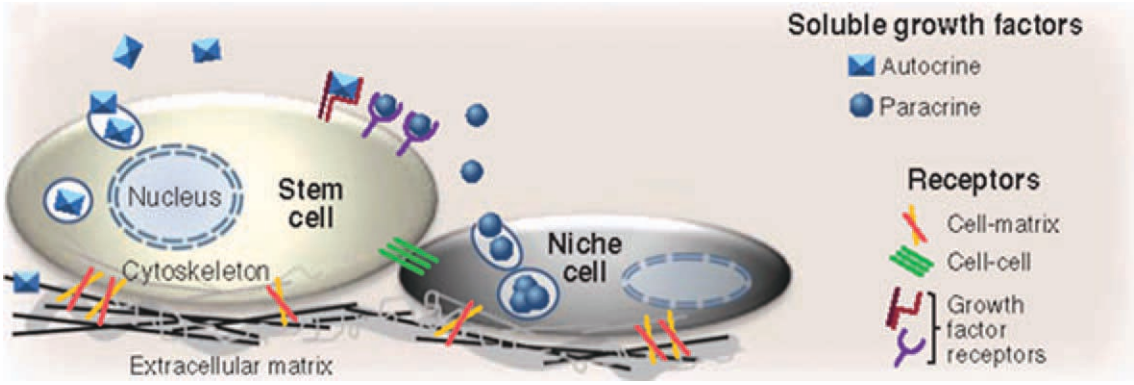

Figure 1. Factors controlling stem cell behavior arise from their interactions with growth factors, ECM, and niche cells (Schematic diagram is modified from ''D. E. Discher, et al. (2009). Growth factors, matrices, and forces combine and control stem cells. Science 324, 1673''; copyright 2009, with permission from The American Association for the Advancement of Science).

their structure and chemical composition, leading to variations in tissue engineering outcomes. Compared to natural polymers, synthetic polymers can be more easily synthesized on a large scale with more precisely controlled molecular weight and addition of functional groups. However, synthetic polymers in their native state can only support cell adhesion and growth to a limited extent since they lack functional groups for cell interaction (Alvarez-Barreto et al., 2011). The synthetic polymers that have

TABLE 1. Major Polymeric Materials for Tissue Regeneration

Polymer name	Main applications and properties	References
Natural polymers		
Alginate	Bone, nerve, and cartilage regeneration; enzymatically degradable	Banerjee et al. (2009), Shanbhag et al. (2010)
Collagen	Bone, heart and cartilage regeneration; enzymatically degradable	Battista et al. (2005)
Hyaluronic acid	Cartilage, nerve regeneration; degradable	Tian et al. (2005), Ren et al., (2009), Park et al. (2010)
Chitosan	Cartilage regeneration; degradable	Chung et al. (2011)
Fibrin	Cartilage regeneration; degradable, injectable	Ahearne et al. (2011)
Synthetic polymers		
Poly(vinyl alcohol)	Cartilage regeneration; nondegradable	Spiller et al. (2011)
Poly(hydroxyethyl methacrylate)	Cartilage regeneration; nondegradable	Singh et al. (2011)
Poly(N-isopropylacrylamide)	Cartilage regeneration; nondegradable	Sa-Lima et al. (2011)
Polyethylene terephthalate	Bone regeneration; nondegradable	Cao et al. (2010)
PGA	Cartilage regeneration; bulk degradable	Bilir et al. (2011)
Poly(lactic acid) PLA	Cartilage, nerve, bone regeneration; degradable	Yang et al. (2005) Liao et al. (2011)
Polyethylene oxide	Cartilage regeneration; injectable, degradable	Akpalo et al. (2011)
PLGA	Bone, nerve regeneration; bulk degradable	Levenberg et al. (2003)
Poly caprolactone	Bone, nerve regeneration; degradable	Mahairaki et al. (2011)
Polypyrrole (PPy)	Nerve regeneration; conductive polymer	Lundin et al. (2011)
Poly (propylene fumarte)	Bone regeneration; bulk degradable, injectable	Shin et al. (2011)
Polyanhydrides	Bone regeneration, surface degradable	Li et al. (2004)
Aminopropylmethacrylamide (APMAAm)	Support self renewal of human embryonic stem cell	Irwin et al. (2011)
Poly(methyl vinyl ether-alt-maleic anhydride)	Support self renewal of human embryonic stem cell	Brafman et al. (2010)
PEG	Heart, bone regeneration; degradable	Kraehenbuehl et al. (2008)

received the most study are poly (L-lactic acid) (PLLA), poly(glycolic acid) (PGA), poly(lactic-co-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), polycaprolactones, polyorthoesters, polyanhydrides, and polycarbonates (Chan and Mooney, 2008).

There are at least two advantages of using polymeric materials for tissue regeneration. First, the structure and composition of polymers can be easily tailored to yield a variety of physical and chemical properties that can elicit certain cellular functions, including proliferation and differentiation in a controlled manner. Second, many of the polymers are biodegradable through either hydrolysis or via activities of enzymes secreted by cells. Therefore, over a prescribed time, the scaffold can be replaced by newly formed tissue. Thus, with degrading polymers, a secondary surgery is not needed to remove the scaffold after implantation.

A drawback of many polymers, however, is that their biocompatibility is lower than other types of biomaterials, such as ceramic materials. Polymeric materials are usually encapsulated by a persistent layer of fibroblasts, collagen, and inflammatory cells in vivo, which is suboptimal for tissue formation (Vergroesen et al., 2011). However, the biocompatibility of polymer materials can be improved by engineering functionality into these materials. The behavior of stem cells can be controlled by engineering functionality into a biomaterial, such as via immobilization of adhesion peptides, modification of surface chemistry, and mineralizing polymer surfaces.

CELL ADHESION AND PROLIFERATION ON POLYMERIC MATERIALS

2D Polymeric Substrates

2D polymeric substrates have been used for in vitro cell culture for decades, and surface properties of roughness and topography can be more easily and precisely controlled over a 2D surface than a 3D scaffold (Naing and Williams, 2011). A well-defined surface can subsequently benefit the study of the interactions between cells and materials, decrease variability in cell response, and lead to less complication in the interpretation of data. Effects of surface properties, such as stiffness and topography, on cell adhesion and proliferation have been extensively investigated (Castellani et al., 1999; Discher et al., 2005; Saha et al., 2008).

The magnitude of surface stiffness affects cell adhesion and proliferation (Pek et al., 2010; Wang et al., 2010a; Chandler et al., 2011; Park et al., 2011a; Schrader et al., 2011). For example, 2D polymer substrates with moduli greater than 1000 Pa favor proliferation of adult NSCs, whereas cell spreading and proliferation are inhibited on substrata with moduli of 10 Pa (Saha et al., 2008). The trend of stiffer surfaces leading to a higher rate of proliferation holds for many other cell types, such as adipose progenitor cells, human MSCs, and hepatocellular carcinoma cells (Wang et al., 2010a; Chandler et al., 2011; Park et al., 2011a; Schrader et al., 2011).

Besides surface stiffness, surface topography is another important factor that controls cell adhesion and proliferation. Various fabrication methods are used to alter surface topography or create microscale and nanoscale features to facilitate cell adhesion (Castellani et al., 1999; Hatano et al., 1999; Deligianni et al., 2001; Anselme et al., 2002; Korovessis et al., 2002; Linez-Bataillon et al., 2002; Zhao et al., 2006b). Surfaces with lower periodicity (e.g., totally random surface) generally favor cell adhesion and proliferation (Anselme et al., 2000; Bigerelle and Iost, 2001). However, most studies only focus on cell responses to static surface topography or patterning. One interesting study designed a dynamic substrate that can communicate active physical cues to cells (Le et al., 2011). In this study, the surface of thermally responsive poly(ε-caprolactone) shape-memory polymers transformed between a $3 \times 5\ \mu m^2$ channel array and a planar surface at 37°C. Correspondingly, the morphology of hMSCs switched from highly aligned to stellate shaped. Meanwhile, cell attachment and detachment can be controlled by thermally responsive polymer substrates (Hatakeyama et al., 2005; Idota et al., 2009; Kumashiro et al., 2010). The detachment of cells from such temperature-responsive surfaces is achieved by lowering the temperature without conventional enzymatic treatment, while keeping the deposited ECM intact (Kumashiro et al., 2010).

Two-dimensional polymeric surfaces have been investigated for supporting self-renewal of pluripotent stem cells, including ESCs and iPSCs (Brafman et al., 2010; Villa-Diaz et al., 2010; Irwin et al., 2011). The successful integration of stem cells into tissue engineering requires large-scale cell expansion without differentiation. Therefore, the precise control the self-renewal of stem cells is important (Irwin et al., 2011).

The motivation for using a 2D polymeric substrate to support self-renewal of pluripotent stem cells is the lack of chemically defined culture system for these cells. Pluripotent stem cells are typically maintained on a feeder layer of mouse cells with a combination of the animal-based products, which are expensive, difficult to isolate, subject to batch-to-batch variations, and unsuitable for translation cell-based therapies to the clinic (Brafman et al., 2010). Therefore, a defined system is needed for better supporting hESC self-renewal. The first attempt to create a fully defined synthetic polymer coating to support hESC self-renewal was done by Villa-Diaz et al. (2010), where poly[2-(methacryloyloxy)ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH) was created by UV-ozone activated polymerization. Cells seeded on PMEDSAH expressed characteristic hESC markers, displayed a normal karyotype and retained pluripotency throughout 25 passages. In this study, however, only human ESCs were evaluated. Just a few months later, a new polymeric sub-

strate, poly(methyl vinylether-alt-maleic anhydride), that can support both hESC and iPSC self-renewal, was identified through a high-throughput screening approach (Brafman et al., 2010). Both cell types exhibited their characteristic morphology and grew as tightly clustered colonies expressing pluripotency markers, such as OCT4, NANOG, and SOX2 over five passages.

A disadvantage of the techniques described earlier is that serum supplemented, chemically undefined cell culture media is used. In these media, fetal bovine serum or similar serum is used to provide growth factors for stem cell adhesion and proliferation. However, the type or concentration of individual growth factors is not fully characterized and often varies between batches. Self-renewal of pluripotent cells on polymeric substrates was advanced by the development of a complete chemically defined cell culture system with serum-free media (Irwin et al., 2011). In this study, the pluripotency of hESCs was maintained on aminopropyl-methacrylamide (APMAAm) for over 20 passages in chemically defined mTeSRTM1 media. This synthetic and defined cell culture system does not require the prior attachment of peptides or proteins to promote cell attachment and is free of complex, undefined culture conditions.

The mechanisms explaining why some polymeric substrates can support self-renewal of pluripotent stem cells are still not clear. It is speculated that the hydrolysis products of the polymers (e.g., carboxyl and sulfonyl groups) may mimic functional features of proteins that support self-renewal (Brafman et al., 2010). An alternative hypothesis is that the existence of specific functional groups on the polymer substrate either stimulates the production of endogenous proteins or promotes the adsorption of exogenous proteins that support self-renewal (Brafman et al., 2010). Indeed, bovine serum albumin in the mTeSRTM1 media was identified to be critical for cell adhesion and potentially self-renewal of pluripotent stem cells on APMAAm surfaces (Irwin et al., 2011).

3D Scaffolds

Cells can behave differently in 2D and 3D systems. For instance, tumor cells grown in 3D culture are relatively more resistant to cytotoxic drugs compared with their response in conventional 2D culture (Li et al., 2010). There has been increasing agreement that 3D matrices provide better model systems for physiologic situations (Weaver et al., 1997; Zhao et al., 2006a) such as enhanced cell–cell contact or communications. Below, we examine how the properties of 3D fibrous scaffolds, hydrogels, and composites mediate cell adhesion, viability, and proliferation.

Electrospun fibers of various natural polymers, including collagen and fibrin, are used to fabricate 3D scaffolds. Fiber diameter ranges from $\sim$100 to 600 nm promote cell adhesion and proliferation. (Kitazono et al., 2004; Bao et al., 2011; Pant et al., 2011; Wei et al., 2011; Wu et al., 2011). Compared to microfibers, cells develop smaller focal adhesion complexes and exhibit higher proliferation on nanofibers (Hsia et al., 2011). Increasing the porosity and surface area of fibrous scaffolds better supports cell migration into the scaffold, increasing the adhesion and proliferation of cells (Rnjak-Kovacina et al., 2011).

The orientation of the fibers can also affect cell adhesion and viability (Hsieh et al., 2010). For example, a physical hydrogel blend of hyaluronan and methylcellulose incorporating electrospun fibers of collagen or poly(3-caprolactone-co-D,L-lactide) was developed to promote cell–synthetic matrix interactions and influence NSC behavior. Although collagen scaffolds facilitate NSC transplantation and help recovery of an injured spinal cord (Hatami et al., 2009), electronspun collagen fibers in HAMC hydrogels inhibit NSC survival and proliferation. The fine, fragmented, and tangled structures of the less oriented collagen fibers are thought to be responsible for these inhibitory effects. Indeed, human neural precursors (NPs) on aligned polycaprolactone fiber scaffolds exhibit a higher viability than on randomly orientated fibers (Mahairaki et al., 2011). Human NPs seeded on aligned fibers acquire a spindle-like shape and extended processes parallel to the fiber axis, whereas NPs on plain tissue culture surfaces or random fiber substrates form non-polarized neurite networks (Mahairaki et al., 2011). These morphological differences are due to the rearrangement of cytoskeletal constituents, a process that in turn can influence cell phenotype and function via established links with intracellular signaling pathways (Mahairaki et al., 2011).

Hydrogels or polymers with high water content ($>$99% water), are another important class of 3D scaffolds. Hydrogels can be crosslinked via chemical bonds, ionic interactions, hydrogen bonds, hydrophobic interactions, or physical bonds, and have been extensively studied platforms because of their 3D nature, biocompatibility, and versatility in processing (Tian et al., 2005; Thonhoff et al., 2008; Banerjee et al., 2009; Ren et al., 2009; Hsieh et al., 2010; Shanbhag et al., 2010). Hydrogels can be synthesized by various methods, such as radical polymerization, Michael addition chemistry, click chemistry, and a variety of functional moieties can be incorporated to enhance biodegradability and biocompatibility. Growth factors, cytokines, and other chemical additives can also be incorporated into hydrogels to mediate cell activity (Liu et al., 2010b).

Adhesion and proliferation of stem cells can be influenced by hydrogel properties, such as hydrogel concentration and stiffness. For example, human MSCs shrink and degenerate on concentrated PF127 and PuraMatrix hydrogels, and the viability of human NSCs decreases as the concentration of PF127 and Puramatrix increases (Thonhoff et al.,

2008). The mechanism controlling adhesion is unclear, but it is possibly due to a complex dynamic between cytotoxicity and growth factor stimulation or the release of harmful or acidic byproducts during degradation of the hydrogel (Thonhoff et al., 2008). Compared to 2D surfaces, the stiffness of 3D hydrogels affects cell proliferation in a more complex way. Increasing the hydrogel stiffness decreases the proliferation of NSCs when encapsulated in 3D alginate hydrogels (Banerjee et al., 2009), whereas hydrogels enhance cell self-organization and subsequent tissue development (Miyajima et al., 2011). In contrast, smooth muscle cell proliferation in 3D PEG-conjugated fibrinogen hydrogels does not depend on gel stiffness (Peyton et al., 2008).

Composite scaffolds offer biological, chemical, and mechanical advantages that go beyond what each individual component can provide. For example, the natural polymer, fibronectin, is known for its ability to promote cell adhesion. Another natural polymer, chitosan (CS), can promote differentiation of stem cells to several lineages. The combination of these two polymers can offer a more versatile scaffold for tissue regeneration (Chen et al., 2011; Chung et al., 2011; Pei et al., 2011). However, one problem that exists in combining natural polymers is that crosslinking molecules, which are used to stabilize the construct, often lead to in vivo complications, including graft failure (Heydarkhan-Hagvall et al., 2008). Another type of composite is the combination of two synthetic polymers, for example, PLGA and polyacrylic acid (PAA), where PLGA is biocompatible and degradable, while PAA provides better adhesive ability (Endres et al., 2003; Cao et al., 2011). The hybridization of synthetic and natural polymers can also offer improved biological and mechanical properties (Jiao et al., 2007; Craciunescu et al., 2008; Heydarkhan-Hagvall et al., 2008; Venugopal et al., 2008; Liu et al., 2009; Liao et al., 2010).

Incorporating polymeric and inorganic materials is another way to create composite materials with superior mechanical and biological properties. Inorganic components, such as hydroxyapatite, can improve protein adsorption and subsequent cell adhesion (Leonova et al., 2006; Zhao et al., 2006a; Dimitrievska et al., 2008; Li et al., 2009; Venugopal et al., 2010; Akkouch et al., 2011). Mineralized polymer surfaces can also be used to provide sustained release of growth factors and genes (Murphy et al., 2000; Luong et al., 2006; Luong et al., 2009; Segvich and Kohn, 2009). Apatites and bioactive glasses can also neutralize the acidic byproducts of polymer degradation, helping to maintain pH within physiological ranges, supporting cell function, and minimizing long-term adverse host responses (Roether et al., 2002; Yang et al., 2006; Zhao et al., 2006a).

Surface Functionalization

As the first step in the sequence of cell-biomaterial interactions, initial adhesion of anchorage-dependent cells is crucial to the subsequent cell proliferation and differentiation. Many methods are used to physically modify biomaterial surfaces to increase cell adhesion, including creating surface roughness, topography, and patterning (Vandrovcova and Bacakova, 2011). In general, nanostructured substrates with irregularities smaller than 100 nm are more favorable to cell adhesion and growth than microstructured substrates (Bacakova et al., 2011). Trends in cell adhesion and proliferation with increased roughness are inconsistent though (Zhang et al., 2010b). Some literature shows that optimal cell adhesion is obtained with small roughness ratios (Ranella et al., 2010), while other literature shows opposite results (Lohmann et al., 2000; Marinucci et al., 2006; Zhao et al., 2006b; Ponader et al., 2008). One of the explanations for these contradictory results is that various methods (e.g., acid-etching vs. sandblasting) used to create different surface roughness ratios on substrates changes surface reactivity or introduced new surface chemistry (Zhang et al., 2010b).

For polymeric materials, immobilization of proteins, such as fibronectin, laminin, and collagen, or peptides, such as RGD and YIGSR, on biomaterial surfaces are the main chemical methods to promote cell adhesion and proliferation (Cheng and Teoh, 2004; Jeong et al., 2005; Shin et al., 2008; Segvich et al., 2009a; Segvich et al., 2009b). These molecules can increase hydrophilicity and surface charge, conditions that facilitate integrin–adhesion molecule interactions and are favorable to cell adhesion. (Shin et al., 2008; Pan et al., 2009; Lundin et al., 2011). However, the stability of these immobilized molecules is dependent on the biomaterial surface. As an example, the anionic ions, tosylate (TsO), perchlorate (ClO_4), and chloride (Cl), doped on polypyrrole (PPy) degrade over time under physiological conditions, resulting in low NSC viability (Lundin et al., 2011).

Surface modification with layer-by-layer assembly can enhance cell adhesion (Boura et al., 2003; D'Britto et al., 2009). The nature of the substrate is largely determined by the characteristics of the outmost layer. For example, bioactive multilayer films composed of PAA-*b*-PLGA and CS assembled on the surface of PLLA films support better attachment and proliferation of human adipose-derived stem cells than PLLA films, because of the more hydrophilic PAA block chains (Cao et al., 2011). Similarly, the interactions of the RGD domain of FN and the receptors on MSCs are responsible for the higher cell mass on CNT/CS/FN surfaces than on CNT/CS and CNT/CS/HA surfaces (Chung et al., 2011).

CONTROL OF CELL DIFFERENTIATION ON POLYMERS

Key components in the cellular microenvironment that influence

TABLE 2. Properties of Polymeric Materials Controlling Stem Cell Differentiation

Physical properties	Roughness, stiffness, topography, pore size, porosity, pore connectivity, fiber diameter, fiber orientation, and surface charge
Chemical properties	Composites, polymer concentration, density of crosslinker, hydrophobicity, surface functionalization with peptides, functional groups, adhesion molecules, and growth factors

stem cell differentiation to more committed lineages include soluble factors, cell–cell contact, and cell–matrix interactions (Discher et al., 2009). One of the goals of biomaterial design in stem cell engineering is to control the differentiation of these cells using substrate properties (Table 2). However, the ability to design novel materials has been limited by a poor understanding of the complex signaling events that influence cell differentiation (Fisher et al., 2010). Furthermore, key components involved in directing cell differentiation often interplay and change temporally and spatially. In response to these dynamic and complex changes in the microenvironment, stem cell responses to the extracellular environment are difficult to predict, and therefore, contribute to contradictory results in the literature.

The type and magnitude of physical, chemical, and biological cues can induce stem cell differentiation into neurogenic, osteogenic, chondrogenic, and myogenic lineages, respectively. Therefore, we will discuss how these different types of cues that can be designed into a biomaterial can dictate stem cell differentiation to specific cell lineages.

Neurogenic Differentiation

Recovery of neuronal networks is limited by the inability of the nervous system to self repair after injury or trauma (Nisbet et al., 2009). Among the polymeric biomaterials, only a subset are suitable for soft tissue engineering, especially nerve regeneration, owing to limitations in mechanical properties such as stiffness (Gu et al., 2010). Polymers with similar mechanical properties to the native tissues they are targeted to replace are preferred for tissue engineering (Subramanian et al., 2009). The stiffness of brain tissue is ~500 Pa (Saha et al., 2008). Therefore, substrates with stiffness in the range of ~100 to 500 Pa are ideal for neural tissue regeneration (Engler et al., 2006; Saha et al., 2008; Banerjee et al., 2009). For example, PGA is relatively rigid and not mechanically suitable for transplantation into neural tissue (Thonhoff et al., 2008).

Polymer hydrogels

An ideal biomaterial for neural transplantation would have the ability to be mixed with stem cells and injected in a fluid form (Thonhoff et al., 2008). It is beneficial if the material is hydrophilic and has a stiffness of ~100 to 500 Pa (Gu et al., 2010). Meanwhile, the porous network and interconnectivity of the scaffold need to be maintained in hydrated conditions, to facilitate the transportation of nutrients, oxygen, and metabolites and tissue ingrowth. Hydrogels, as a class of polymeric materials, meet all of these requirements (Gu et al., 2010).

Hydrogels are soft, elastic, water-swollen polymeric structures cross-linked either by covalent bonds, physical cross-links (e.g., entanglements), hydrogen bonds, or strong van der Waals interactions. Attention has been given to these materials for neuroengineering because of their flexibility in processing and handling.

One of the most common ways to direct neurogenic differentiation through hydrogels is to incorporate growth factors on peptide sequences into the gel via either simple mixing or covalent bonding to the gel network. For example, IKVAV, a peptide-derived from laminin, and brain-derived neurotrophic factor (BDNF), reduce neuronal death, induce neuronal differentiation of hMSCs (Park et al., 2010), and promote neuronal regeneration in several models (Tobias et al., 2001; Tuszynski et al., 2003; Katz and Meiri, 2006). Growth factors can also be delivered by genetically engineering cells. An example of this strategy is the engineering of fibroblasts to act as a controlled delivery system to continuously express the neurotrophic factors BDNF and NT-3 (Shanbhag et al., 2010). By utilizing this strategy, alginate constructs can serve as a microenvironment for neural progenitor cell (NPC) differentiation, representing a promising bioengineered solution for neural repair.

In the studies summarized above, proneurogenic growth factors were immobilized on material surfaces. Polymeric biomaterials can also be used to release factors for the purpose of blocking inhibitors of tissue regeneration. For example, Nogo-66 and NgR are important receptors inhibiting neuronal regeneration. Antibodies (e.g., IgG) covalently attached to biodegradable HA hydrogels have been used to block the function of Nogo-66 and NgR to treat brain injury in rodents (Tian et al., 2005). Although a sustained release of IgG was observed, cell culture experiments showed that NSC differentiation on the same HA substrates coated with the same antibody was similar to that on bare HA surfaces (Pan et al., 2009). These results indicate that cell–material interactions in vitro may not be predictive of cell–material interactions in vivo and that neuronal tissue regeneration requires further investigation.

Besides being used as a delivery vehicle for growth factors, several properties of hydrogels can be tuned to control cell differentiation. For example, lower concentrations (0.8–3%) of Matrigel support migration of human NPCs and neuronal differentiation. However, when the concentration of Matrigel increases to 50%, neurogenic differentiation is inhibited (Katakowski et al., 2005; Flanagan et al., 2006; Thonhoff et al., 2008).

Another property of hydrogels that affects lineage commitment and cell differentiation is stiffness. 2D polymer substrates with moduli ranging from 10 to 10,000 Pa affect differentiation of adult NSCs (Saha et al., 2008), with substrates having moduli similar to brain tissue (100–500 Pa), maximizing NSC differentiation. Similarly, when NSCs are encapsulated within 3D alginate hydrogels, the greatest expression of the neuronal marker β-tubulin III is observed on the softest hydrogel (Banerjee et al., 2009), indicating a modulus value near that of brain tissues best promotes neuronal differentiation. The mechanisms by which the mechanical properties of hydrogels influence stem cell commitment are not clear yet, but it appears that cytoskeletal motors may be involved in matrix-elasticity sensing, which is responsible for neuronal differentiation (Banerjee et al., 2009; Discher et al., 2009). Promotion of neural differentiation on 2D and 3D biomaterials of lower stiffness was confirmed by other studies using other polymer substrates such as polyacrylamide gels, and alginate hydrogels (Engler et al., 2006; Saha et al., 2008; Wang et al., 2010b).

Nonhydrogel polymer substrates

Nanoscale fibers favor neural differentiation of both NSCs and human ES cell-derived NPs compared to microscale fibers (Yang et al., 2005; Mahairaki et al., 2011). The orientation and diameter of polymer fibers also affect neuronal differentiation. For example, the degree of differentiation of NPs is higher on aligned nanopolycaprolactone and micropolycaprolactone fibers than on random fibers and on 2D tissue culture plate substrates (Mahairaki et al., 2011). Differentiation of NSCs on PLLA polymers, however, is independent of fiber alignment (Yang, 2005), suggesting that material chemistry is a covariate with stiffness in controlling differentiation. The signaling pathways responsible for the effects of matrix architecture on stem cell function have yet to be elucidated, but it is hypothesized that a lineage specification mechanism may involve cytoskeletal and nuclear rearrangements induced by the matrix architecture (Mahairaki et al., 2011).

Although a majority of studies have focused on a direct control of biochemical, physical, and mechanical cues on stem cell differentiation, stem cell microenvironments can also be manipulated using conducting polymer scaffolds (Lundin et al., 2011). The general idea behind using conducting polymers is that bulk properties (e.g., volume, conductivity and mechanical properties) and surface properties (e.g., surface tension and chemistry) dynamically change when the redox states of the polymer are reversibly switched (Causley et al., 2005; Robinson et al., 2006). For example, with the conducting polymer PPy used in neural tissue engineering, the addition of anionic dopants of varying molecular weight and chemical character: dodecylbenzenesulfonate, TsO, ClO_4, and Cl is hypothesized to control cell differentiation. PPy doped with the laminin peptide sequence RNIAEIIKDI or nerve growth factor enhances neuronal differentiation of hESCs (Lee et al., 2009b; Zhang et al., 2010a). Various composites made of PPy and other polymers such as PLGA also enhance adhesion, proliferation, and neurogenic differentiation of stem cells (Lee et al., 2009a; Liu et al. 2009, 2010a–d, 2011, Wei et al. 2010).

Osteogenic Differentiation

Surface functionalization

Cell–matrix interactions conducive to osteogenic differentiation can be enhanced by surface functionalization of polymers with different peptide sequences, growth, or differentiation factors. Common techniques of surface functionalization include chemical modifications via cross-linking polymer chains with bioactive factors, physical modifications via physisorption of the molecules onto the surface, or physical entrapment (Alvarez-Barreto et al., 2011).

One strategy to functionalize biomaterials surfaces that has received extensive study is the tethering of RGD peptide sequences (Massia and Hubbell, 1991a; Massia and Hubbell, 1991b; Drumheller and Hubbell, 1994; Hubbell, 1995; Hern and Hubbell, 1998; Kao et al., 2001; Vande-Vondele et al., 2003; Fittkau et al., 2005; Meinhart et al., 2005; Gurav et al., 2007). This peptide motif is found in many bone ECM molecules, including fibronectin, bone sialoprotein, and osteopontin (Lee et al., 2007). The positive role of RGD in cell adhesion has been widely demonstrated with various materials, including glasses, hydroxyapatite, and polymers (De Giglio et al., 2000; Morgan et al., 2008; Alvarez-Barreto et al., 2011). Support of osteogenic differentiation by the presence of RGD is also dose-dependent (Meinel et al., 2004; Shin et al., 2005; Alvarez-Barreto et al., 2011). Other peptides and ligands such as the collagen-mimetic peptide, GFOGER, can accelerate and increase bone formation, and improve osseointegration of bone into an implant in vivo (Reyes et al., 2007; Petrie et al., 2008; Phillips et al., 2008; Wojtowicz et al., 2010).

Although the majority of literature focuses on a single surface functionalization parameter (e.g., RGD concentration) on cell differentiation, combining surface functionalization with other parameters, such as dynamic flow, can have a synergistic effect (Alvarez-Barreto et al., 2011). For example, under flow perfusion, which introduces dynamic shear forces on cells, RGD modification of PLLA scaffolds has a more pronounced effect on

the differentiation of MSCs. The combined effects of flow perfusion and RGD on differentiation are also more prominent on titanium (Holtorf et al., 2005). Another interesting phenomenon is that there is a critical RGD concentration that yields the greatest extent of differentiation, and this optimal concentration is dependent on the flow rate. With increasing flow rate, the optimal concentration of RGD for cell differentiation decreases (Alvarez-Barreto et al., 2011). The dual roles of the integrin receptor $\alpha_v\beta_3$ either in promoting cell adhesion or inhibiting cell differentiation explains the existence of an optimal RGD concentration for cell differentiation. Higher flow rate can enhance the cell–matrix interaction, and therefore, increase the inhibiting effect of receptor $\alpha_v\beta_3$. Consequently, the optimal modification level shifts to a lower concentration.

Beside small peptide motifs, large molecules, such as fibrin and hyaluronic acid that favor cell adhesion, can be coated onto biomaterial surfaces. However, these large molecules do not directly signal cells to undergo osteogenic differentiation. Instead, they create a suitable environment for the activity of inductive factors, such as bone morphogenetic protein (BMP)-2 (Kang et al., 2011). For example, the activity of alkaline phosphatase in human ASCs cultured on fibrin and hyaluronic acid modified scaffolds followed by BMP-2 loading was significantly higher than that of ASCs on scaffolds without BMP-2 or just BMP-2 supplemented cell culture medium.

Composites

Many types of polymeric materials have been investigated for bone tissue engineering. Among these materials, most attention has focused on poly(α-hydroxy) esters, such as PLA, PGA, and PLGA (Cordonnier et al., 2011). However, their biodegradability, soluble factor release kinetics, mechanical properties, and process ability differ depending on stereochemistry and copolymer ratio (Costa-Pinto et al., 2009; Aydin et al., 2011). By combining polymers with different properties, a scaffold with a more desirable combination of properties can be obtained. One example would be the matching of polymeric scaffold degradation rate with that of in situ host site healing by adjusting the relative amount of PGA and PLGA (Hutmacher, 2000). The matching of rates minimizes adverse reactions (e.g., inflammatory) and is critical for the clinical success of tissue engineered substitutes. Other examples of composites used in bone tissue engineering are the mixture of naturally derived materials, including collagen, CS, and hyaluronic acid containing specific ligands for directing cell differentiation, with synthetic polymers whose physical properties are superior and more easily controllable (Chen et al., 2011).

It remains a challenge to separate the various biomaterial parameters that control stem cell differentiation. To partially solve this problem, hydrogels of PEG monomethacrylate, poly(propylene glycol) monomethacrylate, and methacrylic alginate (MA) have been developed (Cha et al., 2011). In this system, scaffold variables of charge density and hydrophobicity are separately controlled by controlling the mass fractions of MA and PPGmM, and porosity is controlled via lyophilization, providing a versatile platform enabling the independent control of the matrix variables. An investigation of poly(N-isopropylacrylamide-co-acrylic acid) hydrogel with independently tuned matrix stiffness and peptide concentration revealed that these matrices induced bone regeneration only when protease degradable crosslinks were used to create the network (Chung et al., 2006). Similar systems with independently tunable properties are also used for systematic optimization of material properties that lead to enhanced cell adhesion, proliferation, and tissue regeneration (Healy, 2004; Wall et al., 2010).

Bone is composed of an organic and inorganic mineral matrix. Therefore, it is also relevant to make a composite material consisting of both organic polymer and inorganic bioactive ceramics, such as tricalcium phosphate, hydroxyapatite, and bioactive glasses. In fact, many in vitro and in vivo studies have already demonstrated that polymeric materials containing ceramic second phases or coated with ceramics exhibit at least three improvements over polymers: enhanced bioactivity, better mechanical properties, and structural integrity, and less adverse host reactions after implantation (Roether et al., 2002). Ceramic materials such as bioactive glasses can form a direct bond to living bone tissue, while most polymeric materials are usually encapsulated by fibrous tissue in vivo. Therefore, the incorporation of a polymer with ceramic materials can improve the bioactivity of the scaffold. Adhesion proteins also more easily adsorb to ceramic surfaces (Zhao et al., 2006a), resulting in increased cell adhesion. Ceramic surfaces also induce the formation of carbonated apatite when placed in physiological media. This calcium phosphate layer plays an important role in mediating cellular responses, including cell differentiation (Murphy et al., 2005). Another advantage of ceramics is that the dissolution products of bioactive glasses and calcium silicate promote the expression of osteogenic genes at a critical concentration of Ca^{2+} and/or Si (Xynos et al., 2000a, b; Zhang et al., 2010b).

To make 3D polymer/ceramic composites, ceramic particles are infiltrated into porous polymeric matrices by solid–liquid phase separation or electrophoretic deposition, or one component is coated on the other using techniques such as slurry-dipping technique or mineral precipitation (Roether et al., 2002; Zhao et al., 2006a). The former way of synthesizing composites is inspired by the hierarchical structure of bone, where calcium phosphate particles of a nano size are embedded into an organic matrix. However, organic/inorganic composites fabricated in this way often fail to distribute the ceramic particles

uniformly through the polymer matrix. Therefore, the original interconnected porous structure can become blocked, and cell proliferation and differentiation are negatively affected. In contrast, making a composite material by coating techniques can create a more uniform and reproducible ceramic layer along the walls of pores, especially if flow is used (Roether et al., 2002; Segvich et al., 2008, 2009b), making the output of cell differentiation more controllable and predictable.

Chondrogenic Differentiation

To develop a stable and efficient strategy for directing differentiation of stem cells into a chondrogenic lineage, various material design approaches have been investigated (Heymer et al., 2009; Liu et al., 2010d; Anderson et al., 2011; Lim et al., 2011; Park et al., 2011b), including manipulating polymeric properties, such as macromer density, incoporating growth and differentiation factors into the polymeric substrate or coating, immobilizing signaling factors on the polymer surface, and making polymer composites.

Substrate Properties

Some polymer substrates intrinsically support and enhance chondrogenic differentiation of stem cells. One example is collagen type II; compared to alginate and collagen type I, collagen type II promotes expression of the chondrogenic genes sox9, collagen type II, aggrecan, and COMP (Bosnakovski et al., 2006). The shape of cells on type II collagen is also more rounded compared to type I collagen. Blocking the cell surface receptor β1 integrin reduces chondrogenic gene expression and also eliminates differences in Rock 1 and Rock 2 gene expression and cell shape. Therefore, collagen type II provides inductive signaling for chondrogenic differentiation by evoking a round cell shape through the β_1 integrin-mediated Rho A/Rock signaling pathway (Lu et al., 2010).

Dynamic loading can affect the movement and distribution of large molecules in dense hydrogels. Therefore, it is relevant to investigate the effect of variations in macromer density on chondrogenesis. For example, chondrogenesis and matrix formation are proportional to macromer density in methacrylated hyaluronic acid hydrogels, due to a greater probability of receptor-mediated interaction with the high density macromer material. However, a higher macromer density yields functionally inferior constructs (Erickson et al., 2009) due to lower permeability of these construct, where only limited matrix expansion by cells occurs.

The majority of studies have focused on the effect of mechanical cues on cell differentiation statically. However, an anionic hydrogel system, PEG-chondroitin sulfate (PEG/CS), undergoes reversible, anisotropic bending in an electric field (Lim et al., 2011). By using this unique property, dynamic mechanical and electrical cues can be simultaneously provided to cells. The magnitude of mechanical cues can be tuned through hydrogel crosslink density. More interestingly, the mechanical and electrical cues can be independently varied, which allows the investigation of one factor while maintaining the other one unchanged.

Growth factors

Chondrogenic differentiation of stem cells can be induced by growth factors and signaling molecules, including transforming growth factor-β (TGFβ)-1, insulin-like growth factor1 (IGF1), BMP2, BMP7, growth and differentiation factor5 (GDF5), glucosamine (GLCN), dexamethasone, vitamin C, and retinoic acid (Hwang et al., 2006; Toh et al., 2010). The induction effect of these factors is dose, temporally, and spatially dependent (Hwang et al., 2006; Erisken et al., 2011). For example, a 2-mM GlcN supplement in standard chondrogenic differentiation medium increases levels of aggrecan mRNA, and tissue-specific ECM accumulation from ESC compared to 0- and 10-mM concentrations (Hwang et al., 2006). As an example of the spatial effect of growth factors, when human MSCs are seeded on graded poly(e-caprolactone) with concentration gradients of two bioactive agents, insulin and β-glycerophosphate (β-GP), chondrogenic differentiation is increased at insulin-rich locations and osteogenic differentiation is increased at β-GP-rich locations (Erisken et al., 2011).

TGF-β1 also promotes chondrogenic differentiation. For example, TGF-β1 can be encapsulated with ASC into carrageenan-based hydrogels to enhance chondrogenic differentiation (RoCha et al., 2011). A coupling of TGF-β1 with bone marrow MSC macroaggregates in a PLGA scaffold also forms cartilaginous tissue (Liu et al., 2010d). Another interesting strategy of using TGF-β1 is to incorporate growth factor-loaded PLGA polymer microspheres within hMSC aggregates themselves. This approach promotes homogeneous cell differentiation across the cell aggregates, which is not usually seen in conventional cell aggregate culture, since the induction effect is limited by the diffusion of the chondrogenic growth factor from the culture medium into the aggregate and peripheral cell layers.

The use of a single growth factor may not provide all of the necessary signals required for differentiation (Mohan et al., 2010). A combination of two or more induction factors can promote chondrogenic differentiation of stem cells more efficiently (Sharma et al., 2007; Mohan et al., 2010; Toh et al., 2010). The design of biomaterials systems to deliver combinations of factors is motivated by the in vivo milieu, where the formation of a proper chondrogenic phenotype is regulated by the combined action of multiple growth factors spatially and temporally (Thorp et al., 1992). Results from both in vitro and in vivo experiments show that a combination of TFGβ-3 and BMP2 (Mohan et al., 2010)

or TGFβ-3 and hyaluronic acid (Sharma et al., 2007) promotes more chondrogenic tissue formation than any of these factors individually. The choice of factors and sequence of delivery play important roles in controlling chondrogenic differentiation. For example, TGF-β1 together with BMP7 yields a more homogenous hyaline-like cartilaginous tissue than a combination of TGF-β1 with IGF1, BMP2, and GDF5 (Toh et al., 2010).

Composites

Similar to directing other stem cell lineages, a combination of different polymeric materials can provide appropriate mechanical strength, biodegradability, biocompatibility, and surface characteristics that promote cell adhesion and chondrogenic differentiation (Moutos et al., 2010; Ragetly et al., 2010). For example, CS, a natural biomaterial, which has adequate mechanical properties for supporting chondrogenesis and cartilage formation, but limited cell adhesion ability, can be coated with type II collagen to increase cell adhesion and chondrogenic differentiation (Ragetly et al., 2010). A thermosensitive hydrogel, CS glycerol-phosphate (CGP) lacks mechanical properties, but the addition of starch to this material improves its storage modulus, and viscoelastic properties. Chondrogenic differentiation of ASCs and cartilage matrix accumulation can be increased on starch incorporated CGP (Sa-Lima et al., 2010).

Another approach to synthesizing composite scaffolds for cartilage regeneration is the fabrication of biphasic or multiphasic scaffolds made of a cartilage layer over a subchondral bone region (Heymer et al., 2009). This concept allows the implementation of variations in mechanical, structural, and chemical properties in each layer to mimic the natural structure of osteochondral tissue (Heymer, 2009). In one example, a multiphasic composite scaffold contains an upper collagen type I fiber layer for articular cartilage repair, separated by a hydrophobic interface from a lower PLA for bone repair. With TGFβ-1, hMSCs secret glycosaminoglycans and express cartilage-specific markers aggrecan and collagen type II. However, the communication mechanisms between the two distinct regions to promote chondrogenesis in the upper layer are not fully understood. Using biphasic strategies, polymers can be integrated with ceramic materials such as hydroxyapatite, to promote the simultaneous growth of bone, cartilage, and a mineralized interface tissue (Taboas et al., 2003; Schek et al., 2004).

Surface functionalization

As previously discussed, growth factors can be supplemented into cell culture medium or encapsulated into microsphere or bulk materials to induce chondrogenic differentiation of stem cells. Alternatively, growth factors like TGF-β3 can be immobilized on the polymer surface. Surface immobilization leads to more controllable spatial distribution, which avoids undesirable side effects in the areas where no growth factor is needed. Besides better spatial control, biomaterial surface engineering can also result in sustained release and reduce the consumption of growth factors in comparison with simple addition of factors to the media (Fan et al., 2011). Among the various ways of immobilizing growth factors, covalent cross-linking can provide long-term growth factor delivery, compared to physical adsorption (Fan et al., 2008; Fan et al., 2011).

Peptide sequences are often tethered on biomaterial surfaces to directly and indirectly affect cell differentiation through receptor-integrin interactions and other mechanisms. Although the peptide RGD is primarily known to facilitate cell adhesion, chondrogenic differentiation of hMSCs has been observed on RGD modified polymer surfaces as well (Liu et al., 2010c; Re'em et al., 2010; Steinmetz and Bryant, 2011; You et al., 2011). However, incorporation of another peptide sequence, KLER, with RGD can lead to more significant type II collagen and aggrecan gene expression and cartilage ECM production. Because the KLER sequence binds strongly to collagen type II and is responsible for matrix organization instead of directly interacting with cell receptors and activating a specific signaling pathway, these results indicate that an indirect cell-ECM interaction is as important as direct cell–material interactions in controlling chondrogenic diferentiation (Salinas and Anseth, 2010).

Functionalized polymeric surfaces can also be used for gene delivery to induce chondrogenic differentiation. For example, polyethylenimine (PEI) modified PLGA nanoparticles are used to deliver SOX5, SOX6, and SOX9 into human MSCs to enhance chondrogenesis. A surface functionalization step is necessary for the incorporation of DNA on these materials, because neither PLGA nor PEI can bind to DNA. The efficiency of gene transfection following surface functionalization approach is sufficient to switch chondrogenic differentiation of stem cells (Park et al., 2011b).

Myogenic and Endothelial Differentiation

Growth factors

Various growth factors can affect the activity of either endothelial cells or their progenitors, including FGF, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor, hepatocyte growth factor, and placental growth factor (Beohar et al., 2010). Utilization of these growth factors can elicit proliferative and angiogenic effects (Richardson et al., 2001). However, delivery of these factors from polymeric biomaterials to induce cell differentiation presents a challenge because the cellular responses to these soluble stimuli are dose-dependent, temporally dependent, and spatially dependent. Without appropriate control of release kinetics, these soluble factors may negatively influence cell differentiation (Beohar et al., 2010).

Therefore, focus has been placed on the design of ''smart'' materials that can release soluble factors in response to cellular needs or changes in physiological conditions. Growth factors can be released when temperature, pH, or even magnetic field changes (Qiu and Park, 2001; Zhang et al., 2004).

In addition to direct immobilization of growth factors on polymer surfaces, transplanted cells can secrete growth factors that mediate function of stem cells. For example, transplanted cord blood mononuclear cells (CBMNC) seeded on a fibrin matrix were used to treat myocardial infarction in a rat model (Cho et al., 2007). Transplanted human umbilical cord blood cells have the ability to produce various angiogenic growth factors, including VEGF, bFGF, and angiopoietin-1, which can induce angiogenesis in vivo (Ma et al., 2005; Yokoyama et al., 2006). Combining CBMNC transplantation and bFGF delivery can enhance neovascularization in the ischemic myocardium. It is also likely that transplanted CBMNCs secrete other angiogenic growth factors, cytokines, and vasoactive factors to enhance angiogenic efficacy, which is also one of the advantages of using biomaterials to transplant cells compared to supplementing individual growth factors (Cho et al., 2007).

3D composite scaffolds

Cardiac cells cultured in 3D not only display distinct features that are more representative of native myocardium than cells in 2D culture (Clause et al., 2010), but also show enhanced cell–cell interactions, increased cardiac-specific protein expression, spontaneous beating cell activity, and contractility (Akins et al., 2007; Anderson et al., 2007). However, there are also limitations to 3D culturing. The differentiation state of skeletal muscle-derived stem cells in 3D scaffolds is hard to define, due to the coexistence of many cardiac and skeletal muscle-specific proteins produced by mature and immature cells in the complex 3D structure (Clause et al., 2010).

One important consideration in 3D scaffold design for cardiac tissue engineering is that the scaffold has to accommodate the contractile function of differentiated cardiomyocytes. Cardiomyocytes embedded in a fibrin matrix lose their contractile function and type I collagen also limits the spontaneous contraction of cardiomyocytes (Zimmermann et al., 2002; Gonen-Wadmany et al., 2004; Huang et al., 2007). Materials such as PEGylated fibrinogen hydrogels can retain the contractile phenotype of cardiomyocytes through the combined biological and structural attributes of scaffold, in which physical properties, including biodegradation and compliance, are controlled by the PEG, while the fibrinogen confers biological activity (Shapira-Schweitzer et al., 2009).

Another class of materials that is mechanically compatible with heart muscle and avoids permanent deformation and failure under exposure to long-term cyclic strain is elastomers. However, the acidic degradation products of some elastomers lead to an inflammatory response and therefore can limit cell function. Incorporation of a lightly alkaline second phase, such as 45S5 Bioglass® particles, can buffer the acidic cytotoxicity of degradation products, and also improve the functional activity of cardiomyocytes (Chen et al., 2010).

Despite these positive results, there is still limited information on the use of physical stimuli to control stem cell differentiation into a cardiac lineage. Biomaterial stiffness can guide cardiac differentiation of stem cells. For example, in semi-interpenetrating polymer networks made of collagen, fibronectin and laminin, stiffness can be controlled by controlling the percentage of collagen. Stiffer scaffolds resulting from higher collagen concentrations inhibit endothelial cell differentiation, possibly because increasing the elastic modulus decreases cell apoptosis (Battista et al., 2005). These results are consistent with other studies (Kraehenbuehl et al., 2008), where the stiffness of poly-(ethyleneglycol) (PEG)-based extracellular matrices varies with the number of cleavable crosslinker matrix metalloproteinase-sensitive peptides. On soft matrices, embryonal carcinoma (EC) cells express more of the early cardiac transcription factor, Nkx2.5, than its control, embryoid bodies in suspension. In contrast, stiffer matrices decrease the number of Nkx2.5-positive cells.

The range of surface stiffness that induces optimal myogenesis of stem cells is cell-dependent. In the range of 10 to 17 kPa, myogenesis of hMSCs occurs maximally (Engler et al., 2006; Lanniel et al., 2011). However, with embryonal carcinoma cells a softer matrix with a modulus of 0.3 kPa enhances cardioprogenitor differentiation more than a stiffer matrix with a modulus of 4 kPa (Kraehenbuehl et al., 2008). With cardiosphere-derived cells, biodegradable poly(N-isopropylacrylamide) hydrogels having a modulus of 30 kPa more significantly upregulate cardiac expression than gels with a modulus of 5 or $\sim$60 kPa (Li et al., 2011).

Besides structure and physical properties, chemical composition of a biomaterial can also be tuned to affect cell myogenic differentiation. When a scaffold is made of collagen, fibronectin, and laminin, the presence of fibronectin stimulates endothelial cell differentiation and vascularization. In contrast, increasing the concentration of laminin enhances cell differentiation into beating cardomyocytes. Because fibronectin and laminin do not induce detectable matrix mechanical and structural modifications, control of differentiation is likely due to the cell adhesion motifs present on these proteins (Battista et al., 2005).

Surface modification

Peptide ligands can interact with integrin receptors on cell surfaces, mediating cell adhesion and a variety of signaling pathways having

essential biological consequences for cardiac tissue engineering (Liu et al., 2011). These peptides include RGD, PGLD, and RGDSP (Kraehenbuehl et al., 2008; Yu et al., 2010; Moura and de Queiroz, 2011). Surface modifications of polymeric scaffolds with these peptide sequences can stimulate the differentiation of cardiac progenitor cells, promote cardiac matrix maturation, and induce angiogenesis in vitro. Despite these promising in vitro data, in vivo experiments show no significant difference in angiogenesis between RGD modified alginate microbeads and unmodified ones (Yu et al., 2010). These contradictory results highlight that microenvironmental factors are different between in vitro and in vivo situations, and conclusions drawn from in vitro experiments on substrates functionalized by peptides may not be extrapolated to and be predictive of in vivo function.

Besides peptide sequence, surface functional groups can be introduced into hydrogels through plasma polymerization. These functional groups include amino, carboxylic, and phosphate groups. Surfaces with carboxylic coatings yield higher levels of expression of the myogenic differentiation marker MyoD1 than the other functional groups (Lanniel et al., 2011). The compatibility of carboxylic groups with directing cardiac myocyte function was observed in another study, where an increased number of beating cardiac myocytes was seen on the carboxylic-functionalized surfaces compared to hydroxylfunctionalized surfaces (Natarajan et al., 2008). In spite of the ability to control cardiomyocyte differentiation by manipulating polymer surface chemistry, the mechanisms by which these surface functional groups affect differentiation is not clear.

It is important to note that the stimulating effect of surface functional groups on cardiac differentiation can vary with other material variables, such as stiffness. For example, the addition of collagen to a thermosensitive hydrogel made of polycaprolactone, N-isopropylacrylamide, 2-hydroxyethyl methacrylate, and dimethyl-γ-butyrolactone acrylate has no effect on the differentiation of cardiosphere-derived cells into a mature cardiac lineage in low modulus hydrogels of $\sim$5 kPa, but enhances expression of the cardiac genes MYH6 and cTnT in medium modulus hydrogels of $\sim$30 kPa (Li et al., 2011). However, carboxylic groups on polyacrylamide hydrogels enhance MyoD1 expression by human MSCs on low modulus surfaces of $\sim$10 kPa, compared to a lower levels of MyoD1 expression on high modulus surfaces of $\sim$ 80 kPa (Lanniel et al., 2011).

SUMMARY AND FUTURE DIRECTIONS

In summary, various factors from the extracellular environment known to control cell adhesion, proliferation, and differentiation have been incorporated into the design of biomaterials to achieve the objective of creating increased communication between biomaterials and their surrounding biological environment. The effects of these material modifications on cell activity are dose-dependent, temporally dependent, and spatially dependent. Biomaterials design and synthesis, as well as other tissue engineering strategies are used to create a controlled microenvironment and mimic the dose-response relations in time and space to favor specific types of cell activity.

Despite the interesting in vitro and in vivo results summarized in this review, the precise control of cell activity by polymeric substrates still represents a major challenge due to complex and dynamic interactions in these multicomponent systems, involving many biological, physical, and chemical processes. For instance, investigations of how polymer stiffness affects cell activity do not account for changes in stiffness in situ over time, as cells lay down ECM to create a ''new'' substrate. When investigating peptide immobilization on polymer surfaces, the distribution of peptide sequences on the surface and how distribution patterns affect cell activity need to be accounted for. Therefore, to gain better understanding of cell–biomaterial interactions, a systematic study that evaluates the role of extracellular factors in a time-dependent manner is required, even if the study is simplified by investigating only one factor. As new methods and strategies become available to fabricate new polymeric substrates with independently controllable properties, and new techniques are able to offer more precise monitoring and characterization of substrate properties and cell activities, better understanding of cell–biomaterial interactions can be achieved in the future. Ultimately, better control of cell–biomaterial interactions will enable advances in tissue engineering to be achieved.

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