DOI: 10.3727/096368910X532756

CT-2302 Accepted for publication in special 2010 PPSSCR issue of "***Cell Transplantation***"

Differentiation of Stem Cells: Strategies for Modifying Surface Biomaterials

Ru-Huei Fu,*†[1] Yu-Chi Wang,‡[1] Shih-Ping Liu,*§ Chin-Mao Huang,* Yun-Han Kang, *

Chang-Hai Tsai,¶# Woei-Cherng Shyu,* †[2] and Shinn-Zong Lin*†‖[2]

*Center for Neuropsychiatry, China Medical University Hospital, Taichung, Taiwan

†Graduate Institute of Immunology, China Medical University, Taichung, Taiwan

‡ Biotechplex corp.,1205 Linda Vista Drive, San Diego, California 92078, USA

§Graduate Institute of Basic Medical Science, China Medical University, Taichung, Taiwan

¶Department of Pediatrics, China Medical University Hospital, Taichung, Taiwan

#Department of Healthcare Administration, Asia University, Taichung, Taiwan

‖China Medical University Beigang Hospital, Yunlin, Taiwan.

[1]These two authors contributed equally to this article.

[2]These two authors contributed equally to this article.

Address correspondence to Shinn-Zong Lin, MD., Ph.D., Center for Neuropsychiatry, China Medical University Hospital, Taichung, Taiwan. Tel: 886-4-22052121 ext. 6034; Fax: 886-4-22080666; E-mail: shinnzong@yahoo.com.tw. Woei-Cherng Shyu, MD., Ph.D., Center for Neuropsychiatry, China Medical University Hospital, Taichung, Taiwan. Tel: 886-4-22052121 ext. 7811; Fax: 886-4-22052121 ext. 7810; E-mail: shyu9423@gmail.com

Running title: Biomaterials for stem cells

ABSTRACT

Stem cells are a natural choice for cellular therapy because of their potential to differentiate into a variety of lineages, their capacity for self-renewal in the repair of damaged organs and tissues in vivo, and their ability to generate tissue constructs in vitro. Determining how to efficiently drive stem cell differentiation to a lineage of choice is critical for the success of cellular therapeutics. Many factors are involved in this process, the extracellular microenvironment playing a significant role in controlling cellular behavior. In recent years, researchers have focused on identifying a variety of biomaterials to provide a microenvironment that is conducive to stem cell growth and differentiation and that ultimately mimics the in vivo situation. Appropriate biomaterials support the cellular attachment, proliferation, and lineage-specific differentiation of stem cells. Tissue engineering approaches have been used to incorporate growth factors and morphogenetic factors—factors known to induce lineage commitment of stem cells—into cultures with scaffolding materials, including synthetic and naturally derived biomaterials. This review focuses on various strategies that have been used in stem cell expansion and examines modifications of natural and synthetic materials, as well as various culture conditions, for the maintenance and lineage-specific differentiation of embryonic and adult stem cells.

Key words: Stem cells; Biomaterials; Tissue engineering; Extracellular matrix (ECM)

INTRODUCTION

Stem cells are simply defined as the progeny of cells that have the potential to differentiate into a variety of different lineages (14,55). Stem cells are characterized by their ability to self-renew and to differentiate into multiple different cell types and tissues (75,82). There are two types of stem cells: embryonic stem (ES) cells derived from the inner cell mass of preimplantation embryos and adult stem cells found in tissue and organs (66).

ES Cells

Mouse ES cells (mESCs) were first isolated in 1981 by Martin Evans and coworkers, who received the Nobel Prize (20) for their work; human ES cells (hESCs) were later isolated in 1998 (82) from preimplantation embryos (blastocysts) (41,45). The pluripotent nature of ES cells gives them the ability to differentiate into any one of the three germ layers: endoderm, ectoderm, and mesoderm. ESCs also have the unique property of indefinite self-renewal, i.e., they can be cultured and maintained in an undifferentiated, pluripotent state. Thus, ES cells have the potential to differentiate into almost all cell types (56). In addition, stem cells are being used to understand the complex molecular and cellular events that occur in early development and disease progression; epigenetics and pathophysiology (14,26,43); and drug development, screening, and toxicology (39,49).

Adult Stem Cells

An adult or postnatal (somatic), whereas mesenchymal stem cells (MSCs) have been found among various adult tissues (51,57) and organs, including the heart (11),

nervous system (38), gut (3), and skin (89). MSCs are thought to be a self-renewing population of cells that can give rise to differentiated cells found in adult tissues. Bone-marrow-derived MSCs are currently undergoing clinical trials for cardiac and orthopedic applications. However, the ability of MSCs to proliferate and differentiate decreases with the age of the donor and culture time (18,60). Other problems, such as their infrequent occurrence, restricted differentiation potential, and poor growth, limit the usefulness of MSCs (28). Stem cells are also found in cord blood, which may have advantages over other sources of stem cells and currently provides an alternative solution for bone marrow reconstitution for childhood leukemia (8). The only real block to the broader application of cord blood transfusion is the need for sufficient cell numbers.

Tissue Engineering or Regenerative Medicine

Of all the applications for stem cells, one of the most exciting may be their use in regenerative medicine. Regenerative medicine is an interdisciplinary field that aims to provide safe and reliable ways to repair, restore, or replace damaged tissues or organs (66,93,98). Stem cell therapy and tissue engineering are the two main components of regenerative medicine. Tissue engineering combines specially designed biomaterials with cells to augment, replace, or reconstruct damaged or diseased tissues (9,17). In recent years, biomaterial design has evolved from basic materials to biofunctional materials that incorporate instructive signals into scaffolds to modulate cellular functions such as proliferation, differentiation, and morphogenesis (14) (Fig. 1). Stem cells present in the extracellular matrix (ECM) respond to change in their environment. Their response to biological stimuli has been well studied, and these physiological stimuli clearly influence

cell behavior and differentiation down tissue- and organ-specific pathways for repair and regeneration (8,11). Various strategies and factors have been used in biomaterials to induce growth and regeneration of specific tissues from stem cells. In this review, we focus on the surface modification of biomaterials to induce growth and regeneration of specific tissues from stem cells (Fig. 2).

BIOMATERIALS AND STEM CELLS

Many studies have been devoted to the regulation of stem cells by specific stimuli in biomaterials, including growth factors, peptides, shape, and size, to modulate cellular differentiation and proliferation (23).

Biomaterials provide scaffolds and initial support that allows cells to attach, proliferate, differentiate, and form an ECM (1). The basic requirements for biomaterials for use as scaffolds are their biocompatibility and appropriate surface properties that mimic the structure and properties of tissue in order to direct the macroscopic process of tissue formation (80). Biomaterials also supply cells with growth factors and other biomolecular signals. Scaffold materials can be synthetic or natural. The most common synthetic biodegradable polymers being used or studied include polylactic acid (PLA) (13), polyglycolic acid (PGA), polyfumarates (87), and polycaprolactones (PCLs) (87). Poly(DL-lactic acid-co-glycolic acid) (PLGA) is a co-polymer of PLA and PGA and is widely used in the fabrication of polyanhydrides for scaffolds (34,40,51,72). Other important synthetic biodegradable polymers include poly(orthoesters) and polyanhydrides (from nonphysiological monomers), which possess biocompatible, well-defined degradation characteristics (57).

Natural biomaterials used for biomaterials may consist of components in the ECM, such as collagen, hydroxyapatite, fibrinogen, and hyaluronic acid. Other natural materials such as chitosan and cellulose are derived from insects or plants and provide favorable microenvironments for stem cell culture. The advantages of natural biomaterials are that they are biocompatible and bioactive and have properties similar to native tissue. Disadvantages of using natural materials over synthetic materials include limited control over physicochemical properties, difficulty in modifying degradation rates, and problems with purification (removal of viruses and other pathogens) when isolating these materials from different sources. However, recently several natural materials, including chitosan and hyaluronic acid, have become commercially available, are well characterized, and have reproducible, controlled properties (14).

Collagen is a major natural ECM component and the main structural element in skin, bone, tendon, cartilage, and blood vessels and heart valves (37,38,62). Collagen has a native surface that induces cellular attachment and is chemotactic to cells. Thus, it has useful biological properties desirable for tissue engineering applications. Matrigel Matrix, a product that is currently available commercially, comprises laminin, collagen IV, and heparan sulfate proteoglycans (5,35). It has been used to extensively improve neovasculature formation when injected with cells isolated from the stromal vascular fraction of adipose tissue (i.e., adipose-derived stem cells) in an ischemic mouse model (50). Chitosan is another natural polymer comprising glucosamine and N-acetylglucosamine isolated and processed from crustacean shells (69,70). One study specifically examined chitosan as a composite scaffold for the study of MSC osteogenesis (26). Other materials that have been extensively used include hyaluronic acid and alginate (derived from algae

cell walls). Hyaluronic acid is a highly attractive biomaterial because it is available as a gel-like material and can be chemically modified for processing into fibers, membranes, or microspheres. A modified type of hyaluronic acid that is commercially available is Hyaff (7). Gerecht et al. showed that hyaluronic acid hydrogels can be used to maintain the pluripotency and undifferentiated state of hESCs (25). Alginate is a natural polysaccharide that has been evaluated for the encapsulation and differentiation of ESCs (13). In one study, the microencapsulated structure of alginate prevented embryoid body (EB) formation and promoted differentiation toward a hepatic lineage without the need for EB formation (52).

Despite their weak mechanical properties and difficulties in their regulation and manufacture, overall, natural biomaterials allow more efficient cellular attachment and production of biological signals for the culture of stem cells In addition to synthetic and natural polymers, a wide variety of biocompatible materials, including ceramics and metals, is available for stem cell culture (36,44). In the next section, we discuss the creation of a biomicroenvironment to mimic the in vivo regulation of stem cells through the attachment of 3D biomaterials.

3D Differentiation Culture

For in vivo tissue structure, cells are organized within the complex molecular framework of the ECM. Molecules in the ECM influence cell migration, proliferation, and differentiation through cell–cell and cell–substrate interactions (13). However, the traditional 2D tissue culture plates do not support a complex biological microenvironment similar to the in vivo environment. To mimic this 3D growth environment in vitro, a variety

of 3D biomaterials have been used as substitutes for the ECM, providing a physical support matrix and increasing cell–cell and cell–substrate interactions (85,96).

Langer et al. and several research groups first hypothesized that porous biodegradable polymer scaffolds can be used to support ES cells because such scaffolds represented a promising system for allowing the formation of complex 3D tissues during differentiation (70,84,86). Not only does the scaffold provide physical cues for cell orientation and spreading, but the pores also provide space for the remodeling of tissue structures. In addition, localized growth factor supplementation can be controlled with the directed degradation of scaffolds. One possible reason for the differences obtained between 2D and 3D cultures could be the scaffold's mechanical stiffness, which is necessary to resist the force of cell contraction. Precise spatial and temporal presentation of the factors that direct stem cell differentiation is critical to homogeneous and efficient differentiation.

Many studies have found significant differences in the differentiation profile of ESCs when cultured in a 3D environment compared with a 2D environment (24,55). Comparison of the differentiation and organization of scaffold-grown constructs with EBs revealed higher expression of differentiation-associated proteins such as cytokeratin, alpha-fetoprotein, and nestin on the scaffolds, which correlated with more organization into defined epithelial tubular structures and neural tube-like rosettes. Researchers have reported that complex structures with features of various committed embryonic tissues can be generated in vitro by using early differentiating hESCs and further inducing their differentiation in a supportive 3D environment such as poly(L-lactic acid) (PLLA)/PLGA polymer scaffolds, leading to the formation of neural, hepatic, and mesenchymal tissues (39).

Roy and coworkers showed results that demonstrate that 3D culture of ES cells, especially under dynamic conditions, promotes cell–cell interactions and entrapment of secreted ECM, resulting in increased signaling and enhanced expression of genes that function in promoting cell differentiation. In their study, more than 80 genes involved in signal transduction were altered in 3D culture (50). 3D culture is sufficient to induce selective differentiation of embryonic-derived cells and to provide structural support for the differentiation of ES cells to higher order tissue organization and remodeling (43). Elisseeff demonstrated that growth factors such as transforming growth factor- β1 (TGF-β1) and bone morphogenetic protein-2 (BMP-2) have a significant impact on the chondrogenic differentiation of mESCs and regulate chondrogenic cell fate through different mechanisms, depending on 2D or 3D culture of EBs (23). These results provide a comprehensive description of the effects of 3D culture conditions on the gene expression and growth factor profiles of ES cells. These studies also describe the dynamic environment and beneficial effects of 3D culture on ES cell differentiation.

Modification of Surface

In recent years, stem cell behavior has been directly influenced through signal transduction pathways by modifying the physical, chemical, and biological characteristics of biomaterials to change substrate properties, surface interactions, and the microenvironment of SCs. Cell–biomaterial interactions can be influenced in many ways, among them, altered gene expression and specificity of the signal pathway. Biomaterials can be designed to control their degradation kinetics, link specific ligand signals, or release biological molecules such as peptides or small molecules to change the microenvironment

between stem cells and the biomaterials. Numerous studies have demonstrated that specific biological responses in stem cells can be introduced by modifying biomaterials and their surfaces. The next section reviews the various methods used to modify biomaterials for the maintenance and differentiation of stem cells.

Surface Chemistry and Topography. The surface chemistry and topography of biomaterials induce in vitro and in vivo cellular responses, including adhesion, survival, cell cycle progression, and expression of differentiated phenotypes (7,21,31,58). The biomaterial surface properties, e.g., hydrophobic/hydrophilic properties and surface charges, play an important role in protein adsorption kinetics and their folded conformation, which in turn influence cellular activities (24). The effects of biomaterial surface properties on cellular responses are generally attributed to material-dependent differences in adsorbed protein species, concentration, and/or biological activity (41). On the other hand, many resorbable materials used for tissue engineering are hydrophobic in their native state and require surface modification to become hydrophilic before cell seeding (92). Mikos et al. have precisely decorated the biomaterial surface with bioactive molecules to enhance bioactivity (76). The results demonstrated surface-dependent differences in integrin binding as a mechanism to regulate differential cellular responses to biomaterial surfaces and improve the performance of biotechnological culture supports (41). In addition, another study demonstrated that surface chemistry and the binding of integrin adhesion receptors to ECM components, such as fibronectin and type I collagen, activate signaling pathways that direct osteoblast survival, cell-cycle progression, gene expression, and matrix mineralization (23). Surface engineering approaches, including modification of topography,

provide promising strategies that could be used as powerful tools in directing the ES cell–matrix interactions and their subsequent differentiation.

Chemical and Biological Signals. In 2000, Schuldiner et al. investigated a number of different growth factors; depending on their type (biological activity), EBs differentiated selectively into mesodermal, endodermal, or ectodermal lineages. This study was conducted as a first screening of growth factors to understand the differentiation of hESCs (74). Many studies have shown increased self-renewal of MSCs by fibroblast growth factor-2 (FGF-2) and the maintenance of their multilineage differentiation potential (5,83). Recently, it was also demonstrated that FGF-2 could allow long-term self-renewal of hESCs and maintain their pluripotent status (45). In addition, BMPs play a significant role by initiating chondro-progenitor cell determination and differentiation of bone (68). Other growth factors include retinoic acid (RA), TGF-β, activin-A, and insulin-like growth factor 1. Both RA and TGF-β are involved in pancreatic formation (47). Activin-A has been added to EB cultures to create lung epithelial progenitor cells (71). Activin-A has also been shown to promote the differentiation of mESC-derived EBs toward the endoderm germ layer (71). Studies have demonstrated success with RA and activin-A in promoting in vitro differentiation of mESCs into α, β, γ, and δ cells, all of which are pancreatic endocrine cells (59). In 1984, researchers found that, besides growth factors and cytokines, RGD peptides (R: arginine; G: glycine; D: aspartic acid) promote cell adhesion (64). Piershbacher et al. determined that the complete primary structure of a site in the fibronectin molecule contains this Arg-Gly-Asp-Ser sequence, which interacts with cell surfaces (65) and promotes cell attachment when insolubilized on a surface; the arginine, glycine, and

aspartate residues cannot be replaced even with closely related amino acids, but several amino acids can replace serine without loss of activity.

The impact of RGD peptide surface density and spatial arrangement, as well as integrin affinity and selectivity, on cell responses such as adhesion and migration have been discussed (36). According to various studies, SC differentiation can be directly mediated by presenting appropriate biological or chemical signals, specific growth factors, hormones, peptides, and cytokines in their microenvironment. However, studies in which growth factors, hormones, and chemicals were directly added to the culture medium did not result in homogeneous differentiation of ES cells. Hence, the current challenge is to find an optimized combination of these various cytokines and growth factors.

Controlled release and delivery of growth factors responsible for different stages of differentiation may provide instructive signals for guided differentiation of ES cells. In tissue engineering, many methods have been used to deliver biological signals by simple physical adsorption on the biomaterial or scaffold surface or by direct incorporation of biomolecules within the scaffold structure or into the scaffold biomaterial in a variety of ways. Soluble growth factors can be directly encapsulated or incorporated during the scaffold fabrication process (70) and this technique has been used widely. To mimic the patterned distribution of growth factors and ECM molecules in the in vivo process, it is necessary to develop biomaterial delivery systems to sequester these factors in a localized microenvironment and prevent their diffusion into other regions of the scaffolds. In the next section, we summarize the various methods that have been used.

Altering the material surface physically. For many years, alterations have been stimulated in the microenvironment by modifying the biomaterial surface coatings, submerging the biomaterial in various chemicals, using protein absorption, and charging the biomaterials or scaffold surface. One mechanism used to enhance the cellular response to hydrophilic surfaces is to alter the array of proteins adsorbed to the hydrophilic and hydrophobic materials (19). After coating TiO(2) nanotube layers with a self-assembled monolayer (octadecylphosphonic acid) researchers showed that there was super-hydrophilic behavior. MSC adhesion and proliferation are strongly affected in the super-hydrophobic range (4). By altering the hydrophobicity of peptides used to create scaffolds, it may be possible to encourage a variety of cellular interactions (25,54). Submerging the scaffolds in various concentrations of potassium hydroxide can change the hydrophilicity of poly (DL-lactide), PLA, PGA, and PLGA. Scaffolds such as poly(α-hydroxyl ester) treated with potassium hydroxide (0.1 M), as compared with non-surface-treated scaffolds, were able to support a larger number of mESCs (25). All of these methods indicate that, for each polymer used, an optimized hydrophobicity will best promote cellular growth.

The mode of cell adhesion is distinct for positive and negative charges. Konno et al. studied the effects of electrostatic charge on mESCs by culturing mESCs on photoimmobilized polymers with leukemia inhibitory factor (LIF) (44). In another study, titanium fiber mesh scaffolds were coated with aRGD (30), a cell adhesive, integrin-binding peptide found in fibronectin and laminin. MSCs were shown to attach more strongly to these RGD-coated scaffolds.

Protein adsorption to scaffold surfaces can be an effective route for the presentation of bioactive molecules (94), but difficulty in controlling the concentration and degradation of protein and poor reproducibility of this process limits its applicability.

Covalent conjugation of bioactive molecules to the biomaterial surface. Covalent conjugation of bioactive molecules to the biomaterial surface should be more reproducible because both biofactor amount and density can be controlled. In addition, incorporation of bioactive molecules in biomaterials is an important way to regulate cell differentiation and enhance the functionality of differentiated cells by providing adequate signaling and concentration of biofactors.

Numerous materials have been incorporated with RGD-containing peptide sequences covalently conjugated to polyethylene glycol (PEG) macromers. N-hydroxysuccinimidyl-ester chemistry (6,35), widely used in academic studies and medical applications, has been applied by functionalizing the amine terminus of the peptide, thereby enabling the adhesion peptide to co-polymerize rapidly with hydrogels (35). Freudenberg et al. showed that biohybrid hydrogels were based on covalently cross-linked heparin and RGD peptides (22). Other peptide sequences have been discussed and applied since the discovery of RGD peptides. Neonatal mouse cerebellum stem cells were seeded on thelaminin-derived peptides, CYIGSR (Cys-Tyr-Ile-Gly-Ser-Arg) and CSIKVAV (Cys-Ser-Ile-Lys-Val-Ala-Val), grafted on PLLA/PLLA films. Improved viability and longer neurites were obtained than when using PLLA film over the cultivation period. This study highlights the potential of using the lysine-capped PLLA with laminin-derived peptides for promoting nerve regeneration (33). Cell–matrix interactions were enhanced in RGD-

functionalized hydrogels, leading to increased MSC viability. Nuttelman and colleagues showed that the viability of the encapsulated hMSCs increases from 15% to 75% when RGD is incorporated into hydrogels (61). Not only did the hESC-derived cells morphologically resemble chondrocytes, but, as indicated by RT-PCR analysis, the cells also expressed a number of chondrocytic markers and demonstrated the ability to produce 7% w/v of GAG (arginine-glycine-aspartate) after 3 weeks in culture (38). MSCs cultured under similar conditions without RGD produced 3.5% w/v of GAG accumulation (90); however, RGD remained the dominating peptide for cell attachment (77).

In addition to presenting peptides to control differentiation via covalent linkage with biomaterials, it may be possible to use other materials to mimic the cellular environment. Most cellular interactions are complex processes that are controlled by the surrounding matrix, growth and differentiation factors, and other environmental factors that initiate or suppress cellular signaling pathways and transcription of specific genes in a temporal–spatial manner. For this purpose, physical or covalently modified biomaterials, in combination with multibiofactors such as growth factors, can be released via diffusion, cell-mediated proteolysis, or in response to mechanical stimuli that have been successfully incorporated for in vitro and in vivo regenerative applications (53,59). Controlled delivery of multiple growth factors via different release kinetics has been shown to promote enhanced differentiation and tissue formation. For example, gelatin-based semi-interpenetrating networks (sIPNs) containing soluble and covalently linked bioactive factors have been shown to aid in wound healing. In one study, modulation of wound healing by sIPNs grafted with PEGylated fibronectin-derived peptides and used as platforms for the delivery of exogenous keratinocyte growth factor induced the release of

other key cytokines involved in tissue repair (2). In recent years, many versatile biomaterial-based methods have been developed to quantitatively present ligands to SCs in order to direct lineage-specific differentiation and study cellular processes during cell differentiation. Scaffold mineralization during osteogenic differentiation of MSCs can also be enhanced through chemical modification of the biomaterial structure. In one study, functionalized PLGA scaffolds incorporated two chondrogenic factors, Dex and TGF-β1, to provide an appropriate niche for the chondrogenic differentiation of MSCs without a constant supply of Dex and TGF-β1 in the medium (62).

Synthetic polymers have been widely used, but natural materials can also be chemically altered to improve specific properties for tissue engineering. For example, Zhang and colleagues created PEGylated fibrin patches to study in vitro differentiation of MSCs into endothelial cell lineage for potential use in myocardial repair (97). Human MSCs cultured in the RGD/BMP-2-immobilized hydrogels showed proliferation rates higher than that of control or RGD-immobilized hydrogels. Real-time RT-PCR showed that the expression of osteoblast marker genes such as CBFalpha1 and alkaline phosphatase was increased in hyaluronic acid-based hydrogel, and the expression level was dependent on the molecular weight of hyaluronic acid, RGD peptide, and BMP-2 (42). Another example of a natural biomaterial, chitosan, a polysaccharide, has been modified to be thermo-responsive (12) by incorporating hydroxybutyl groups in the polymer backbone.

APPLICATION OF BIOMATERIALS IN STEM CELL BIOLOGY

Expansion and Maintainence of Stem Cells

For multiple clinical and biotechnological applications, ES cells that are free of foreign proteins and antigens hold promise as an untapped cell source. In a recent report, ES cells were seeded on the feeder layer to maintain their undifferentiated state and to support their expansion. The serum or feeders were replaced by BMPs combined with LIF to sustain self-renewal and preserve multilineage differentiation, chimera colonization, and germline transmission properties of mESCs (95). Various laboratories are able to expand mESCs in vitro by using biomaterials in conjunction with LIF (31,60). Unfortunately, hESCs do not work when supplemented with LIF or BMP; therefore, developing feeder-free culture conditions for hESC expansion has been an active area of research.

The first successful feeder-free culture for hESCs was reported by Xu et al. (91). In their study, they demonstrated a successful feeder-free hES culture system in which undifferentiated cells can be maintained for at least 130 population doublings. In this system, hES cells are grown on culture dishes coated with various biologically active materials such as laminin, collagen, and Matrigel Matrix, with 100% mouse embryonic fibroblast conditioned medium supplemented with serum replacement and growth factors such as FGF. The hES cells maintained on or off feeders express integrin alpha6, and beta1, which may form a laminin-specific receptor. The authors report that the cells retain fundamental characteristics such as normal karyotype, stable proliferation rate, and high telomerase activity of hES cells in this culture system and are suitable for scale-up production. Biomaterials-based expansion of hESCs has now become a distinct possibility, as has large-scale culture of hESCs in bioreactors, which would offer numerous advantages

such as a risk-free environment, ease of scale up, a fully defined microenvironment, and control over biochemical and biomechanical properties (39).

Biomaterials for Differentiation of Stem Cells

Achieving production of specific tissues from stem cells will require precise control of their differentiation. Many factors are involved, both physical and biochemical, as discussed in the aforementioned studies. In this review, our main focus is on chondrogenic and neuron stem cell differentiation and how a biomaterials approach can influence their differentiation.

Biomaterials for Chondrogenic Differentiation of MSCs. Cartilage defects are common features of joint diseases, but current treatments rarely restore the full function of native cartilage (16). A wide spectrum of natural and synthetic biomaterials has been investigated for chondrogenic differentiation of MSCs. Many natural polymers have been studied, including silk (54), cellulose (58), hyaluronan (48), hyaluronic acid (49), agarose (37), and marine sponge fiber skeleton (27). In addition, synthetic hybrid polymers (synthetic and natural polymer blends) and other natural polymers and their derivatives have been tested.

Synthetic polymers. Compared with PLGA scaffolds, PLGA-gelatin/chondroitin/hyaluronate scaffolds have been proven as carriers of autologous MSCs in repairing full-thickness cartilage defects in rabbits (38). Two chondrogenic factors, Dex and TGF-β1, were incorporated into PLGA scaffolds with rabbit MSCs and cultured for 4 weeks. The results show that the scaffolds that included chondrogenic factors strongly up-

regulated the expression of cartilage-specific genes and clearly displayed type-II collagen immunofluorescence (62). Pure poly(epsilon-caprolactone) (PCL) and PCL mixed with PLGA demonstrated chondrogenic potential. Of all materials tested, PCL 7 (70 wt % PCL, 30 wt % PLGA) demonstrated the greatest chondrogenic differentiation potential (14). In another study, MSC/PLGA scaffold composites were pretreated with TGF-β3 before transplantation into 12 rabbits. After 12 weeks, 10 rabbits showed cartilaginous regeneration. Improved hyaline-like cartilage was successfully regenerated and the feasibility of treating damaged articular cartilage determined (30). Richardson et al. demonstrated a biodegradable PLLA scaffold as a potential of chondroactive substrate (69). Guo et al. reported the repair of large articular cartilage defects with implants of autologous MSCs seeded on β-tricalcium phosphate scaffolds (29).

Natural polymers. An injectable, biodegradable hydrogel composite of oligo(poly(ethylene glycol) fumarate) (87) and gelatin microparticles has been investigated for applications in cartilage tissue engineering (62). Another injectable thermosensitive hydrogel with a co-polymer of water-soluble chitosan and poly (*N*-isopropylacrylamide) was developed by Cho et al. When injected into the submucosal layer of the bladder of rabbits, cells entrapped in the co-polymer underwent further chondrogenesis and formed tissue resembling articular cartilage composed of a mixture of hyaline and fibrous cartilage and other tissue components (12).

Neural Stem Cells (NSCs). The central nervous system loses its proliferative potential in mammals. It has limited regenerative capacity when lesions form as a result of

trauma, stroke, neuropathological conditions, or neurodegenerative diseases such as Parkinson disease (79). Tissue engineering with NSCs provides an alternative way to regenerate tissue for the central and peripheral nervous systems (47). Many biomaterial substrates have been developed to culture, transplant, and influence the differentiation and integration of NSCs.

Approaches with biomimetic materials to modulate NSCs responses include scaffold modification with bioactive components, such as proteins, adhesive peptide sequences, and growth factors, as well as modification of the local environment of the transplantation site (67). Li et al. suggest that neurotrophin-3-chitosan maintains the viability of NSCs and increases the percentage of cells that differentiate into neurons (46). As shown by fluorescence image analysis, the efficiency of neuron differentiation on poly(acrylic acid)-grafted carbon nanotube thin films is significantly greater than that on poly(acrylic acid) thin films used for neuron culture. This thin film scaffold shows enhanced neuron differentiation (10). Teng et al. fabricated a bilayered scaffold by using a biodegradable blend of 50:50 PLGA and a block co-polymer of PLGA-polylysine with outer and inner microarchitectures to mimic the white and gray matter of the spinal cord, respectively. NSCs were seeded in the inner layer of the scaffold and inserted into a lateral lesion of the rat spinal cord. Animals implanted with the scaffold-NSC constructs displayed improved recovery of hindlimb locomotor functions compared with controls (81). Interaction feedback occurred between an implanted poly(glycolide)-based scaffold-NSC construct with the brain in a reciprocal manner to mediate repair of an ischemia-induced lesion (63). A self-assembling peptide nanofibrous scaffold designed with the neurite-

promoting laminin epitope IKVAV could rapidly induce differentiation of cells into neurons (78). Eillis-Behnke et al. designed a self-assembling peptide nanofiber scaffold that created a permissive environment for axons not only to regenerate through the site of an acute injury, but also to knit the brain tissue together for tissue repair and restoration. This also raises the possibility of an effective treatment for central nervous system and other tissue or organ trauma (18). Another study focused on collagen type-1 as a scaffold for neural stem/precursor cell (NSPC) transplantation into the injured spinal cord. The optimal conditions for NSPC culture in 3D collagen gel were a cell density between 1×10^7 and 5×10^7cells/ml and a collagen concentration between 0.5 and 0.75 mg/ml. Under these conditions, NSPCs could differentiate into neurons, astrocytes, and oligodendrocytes (88). Understanding neural differentiation and the development of complex neurite networks in 3D matrices is critical for neural tissue engineering in vitro. Heyman et al. described the growth of human stem cell-derived neurons on solid polystyrene matrices. Highly porous foams were prepared from poly (styrene/divinylbenzene) coated with bioactive molecules, including poly-d-lysine and laminin (32). These data clearly demonstrate the potential use of biomaterial scaffolds to promote neurite outgrowth from hESC-derived neurons.

Other Stem/Progenitor Cells With Biomaterials. Recently, many publications have identified interesting subjects of research related to biomaterials and induced stem/progenitor cells. Neovasculogenesis, or the formation of blood vessels postnatally, is now thought to be attributed mainly to the activity of endothelial progenitor cells. Various bioengineering research strategies include linking proteins and peptides on the surface of biomaterials, thereby inducing endothelialization of graft surfaces before implantation or as

a result of accelerating in situ graft endothelialization (15). Many reports have been published about types of biomaterials and combinations of different materials to form new biomaterials. For example, in a canine implantation model, grafts molded from type-I collagen and strengthened with segmented polyurethane film remained patent for up to 3 months. Schmidt and coworkers combined umbilical cord myofibroblasts and endothelial progenitor cells that were seeded on poly(glycolide)/P4HB mesh scaffolds for potential application in pediatric cardiovascular repair (73). The creation of a functional pancreas from stem cells and biomaterials has been an area of active research and may be a promising therapeutic option for future diabetes treatment (21).

CONCLUSIONS

Stem cell research offers enormous prospects in many fields and has opened a new page in which potentially powerful tools can be used for therapeutic application. The convergence of two important disciplines—biomaterials engineering and stem cell research—promises to revolutionize regenerative medicine. Ultimately, despite the optimism, researchers must overcome a number of challenges before this potential is reached. We know that the cellular microenvironment has a significant role in determining progenitor cell fate and function. However, we have little understanding about the specific molecular mechanisms in the cellular microenvironment and the signaling pathways that lead to efficient differentiation of stem cells and tissue formation. A number of biomaterials and biofactors have been investigated as microenvironments for the commitment and differentiation of stem cells. Given the complexity of stem cell control systems, as we learn more about how the microenvironment directs stem cell fate, these factors can be

incorporated into new biomaterial culture conditions to better control stem cell differentiation.

ACKNOWLEDGEMENT: *The authors have no conflict of interest to this review paper. This study is supported in part by the National Science Council (Taiwan) (NSC98-2320-B-039-043-MY2), China Medical University (CMU98-N2-01), and Taiwan Department of Health Clinical Trial and Research Center of Excellence (DOH99-TD-B-111-004).*

REFERENCES

1. Agrawal, C. M.; Ray, R. B. Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. J. Biomed. Mater. Res. 55:141-150; 2001.
2. Bader, R. A.; Kao, W. J. Modulation of the keratinocyte-fibroblast paracrine relationship with gelatin-based semi-interpenetrating networks containing bioactive factors for wound repair. J. Biomater. Sci. Polym. Ed. 20:1005-1030; 2009.
3. Barker, N.; van Es, J. H.; Kuipers, J.; Kujala, P.; van den Born, M.; Cozijnsen, M.; Haegebarth, A.; Korving, J.; Begthel, H.; Peters, P. J. Clevers, H. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature 449:1003-1007; 2007.
4. Bauer, S.; Park, J.; von der Mark, K.; Schmuki, P. Improved attachment of mesenchymal stem cells on super-hydrophobic TiO2 nanotubes. Acta Biomater. 4:1576-1582; 2008.
5. Bianchi, G.; Banfi, A.; Mastrogiacomo, M.; Notaro, R.; Luzzatto, L.; Cancedda, R.; Quarto, R. Ex vivo enrichment of mesenchymal cell progenitors by fibroblast growth factor 2. Exp. Cell Res. 287:98-105; 2003.
6. Brandley, B. K.; Schnaar, R. L. Covalent attachment of an Arg-Gly-Asp sequence peptide to derivatizable polyacrylamide surfaces: support of fibroblast adhesion and long-term growth. Anal. Biochem. 172:270-278; 1988.
7. Brun, P.; Abatangelo, G.; Radice, M.; Zacchi, V.; Guidolin, D.; Daga Gordini, D.; Cortivo, R. Chondrocyte aggregation and reorganization into three-dimensional scaffolds. J. Biomed. Mater. Res. 46:337-346; 1999.

8. Brunstein, C.; Barker, J. N.; Weisdorf, D. J.; DeFor, T. E.; Miller J. S.; Blazar, B.; McGlave, P. B.; Wagner, J. E. Umbilical cord blood transplantation after nonmyeloablative conditioning: impact on transplantation outcomes in 110 adults with hematologic disease. Blood 110:3064-3070; 2007.
9. Chai, C.; Leong, K. W. Biomaterials approach to expand and direct differentiation of stem cells. Mol. Ther. 15:467-480; 2007.
10. Chao, T. I.; Xiang, S.; Chen, C. S.; Chin, W. C.; Nelson, A. J.; Wang, C.; Lu, J. Carbon nanotubes promote neuron differentiation from human embryonic stem cells. Biochem. Biophys. Res. Commun. 384:426-430; 2009.
11. Chien, K. R.; Moretti, A.; Laugwitz, K. L. Development. ES cells to the rescue. Science 306:239-240; 2004.
12. Cho, J. H.; Kim, S. H.; Park, K. D.; Jung, M. C.; Yang, W. I.; Han, S. W.; Noh, J. Y.; Lee, J. W. Chondrogenic differentiation of human mesenchymal stem cells using a thermosensitive poly(N-isopropylacrylamide) and water-soluble chitosan copolymer. Biomaterials 25:5743-5751; 2004.
13. Choueka, J.; Charvet, J. L.; Koval, K. J.; Alexander, H.; James, K. S.; Hooper, K. A.; Kohn, J. Canine bone response to tyrosine-derived polycarbonates and poly(L-lactic acid). J. Biomed. Mater. Res. 31:35-41; 1996.
14. Dawson, E.; Mapili, G.; Erickson, K.; Taqvi, S.; Roy, K. Biomaterials for stem cell differentiation. Adv. Drug Deliv. Rev. 60:215-228; 2008.
15. de Mel, A.; Jell, G.; Stevens, M. M.; Seifalian, A. M. Biofunctionalization of biomaterials for accelerated in situ endothelialization: a review. Biomacromolecules 9:2969-2979; 2008.

16. Djouad, F.; Mrugala, D.; Noel, D.; Jorgensen, C. Engineered mesenchymal stem cells for cartilage repair. Regen. Med. 1:529-537; 2006.
17. Dvir, T.; Benishti, N.; Shachar, M.; Cohen, S. A novel perfusion bioreactor providing a homogenous milieu for tissue regeneration. Tissue Eng. 12:2843-2852; 2006.
18. Ellis-Behnke, R. G.; Liang, Y. X.; You, S. W.; Tay, D. K.; Zhang, S.; So, K. F.; Schneider, G. E. Nano neuro knitting: peptide nanofiber scaffold for brain repair and axon regeneration with functional return of vision. Proc. Natl. Acad. Sci. USA 103:5054-5059; 2006.
19. Elwing, H.; Li, L.; Askendal, A.; Nimeri, G.; Brash, J. Protein displacement phenomena in blood plasma and serum studied by the wettability gradient method and the lens-on-surface method. In: Horbett, T. A.; Brash, J. L., eds. Protein at interfaces II. Fundamentals and applications. Washington, DC: American Chemical Society; 1995:138-149.
20. Evans, M. J.; Kaufman, M. H. Establishment in culture of pluripotential cells from mouse embryos. Nature 292:154-156; 1981.
21. Foster, J. L.; Williams, G.; Williams, L. J.; Tuch, B. E. Differentiation of transplanted microencapsulated fetal pancreatic cells. Transplantation 83:1440-1448; 2007.
22. Freudenberg, U.; Hermann, A.; Welzel, P. B.; Stirl, K.; Schwarz, S. C.; Grimmer, M.; Zieris, A.; Panyanuwat, W.; Zschoche, S.; Meinhold, D. ; Storch, A.;Werner, C. A star-PEG-heparin hydrogel platform to aid cell replacement therapies for neurodegenerative diseases. Biomaterials 30:5049-5060; 2009.

23. Garcia, A. J.; Reyes, C. D. Bio-adhesive surfaces to promote osteoblast differentiation and bone formation. J. Dent. Res. 84:407-413; 2005.

24. Garcia, A. J.; Vega, M. D.; Boettiger, D. Modulation of cell proliferation and differentiation through substrate-dependent changes in fibronectin conformation. Mol. Biol. Cell 10:785-798; 1999.

25. Gerecht, S.; Burdick, J. A.; Ferreira, L. S.; Townsend, S. A.; Langer, R.; Vunjak-Novakovic, G. Hyaluronic acid hydrogel for controlled self-renewal and differentiation of human embryonic stem cells. Proc. Natl. Acad. Sci. USA 104:11298-11303; 2007.

26. Gravel, M.; Gross, T.; Vago, R.; Tabrizian, M. Responses of mesenchymal stem cell to chitosan-coralline composites microstructured using coralline as gas forming agent. Biomaterials 27:1899-1906; 2006.

27. Green, D.; Howard, D.; Yang, X.; Kelly, M.; Oreffo, R. O. Natural marine sponge fiber skeleton: a biomimetic scaffold for human osteoprogenitor cell attachment, growth, and differentiation. Tissue Eng. 9:1159-1166; 2003.

28. Guillot, P.; Cui, W.; Fisk, N.; Polak, D. Stem cell differentiation and expansion for clinical applications of tissue engineering. J. Cell. Mol. Med. 11:935-944; 2007.

29. Guo, X.; Wang, C.; Zhang, Y.; Xia, R.; Hu, M.; Duan, C.; Zhao, Q.; Dong, L.; Lu, J.; Qing Song, Y. Repair of large articular cartilage defects with implants of autologous mesenchymal stem cells seeded into beta-tricalcium phosphate in a sheep model. Tissue Eng. 10:1818-1829; 2004.

30. Han, S. H.; Kim, Y. H.; Park, M. S.; Kim, I. A.; Shin, J. W.; Yang, W. I.; Jee, K. S.; Park, K. D.; Ryu, G. H.; Lee, J. W. Histological and biomechanical properties of

regenerated articular cartilage using chondrogenic bone marrow stromal cells with a PLGA scaffold in vivo. J. Biomed. Mater. Res. A 87:850-861; 2008.

31. Harrison, J.; Pattanawong, S.; Forsythe, J. S.; Gross, K. A.; Nisbet, D. R.; Beh, H.; Scott, T. F.; Trounson, A. O.; Mollard, R. Colonization and maintenance of murine embryonic stem cells on poly(alpha-hydroxy esters). Biomaterials 25:4963-4970; 2004.
32. Hayman, M. W.; Smith, K. H.; Cameron, N. R.; Przyborski, S. A. Growth of human stem cell-derived neurons on solid three-dimensional polymers. J. Biochem. Biophys. Methods 62:231-240; 2005.
33. He, L.; Liao, S.; Quan, D.; Ngiam, M.; Chan, C. K.; Ramakrishna, S.; Lu, J. The influence of laminin-derived peptides conjugated to Lys-capped PLLA on neonatal mouse cerebellum C17.2 stem cells. Biomaterials 30:1578-1586; 2009.
34. Hedberg, E. L.; Kroese-Deutman, H. C.; Shih, C. K.; Crowther, R. S.; Carney, D. H.; Mikos, A. G.; Jansen, J. A. In vivo degradation of porous poly(propylene fumarate)/poly(DL-lactic-co-glycolic acid) composite scaffolds. Biomaterials 26:4616-4623; 2005.
35. Hern, D. L.; Hubbell, J. A. Incorporation of adhesion peptides into nonadhesive hydrogels useful for tissue resurfacing. J. Biomed. Mater. Res. 39:266-276; 1998.
36. Hersel, U.; Dahmen, C.; Kessler, H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomaterials 24:4385-4415; 2003.
37. Huang, C. Y.; Reuben, P. M.; D'Ippolito, G.; Schiller, P. C.; Cheung, H. S. Chondrogenesis of human bone marrow-derived mesenchymal stem cells in agarose culture. Anat. Rec. A Discov. Mol. Cell Evol. Biol. 278:428-436; 2004.

38. Hwang, N. S.; Varghese, S.; Zhang, Z.; Elisseeff, J. Chondrogenic differentiation of human embryonic stem cell-derived cells in arginine-glycine-aspartate-modified hydrogels. Tissue Eng. 12:2695-2706; 2006.

39. Ilic, D. Culture of human embryonic stem cells and the extracellular matrix microenvironment. Regen. Med. 1:95-101; 2006.

40. Kempen, D. H.; Lu, L.; Kim, C.; Zhu, X.; Dhert, W. J.; Currier, B. L.; Yaszemski, M. J. Controlled drug release from a novel injectable biodegradable microsphere/scaffold composite based on poly(propylene fumarate). J. Biomed. Mater. Res. A 77:103-111; 2006.

41. Keselowsky, B. G.; Garcia, A. J. Quantitative methods for analysis of integrin binding and focal adhesion formation on biomaterial surfaces. Biomaterials 26:413-418; 2005.

42. Kim, J.; Park, Y.; Tae, G.; Lee, K. B.; Hwang, C. M.; Hwang, S. J.; Kim, I. S.; Noh, I.; Sun, K. Characterization of low-molecular-weight hyaluronic acid-based hydrogel and differential stem cell responses in the hydrogel microenvironments. J. Biomed. Mater. Res. A 88:967-975; 2009.

43. Kim, M. S.; Hwang, N. S.; Lee, J.; Kim, T. K.; Leong, K.; Shamblott, M. J.; Gearhart, J.; Elisseeff, J. Musculoskeletal differentiation of cells derived from human embryonic germ cells. Stem Cells 23:113-123; 2005.

44. Konno, T.; Kawazoe, N.; Chen, G.; Ito, Y. Culture of mouse embryonic stem cells on photoimmobilized polymers. J. Biosci. Bioeng. 102:304-310; 2006.

45. Levenstein, M. E.; Ludwig, T. E.; Xu, R. H.; Llanas, R. A.; VanDenHeuvel-Kramer, K.; Manning, D.; Thomson, J. A. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. Stem Cells 24:568-574; 2006.

46. Li, X.; Yang, Z.; Zhang, A. The effect of neurotrophin-3/chitosan carriers on the proliferation and differentiation of neural stem cells. Biomaterials 30:4978-4985; 2009.

47. Lindvall, O.; Kokaia, Z. Stem cells for the treatment of neurological disorders. Nature 441:1094-1096; 2006.

48. Lisignoli, G.; Cristino, S.; Piacentini, A.; Toneguzzi, S.; Grassi, F.; Cavallo, C.; Zini, N.; Solimando, L.; Mario Maraldi, N.; Facchini, A. Cellular and molecular events during chondrogenesis of human mesenchymal stromal cells grown in a three-dimensional hyaluronan based scaffold. Biomaterials 26:5677-5686; 2005.

49. Lisignoli, G.; Cristino, S.; Piacentini, A.; Zini, N.; Noel, D.; Jorgensen, C.; Facchini, A. Chondrogenic differentiation of murine and human mesenchymal stromal cells in a hyaluronic acid scaffold: differences in gene expression and cell morphology. J. Biomed. Mater. Res. A 77:497-506; 2006.

50. Liu, H.; Lin, J.; Roy, K. Effect of 3D scaffold and dynamic culture condition on the global gene expression profile of mouse embryonic stem cells. Biomaterials 27:5978-5989; 2006.

51. Luu, Y. K.; Kim, K.; Hsiao, B. S.; Chu, B.; Hadjiargyrou, M. Development of a nanostructured DNA delivery scaffold via electrospinning of PLGA and PLA-PEG block copolymers. J. Control. Release 89:341-353; 2003.

52. Maguire, T.; Novik, E.; Schloss, R.; Yarmush, M. Alginate-PLL microencapsulation: effect on the differentiation of embryonic stem cells into hepatocytes. Biotechnol. Bioeng. 93:581-591; 2006.
53. Mapili, G.; Lu, Y.; Chen, S.; Roy, K. Laser-layered microfabrication of spatially patterned functionalized tissue-engineering scaffolds. J. Biomed. Mater. Res. B Appl. Biomater. 75:414-424; 2005.
54. Meinel, L.; Hofmann, S.; Karageorgiou, V.; Zichner, L.; Langer, R.; Kaplan, D.; Vunjak-Novakovic, G. Engineering cartilage-like tissue using human mesenchymal stem cells and silk protein scaffolds. Biotechnol. Bioeng. 88:379-391; 2004.
55. Mimeault, M.; Batra, S. K. Concise review: recent advances on the significance of stem cells in tissue regeneration and cancer therapies. Stem Cells 24:2319-2345; 2006.
56. Mitjavila-Garcia, M. T.; Simonin, C.; Peschanski, M. Embryonic stem cells: meeting the needs for cell therapy. Adv. Drug Deliv. Rev. 57:1935-1943; 2005.
57. Muggli, D. S.; Burkoth, A. K.; Anseth, K. S. Crosslinked polyanhydrides for use in orthopedic applications: degradation behavior and mechanics. J. Biomed. Mater. Res. 46:271-278; 1999.
58. Muller, F. A.; Muller, L.; Hofmann, I.; Greil, P.; Wenzel, M. M.; Staudenmaier, R. Cellulose-based scaffold materials for cartilage tissue engineering. Biomaterials 27:3955-3963; 2006.
59. Nakanishi, M.; Hamazaki, T. S.; Komazaki, S.; Okochi, H.; Asashima, M. Pancreatic tissue formation from murine embryonic stem cells in vitro. Differentiation 75:1-11; 2007.

60. Nur, E. K. A.; Ahmed, I.; Kamal, J.; Schindler, M.; Meiners, S. Three-dimensional nanofibrillar surfaces promote self-renewal in mouse embryonic stem cells. Stem Cells 24:426-433; 2006.

61. Nuttelman, C. R.; Tripodi, M. C.; Anseth, K. S. Synthetic hydrogel niches that promote hMSC viability. Matrix Biol. 24:208-218; 2005.

62. Park, H.; Guo, X.; Temenoff, J. S.; Tabata, Y.; Caplan, A. I.; Kasper, F. K.; Mikos, A. G. Effect of Swelling Ratio of Injectable Hydrogel Composites on Chondrogenic Differentiation of Encapsulated Rabbit Marrow Mesenchymal Stem Cells In Vitro. Biomacromolecules 10:541–546; 2009.

63. Park, K. I.; Teng, Y. D.; Snyder, E. Y. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat. Biotechnol. 20:1111-1117; 2002.

64. Pierschbacher, M. D.; Ruoslahti, E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 309:30-33; 1984.

65. Pierschbacher, M. D.; Ruoslahti, E. Variants of the cell recognition site of fibronectin that retain attachment-promoting activity. Proc. Natl. Acad. Sci. USA 81:5985-5988; 1984.

66. Polak, J. M.; Mantalaris, S. Stem cells bioprocessing: an important milestone to move regenerative medicine research into the clinical arena. Pediatr. Res. 63:461-466; 2008.

67. Potter, W.; Kalil, R. E.; Kao, W. J. Biomimetic material systems for neural progenitor cell-based therapy. Front. Biosci. 13:806-821; 2008.

68. Reddi, A. H. Bone morphogenetic proteins: from basic science to clinical applications. J. Bone Joint Surg. Am. 83-A Suppl 1(Pt 1):S1-6; 2001.

69. Richardson, S. M.; Curran, J. M.; Chen, R.; Vaughan-Thomas, A.; Hunt, J. A.; Freemont, A. J.; Hoyland, J. A. The differentiation of bone marrow mesenchymal stem cells into chondrocyte-like cells on poly-L-lactic acid (PLLA) scaffolds. Biomaterials 27:4069-4078; 2006.

70. Richardson, T. P.; Peters, M. C.; Ennett, A. B.; Mooney, D. J. Polymeric system for dual growth factor delivery. Nat. Biotechnol. 19:1029-1034; 2001.

71. Rippon, H. J.; Polak, J. M.; Qin, M.; Bishop, A. E. Derivation of distal lung epithelial progenitors from murine embryonic stem cells using a novel three-step differentiation protocol. Stem Cells 24:1389-1398; 2006.

72. Schek, R. M.; Wilke, E. N.; Hollister, S. J.; Krebsbach, P. H. Combined use of designed scaffolds and adenoviral gene therapy for skeletal tissue engineering. Biomaterials 27:1160-1166; 2006.

73. Schmidt, D.; Mol, A.; Neuenschwander, S.; Breymann, C.; Gossi, M.; Zund, G.; Turina, M.; Hoerstrup, S. P. Living patches engineered from human umbilical cord derived fibroblasts and endothelial progenitor cells. Eur. J. Cardiothorac. Surg. 27:795-800; 2005.

74. Schuldiner, M.; Yanuka, O.; Itskovitz-Eldor, J.; Melton, D. A.; Benvenisty, N. Effects of eight growth factors on the differentiation of cells derived from human embryonic stem cells. Proc. Natl. Acad. Sci. USA 97:11307-11312; 2000.

75. Shamblott, M. J.; Axelman, J.; Wang, S.; Bugg, E. M.; Littlefield, J. W.; Donovan, P. J.; Blumenthal, P. D.; Huggins, G. R.; Gearhart, J. D. Derivation of pluripotent

stem cells from cultured human primordial germ cells. Proc. Natl. Acad. Sci. USA 95:13726-13731; 1998.

76. Shin, H.; Jo, S.; Mikos, A. G. Biomimetic materials for tissue engineering. Biomaterials 24:4353-4364; 2003.
77. Shin, H.; Zygourakis, K.; Farach-Carson, M. C.; Yaszemski, M. J.; Mikos, A. G. Attachment, proliferation, and migration of marrow stromal osteoblasts cultured on biomimetic hydrogels modified with an osteopontin-derived peptide. Biomaterials 25:895-906; 2004.
78. Silva, G. A.; Czeisler, C.; Niece, K. L.; Beniash, E.; Harrington, D. A.; Kessler, J. A.; Stupp, S. I. Selective differentiation of neural progenitor cells by high-epitope density nanofibers. Science 303:1352-1355; 2004.
79. Snyder, B. J.; Olanow, C. W. Stem cell treatment for Parkinson's disease: an update for 2005. Curr. Opin. Neurol. 18:376-385; 2005.
80. Tabata, Y. Biomaterial technology for tissue engineering applications. J. R. Soc. Interface 6 Suppl 3:S311-324; 2009.
81. Teng, Y. D.; Lavik, E. B.; Qu, X.; Park, K. I.; Ourednik, J.; Zurakowski, D.; Langer, R.; Snyder, E. Y. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc. Natl. Acad. Sci. USA 99:3024-3029; 2002.
82. Thomson, J. A.; Itskovitz-Eldor, J.; Shapiro, S. S.; Waknitz, M. A.; Swiergiel, J. J.; Marshall, V. S.; Jones, J. M. Embryonic stem cell lines derived from human blastocysts. Science 282:1145-1147; 1998.

83. Tsutsumi, S.; Shimazu, A.; Miyazaki, K.; Pan, H.; Koike, C.; Yoshida, E.; Takagishi, K.; Kato, Y. Retention of multilineage differentiation potential of mesenchymal cells during proliferation in response to FGF. Biochem. Biophys. Res. Commun. 288:413-419; 2001.

84. Vacanti, J. P.; Langer, R. Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation. Lancet 354 Suppl 1:SI32-34; 1999.

85. Vallier, L.; Rugg-Gunn, P. J.; Bouhon, I. A.; Andersson, F. K.; Sadler, A. J.; Pedersen, R. A. Enhancing and diminishing gene function in human embryonic stem cells. Stem Cells 22:2-11; 2004.

86. Van Zant, G.; Rummel, S. A.; Koller, M. R.; Larson, D. B.; Drubachevsky, I.; Palsson, M.; Emerson, S. G. Expansion in bioreactors of human progenitor populations from cord blood and mobilized peripheral blood. Blood Cells 20:482-490; discussion 491; 1994.

87. von Burkersroda, F.; Schedl, L.; Gopferich, A. Why degradable polymers undergo surface erosion or bulk erosion. Biomaterials 23:4221-4231; 2002.

88. Watanabe, K.; Nakamura, M.; Okano, H.; Toyama, Y. Establishment of three-dimensional culture of neural stem/progenitor cells in collagen Type-1 Gel. Restor. Neurol. Neurosci. 25:109-117; 2007.

89. Watt, F. M.; Lo Celso, C.; Silva-Vargas, V. Epidermal stem cells: an update. Curr. Opin. Genet. Dev. 16:518-524; 2006.

90. Williams, C. G.; Kim, T. K.; Taboas, A.; Malik, A.; Manson, P.; Elisseeff, J. In vitro chondrogenesis of bone marrow-derived mesenchymal stem cells in a photopolymerizing hydrogel. Tissue Eng. 9:679-688; 2003.
91. Xu, C.; Inokuma, M. S.; Denham, J.; Golds, K.; Kundu, P.; Gold, J. D.; Carpenter, M. K. Feeder-free growth of undifferentiated human embryonic stem cells. Nat. Biotechnol. 19:971-974; 2001.
92. Yang, J.; Shi, G.; Bei, J.; Wang, S.; Cao, Y.; Shang, Q.; Yang, G.; Wang, W. Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic-co-glycolic acid) (70/30) cell scaffolds for human skin fibroblast cell culture. J. Biomed. Mater. Res. 62:438-446; 2002.
93. Yang, T.; Tsang, K. S.; Poon, W. S.; Ng, H. K. Neurotrophism of bone marrow stromal cells to embryonic stem cells: noncontact induction and transplantation to a mouse ischemic stroke model. Cell Transplant. 18:391-404; 2009.
94. Yang, X. B.; Roach, H. I.; Clarke, N. M.; Howdle, S. M.; Quirk, R.; Shakesheff, K. M.; Oreffo, R. O. Human osteoprogenitor growth and differentiation on synthetic biodegradable structures after surface modification. Bone 29:523-531; 2001.
95. Ying, Q. L.; Nichols, J.; Chambers, I.; Smith, A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. Cell 115:281-292; 2003.
96. Zaehres, H.; Lensch, M. W.; Daheron, L.; Stewart, S. A.; Itskovitz-Eldor, J.; Daley, G. Q. High-efficiency RNA interference in human embryonic stem cells. Stem Cells 23:299-305; 2005.

97. Zhang, G.; Wang, X.; Wang, Z.; Zhang, J.; Suggs, L. A PEGylated fibrin patch for mesenchymal stem cell delivery. Tissue Eng. 12:9-19; 2006.
98. Zou, X. H.; Cai, H. X.; Yin, Z.; Chen, X.; Jiang, Y. Z.; Hu, H.; Ouyang, H. W. A novel strategy incorporated the power of mesenchymal stem cells to allografts for segmental bone tissue engineering. Cell Transplant. 18:433-441; 2009.

Figure legends

Figure 1. Provides a schematic representation how biomaterials in incorporating with stem cells. Derived stem cells can be maintained and expanded by biomaterials-base culture methods. Interactions between scaffolds, bioactive factors include peptides, protein, biofactors and stem cells would result ECM synthesis, stem cells differentiation and cell-organization to be special tissue construct. Biomaterials can be tailored by many ways to differentiate stem cells according intended therapy.

Figure 2. Examples of various techniques include chemical, physical ways to modify biomaterials for SC culture and differentiated SC as well as their specific applications.

Figure 1.

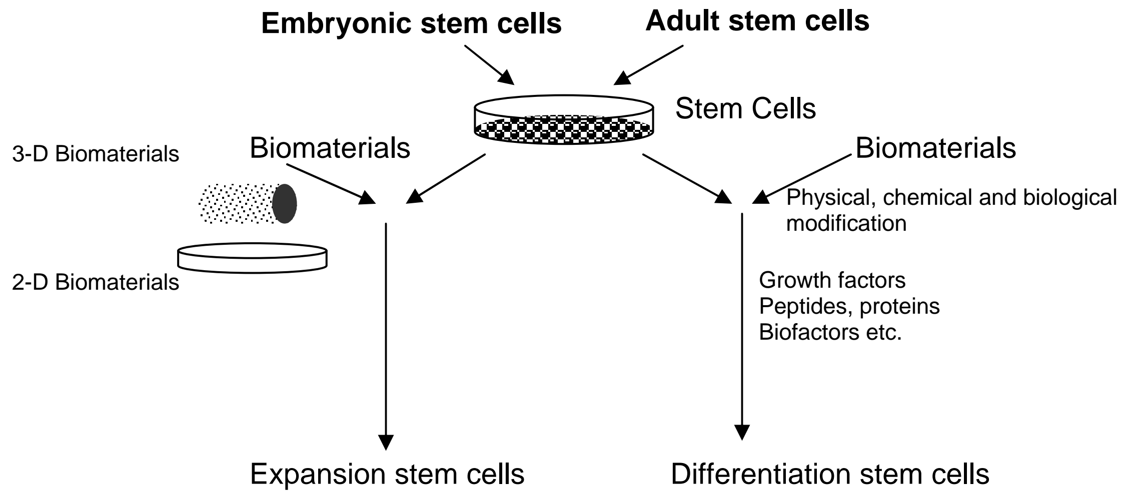

Figure 2.