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SPECIAL ISSUE

Modulation of Stem Cell Differentiation with Biomaterials

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Differentiation of stem cells can be controlled with interactions with microenvironments of the stem cells. The interactions contain various signals including soluble growth factor signal, cell adhesion signal, and mechanical signal, which can modulate differentiation of stem cells. Biomaterials can provide these types of signals to induce desirable cellular differentiation. Biomaterials can deliver soluble growth factors locally to stem cells at a controlled rate for a long period. Stem cell adhesion to specific adhesion molecules presented by biomaterials can induce specific differentiation. Mechanical signals can be delivered to stem cells seeded onto biomaterial scaffolds. These approaches would be invaluable for direction of stem cell differentiation and *in vivo* tissue regeneration using stem cells.

Keywords: Biomaterial, Differentiation, Growth factor, Scaffold, Stem cells

Introduction

Stem cells have potential to differentiate into various types of cells and demonstrate capability of regenerating various tissues both experimentally and clinically. Differentiation of stem cells can be modulated by various signals, including soluble growth factor signal, cell adhesion signal, and mechanical signal. These signals can be incorporated into biomaterials to induce desirable cellular differentiation.

Cell adhesion ligand presentation by biomaterials

Cells adhere to scaffolds based on interactions between cell surface receptors (i.e., integrin) and cell adhesion peptides (e.g., arginine-glycine-aspartic acid (RGD), an integrin-binding peptide found in fibronectin, laminin and collagen). Stem cell adhesion based on interactions between specific integrin and adhesion peptide can modulate stem cell behavior. Scaffolds derived from natural polymers such as collagen contain cell adhesion peptides that allow the direct adhesion of cells. In contrast, scaffolds derived from synthetic polymers do not contain cell adhesion peptides. Thus, cells adhere to synthetic polymers through conjugated cell adhesion peptides or through cell adhesion proteins (e.g., fibronectin, vitronectin, collagen and laminin) adsorbed on the polymers from serum. Although the adsorption of cell adhesion proteins containing cell adhesion peptides to scaffold surfaces facilitates cell adhesion, desorption of the proteins as well as poor reproducibility of the protein adsorption process can limit their applicability. Covalent conjugation of cell adhesion peptides to scaffolds would be preferential, since it is more reproducible and can control the density of peptide conjugation. Therefore, stem cell adhesion to specific peptides conjugated to the polymers of scaffolds can modulate cell behavior.

Cell adhesion peptide presentation by scaffolds can promote stem cell differentiation. RGD peptides covalently conjugated to polyethylene glycol (PEG) diacrylate hydrogels promoted the osteogenic differentiation of human mesenchymal stem cells (MSCs) (1). RGD peptide presentation by oligo (PEG-fumarate) hydrogels also promoted osteogenic differentiation of MSCs (2). The osteogenic differentiation was dependent on the concentration

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of the RGD peptide. The higher RGD peptide concentration showed significantly greater alkaline phosphatase activity of the MSCs. RGD peptide incorporation also stimulated chondrogenic differentiation of stem cells. Compared to PEG diacrylate hydrogels, RGD peptide-conjugated PEG diacrylate hydrogels exerted enhanced gene expression of cartilage-specific markers (i.e., aggrecan and collagen type II) of human embryonic stem cells (ESCs) (3).

Soluble factor delivery using biomaterials

Growth factors entrapped in delivery vehicle made of biomaterials can be controlled released via diffusion through or degradation of delivery systems, or even a combination of these two mechanisms (4, 5). Vascular endothelial growth factor (VEGF) released from a hydrogel delivery system induced vascular differentiation of human ESCs (6). Insulin-like growth factor-1 and transforming growth factor-β1 (TGF-β1) released from gelatin microparticles induced chondrogenic differentiation of MSCs (7).

Growth factor protein delivery systems using polymers often have difficulty preserving growth factor bioactivity. The loss of protein bioactivity results mainly from denaturation of the protein during the protein loading process of the polymer carrier (8). When exposed to harsh environments, such as heat and exposure to sonication and organic solvents, a growth factor protein is often denatured and loses its biological activity (9). The affinity of heparin for scaffolds thus avoids the use of organic solvents, which are generally used for the incorporation of growth factors into polymeric delivery systems and may be detrimental to the bioactivity of loaded growth factor proteins. An additional benefit to using heparin in growth factor delivery systems could be the delivery of dual or multiple growth factors with high affinities for heparin, including VEGF, fibroblast growth factor 2 (FGF2) and platelet-derived growth factor (PDGF), that are capable of synergistically enhancing tissue regeneration, as in osteogenesis (10, 11). Delivery of multiple soluble factors (neurotrophin-3, PDGF and sonic hedgehog) using heparin-conjugated fibrin has been performed to induce differentiation of ESC-derived neuronal progenitor cells into neurons and oligodendrocytes (12). Moreover, heparin itself contained in the delivery system can potentiate the bioactivity of growth factors such as bone morphogenetic protein 2 (BMP-2) (13).

Dual or multiple growth factors can be delivered using biomaterials for the enhancement of stem cell-mediated tissue regeneration. Bone formation by osteogenic precursor cells can be enhanced by BMP signaling as well as by molecules initiating vascularization. Previously, Mooney and colleagues showed that the combined delivery of BMP-4 and VEGF enhanced bone formation driven by human MSCs (14). The combined delivery of BMP-4, VEGF and MSCs resulted in much more extensive bone formation than combined delivery of either BMP-4/MSCs or VEGF/MSCs. Controlled delivery of dual growth factors with different release kinetics has been shown to promote blood vessel function (4). Dual delivery of VEGF and PDGF-BB using a polymeric delivery system with distinct kinetics for each growth factor resulted in more effective neovascularization than delivery of either growth factor. Another study showed that dual delivery of FGF2 and PDGF-BB resulted in formation of neovessels that remain stable for a much longer period than delivery of either growth factor individually (15).

The local delivery of soluble factors using biomaterials to stem cells transplanted for tissue regeneration may induce *in situ* differentiation. This approach would allow tissue regeneration through transplantation of undifferentiated stem cells and would eliminate the *in vitro* culture of stem cells prior to transplantation. For bone regeneration, osteogenically differentiated bone marrow-derived MSCs have traditionally been utilized for transplantation more than undifferentiated bone marrow-derived MSCs (16, 17). Previous studies have demonstrated that transplantation of MSCs that were induced to differentiate into osteoblasts by dexamethasone resulted in more extensive *in vivo* bone formation than transplantation of undifferentiated MSCs (18). However, *in vitro* osteogenic differentiation requires additional culturing, which would increase the treatment cost and therefore not be appropriate for the treatment of urgent patients. Kim and colleagues demonstrated that human MSCs, isolated from bone marrow, cord blood and adipose tissue, that are not osteogenically differentiated prior to transplantation, can extensively regenerate bone *in vivo* when exogenous BMP-2 is delivered locally to the transplantation site using a polymeric delivery system (19, 20). In their study, a heparin-conjugated poly(L-lactic-co-glycolic acid) scaffold (21) was used as a localized, timed delivery system for biologically active BMP-2 at the bone regeneration site. Human MSCs cultured on BMP-2-loaded scaffolds underwent osteogenic differentiation *in vitro*. Upon implantation, undifferentiated MSCs on BMP- 2-loaded scaffolds induced extensive *in vivo* bone formation compared to both undifferentiated MSCs on BMP-2-unloaded scaffolds and osteogenically differentiated MSCs on BMP-2-unloaded scaffolds (20). This method does not require additional culturing for either stem cell differ-

entiation or the treatment of urgent patients.

The response of stem cells to growth factors could vary depending on the culture conditions. Most studies investigating the stem cell response to growth factors have been performed on two-dimensional (2D) culture plates. However, stem cell culture in a 2D environment does not mimic an *in vivo* environment. In 2D culture, cells interact mainly with the culture surface. In three-dimensional (3D) culture, however, cell-cell interactions and cell-extracelluar matrix interactions are promoted. Thus, 2D culture may not result in efficient control of stem cells in response to growth factors. Embryoid bodies (EBs) cultured in 2D and 3D culture systems respond to growth factors differently depending on the culture system (22). Furthermore, in 2D culture, BMP-2 promotes the aggrecan expression of EBs, whereas TGF-β1 promotes the aggrecan expression of EBs in 3D hydrogel culture.

Mechanical signal delivery using biomaterials

Cells in tissues or organs that are in mechanically dynamic movements are subjected to various forms of mechanical forces *in vivo*. Thus, mechanical signals can influence stem cell differentiation. For example, shear force (14) or cyclic strain (23) induces vascular differentiation of ESCs. Biomaterials acting as a cell adhesion substrate can be used to deliver mechanical signals to cells seeded on the biomaterials. Mechanical forces applied to biomaterial scaffolds can be transmitted to the seeded cells via cell surface receptors (i.e., integrin), which serve as mechanical transducers between the biomaterial scaffolds and internal cytoskeleton of the cell. The transmitted mechanical signals are converted into biochemical responses and activate specific genes that modulate stem cell differentiation (24).

Mechanical signal alone, without soluble growth factor signal, can induce the differentiation of stem cells or progenitor cells. A study has demonstrated that cyclic strain can promote cardiomyogenesis of embryonic stem cell-derived cardiomyocytes (ESCCs) (25). ESCCs cultured on elastic polymer [poly(lactide-co-caprolactone), PLCL] scaffolds and subjected to cyclic strain *in vitro* upregulated cardiac gene expression compared to unstrained controls. Upon implantation of ESCCs seeded on elastic scaffolds or non-elastic scaffolds onto the surface of rat beating heart, cardiac gene expression was upregulated in the elastic scaffolds compared to unstrained control scaffolds, suggesting enhanced cardiomyogenesis by cyclic strain.

Cells in knee cartilage are exposed to cyclic compressional forces *in vivo*. Thus, cyclic compression can promote the chondrogenic differentiation of stem cells. Hydrogels can be used as a scaffold for the transduction of compressive mechanical signals to cells. Bone marrow-derived MSCs were cultured in PEG diacrylate hydrogels and application of cyclic compressive strain to the hydrogels *in vitro* upregulated the gene expression of cartilage-related markers such as Sox-9, type II collagen and aggrecan even in the absence of TGF-beta1 (26).

Combination of mechanical signals and soluble signals may stimulate the differentiation of stem cells at higher extent than either mechanical signals or soluble signals do. Either cyclic strain (25) or TGF-β1 (27) alone can stimulate the cardiomyogenic differentiation of stem cells. A study has shown that combination of TGF-β1 supplementation and cyclic strain application upregulated the expression of cardiac-specific markers in MSCs than TGF-β1 supplementation alone (28). The combination of cyclic strain and TGF-β1 induced the expression of troponin-I or cardiac myosin heavy chain in an average of 44.5% of treated MSCs (28). Previous studies reported that cardiomyogenic differentiation of MSCs can be achieved by co-culturing with rat cardiomyocytes (29, 30) or treatment with 5-azacytidine (31). In comparison, only 1.9% of MSCs co-cultured with cardiomyocytes were troponin-I-positive, and the population of cardiomyocytes in 5-azacytidine-exposed cells was less than 30% of spontaneously beating clones screened from bone marrow cell cultures.

Biomaterial architecture

Microfabrication techniques for the fabrication of micropatterned culture surfaces have enabled the study of stem cells behavior in response to cell aggregation. ESCs can differentiate in various types of tissues or organs by forming EBs (32). However, the inability to homogeneously direct ESC lineage commitment limits the clinical application of ESCs. One reason for the heterogeneity of ESC lineage commitment is the wide variation in EB size (33). Precise control of EB size can precisely modulate ESC differentiation. Conventional techniques for the formation of EBs, such as hanging drop and suspension cultures, do not allow for precise control of EB size. Uniformly sized EB formation has been achieved using a microwell array system created by photolithography and plasma etching techniques (34). EBs were formed by culture human ESCs in the microwells, and 78% of the EBs harvested from the culture were 280 to 359 μm in size. By comparison, only 31% of EBs formed by culture on plastic were of the same size range. Precise control of EB

size using a microwell array system can regulate ESC lineage commitment. Culture of mouse ESCs in non-adhesive PEG hydrogel microwells of various diameter resulted in homogeneous formation of EBs with different sizes (35). The resulting ESC differentiation was highly size-dependent; for example, cardiogenic differentiation was dominant in larger EBs (450 μm in diameter). In contrast, endothelial cell differentiation was enhanced in smaller EBs (150 μm in diameter). EB size-mediated differentiation was due to the differential expression of WNT5a and WNT11, two members of the noncanonical WNT pathway. Another method for the production of uniformly-sized EBs involves microfabricated adhesive stencils (36). EBs with sizes ranging from 100 to 500 μm in diameter were formed using this method, and EB size was found to influence EB lineage commitment. Smaller (100 μm) EBs exhibited increased expression of ectodermal markers compared to larger (500 μm) EBs, whereas the 500 μm-sized EBs showed increased expression of mesodermal and endodermal markers compared to the 100 μm-sized EBs.

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Potential conflict of interest

The authors have no conflicting financial interest.

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