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REVIEW ARTICLE

Biomimetic Polymer Scaffolds to Promote Stem Cell-Mediated Osteogenesis

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Bone tissue engineering using stem cells with osteogenic potential is a promising avenue of research for bone defect reconstruction. Organic, inorganic, and composite scaffolds have all been engineered to provide biomimetic microenvironments for stem cells. These scaffolds are designed to promote stem cell osteogenesis. Here, we review current technologies for developing biomimetic, osteoinductive scaffolds for stem cell applications. We summarize the reported *in vitro* and *in vivo* osteogenic effects of these scaffolds on stem cells.

Keywords: Bone tissue engineering, Biomimetic scaffolds, Stem cells, Osteoinductivity

Introduction

Regeneration of bone defects caused by an acquired injury or inherent genetic disorder is a challenge in the clinic. However, bone tissue engineering provides promising solutions for reconstructing bone tissue, some of which could replace conventional bone cement and metal implant treatments that lack osteogenic activity (1). Primary osteogenic cells (e.g., osteoblasts) are major cell sources for bone tissue engineering; however, stem cells have been shown to readily undergo osteogenic differentiation due to their high proliferative and differentiation potential (2-5). Multipotent mesenchymal stem cells (MSCs) isolated from autologous sources such as the bone marrow and adipose tissue are the most commonly used stem cells for bone tissue engineering (4-6). Pluripotent stem cells, including embryonic stem cells (ESCs) (7) and induced pluripotent stem cells (8), can also produce mesenchymal lineage cells capable of further differentiation into osteogenic cells. Thus, they can also contribute to bone formation in the bone defects.

Despite the potential of stem cells to undergo osteogenesis, bone formation by stem cell transplantation can be further improved with functional biomaterial scaffolds. Highly osteoinductive biomaterial scaffolds can promote osteogenesis of transplanted stem cells by stimulating osteogenic signaling pathways that enhance osteogenic differentiation (9, 10). Osteoinductive scaffolds should be fabricated to mimic native bone tissue morphology, structure, and biochemical properties (11). Fabrication methods such as electrospinning and patterning have been used to prepare functional scaffolds capable of providing biophysical cues for improving cellular alignment and providing mechanical signals to promote bone formation (12-14). Biomimetic functional scaffolds can be developed by modifying scaffolds with osteoinductive, bioactive molecules including inorganic particulates and osteogenic growth factors/peptides and by incorporating these molecules into the scaffolds (10, 13, 15). This type of direct and indirect scaffold modification provides biochemical cues for promoting stem cell osteogenic commitment.

In this article, we review various strategies for creating biomimetic functional scaffolds that emulate the biochemical and biophysical signals as well as the mechanical properties of native bone tissue. Studies that describe

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highly osteoinductive scaffolds for enhanced stem cell-mediated osteogenesis *in vitro* and *in vivo* are also discussed.

Biomimetic Hydrogels

Hydrogels have shown great potential as scaffolds for bone tissue engineering (16-20). They are typically injectable and can form three-dimensional (3D) extracellular matrix (ECM) structures *in situ* within irregular bone defects (21). A range of molecules with osteogenic activity (e.g., growth factors, peptides, and chemicals) can be easily incorporated into or conjugated to the hydrogel for enhanced osteoinductivity (21). Internal structures and mechanical properties of hydrogels are easily modulated by selecting a polymer with appropriate properties and molecular weight and by altering the concentration the polymer solution (21). Therefore, hydrogel constructs can be tailored to enhance stem cell osteogenesis by optimizing the biochemical and biophysical environment to create an *in vivo*-like microenvironment that closely resembles the osteogenic stem cell niche.

One recently developed approach for preparing biomimetic bone tissue engineering hydrogels is through chemical covalent modification of the hydrogel construct to enhance the osteogenic potential of stem cells. Techniques for covalent modification include attachment of various ECM peptides, proteins, and chemical functional groups to the hydrogel backbone (22-25). Benoit et al. reported that charged phosphate groups responsible for mineralization and sequestering of osteopontin bound to poly (ethylene glycol) (PEG) hydrogels effectively enhance MSC osteogenesis (23). This same research group recently reported that phosphate-functionalization of PEG hydrogels promotes serum ECM protein adsorption, and thereby, contributes to enhanced osteogenic differentiation of human MSCs (hMSCs) (26). Another study performed by Kisiel et al. demonstrated that covalent grafting of an integrin-specific fibronectin fragment to hyaluronic acid (HA) remarkably improved MSC attachment and spreading on the HA hydrogel (27). They show that the modified hydrogel enhanced the osteogenic potential of bone morphogenetic protein-2 (BMP-2) compared to non-functionalized HA hydrogels (27). Chemical grafting of BMP-2 mimetic peptides to poly(acrylamide-co-acrylic acid) hydrogels, of varying stiffness, was also shown to modulate the fate of MSCs (28). BMP-2 peptides on stiff hydrogel matrices were most effective at enhancing osteogenic differentiation of MSCs (28).

Physical entrapment of ECM components in biomimetic hydrogels can also promote stem cell osteogenesis. To create these hydrogels, ECM proteins are simply entrapped with stem cells in the hydrogel constructs during cell encapsulation to provide a biomimetic microenvironment for stem cell differentiation. Hwang et al. encapsulated MSCs into PEG-based hydrogels supplemented with ECM proteins collagen type I, collagen type II, or HA; they reported that PEG hydrogels containing HA efficiently induced osteogenic differentiation of MSCs (29). Yang et al. described a combinatorial 3D ECM approach for creating type I collagen hydrogels supplemented with additional ECM components (fibronectin and/or laminin) at different concentrations for human ESC (hESC) differentiation (30). The approach was used to identify optimal ECM hydrogel compositions for promoting osteogenic differentiation of hESCs (30).

Incorporation of osteoinductive factors BMP and ceramics can also produce highly osteogenic hydrogel scaffolds that enhance stem cell-mediated bone formation (31-34). Thermo-reversible hydrogels containing hydroxyapatite and BMP-2 were found to increase MSC alkaline phosphatase activity, osteogenic marker gene expression, mineralization, and ectopic bone formation in the subcutaneous space of mice (32). Phadke et al. reported a mineralized hydrogel containing both organic and inorganic components that supported efficient hMSC adhesion, spreading, and proliferation (35). This PEG-based hydrogel significantly promoted hMSC osteogenic differentiation because of chemical topological cues originating from calcium phosphate bound to the hydrogel construct (35).

Biomimetic Nanofibrous Scaffolds

Fibrous polymer scaffolds that mimic the alignment of natural ECM proteins can be fabricated by electrospinning. Electrospun nanofibrous scaffolds are effective for tissue engineering of various types of tissues (36-38), including bone (39, 40). This method is a simple, efficient way to create 3D scaffolds with micro- or nanoscale fibrous structures using various types of synthetic or natural polymers (41, 42). With this method, fiber diameter and alignment is easily controlled by modulating polymer type and concentration. Promotion of cell alignment and orientation by electrospun nanofibrous structures induces favorable cell-cell and cell-matrix interactions for cell differentiation and tissue morphogenesis (43, 44).

Electrospun nanofibrous polymer scaffolds have been successfully used to enhance stem cell-mediated osteogenesis by providing ECM-like topography for stem cells.

In fact, one such scaffold, a highly porous, electrospun nanofibrous scaffold made of biodegradable poly (ε-caprolactone) seeded with rat MSCs was maintained in a rotating bioreactor for 4 weeks (45). This scaffold facilitated stem cell osteogenesis after implantation into rat omentum for an additional 4 weeks (45). At the end of the 4 weeks, the tissue constructs with bone-like appearance were retrieved. Mineralization and bone-specific matrix deposition were detected in the polymer constructs (45). Xin et al. also demonstrated the usefulness of electrospun poly(lactide-co-glycolide) nanofibers for stem cell-mediated osteogenesis by showing that these scaffolds supported growth and osteogenic differentiation of hMSCs (46). Electrospun nanofibrous scaffolds made of type I collagen also exhibited fiber diameter-dependent control over adhesion, growth, and osteogenic differentiation of hMSCs (47).

As previously mentioned, the nanofiber structure itself can induce osteogenic differentiation of stem cells, but biomimetic modification of nanofiber scaffolds is expected to further enhance stem cell osteogenic potential. In fact, Ko et al. showed that a combination of inorganic components and a polymer nanofiber construct could enhance osteogenic differentiation of hMSCs (48). Additionally, nanofibrous poly(L-lactic acid) (PLLA) composite scaffolds containing demineralized bone powder induced greater bone regeneration in critical-sized calvarial bone defects than PLLA scaffolds without demineralized bone powder (48). Schofer et al. also reported that BMP-2-incoporated PLLA nanofiber scaffolds could improve *in vitro* osteogenic differentiation of MSCs (49). Electrospun silk fibroin nanofiber scaffolds containing BMP-2 and inorganic hydroxyapatite particulates induced higher calcium deposition and increased transcript levels of bone-specific markers in hMSCs compared to control scaffolds *in vitro* (50).

Micro/Nanopatterned Matrices

Topographical features of tissue engineered substrates and scaffolds have recently been highlighted for their roles in controlling stem cell proliferation and differentiation. Micro- and nanoscale topographical cues modulate focal adhesion of stem cells, and accordingly, affect integrin clustering and cytoskeleton reorganization, which may result in alterations to cytoskeletal tension and mechanosensitive signaling pathways (51). These cues may alter transcription of genes responsible for proliferation and lineage specification of stem cells, leading to altered phenotype, morphology, and function (51-53). Functional substrates and scaffolds for stem cell-based bone tissue engineering should be designed to mimic the ECM's topographical cues to allow for enhanced osteogenic differentiation.

Micro- and nanoscale patterned surfaces with specific shapes and dimensions promote osteogenic differentiation of stem cells. Dalby et al. demonstrated that nanopatterned polymer surfaces, especially those patterned in a random manner, stimulated hMSCs to express bone-specific genes and produce bone mineral, even in the absence of osteogenesis-inducing factors (54). Watari et al. also reported modulation of hMSC osteogenic differentiation by submicron scale patterned grooves and ridges (55). Culturing of hMSCs on patterned substrates was found to upregulate the expression of osteogenic markers and downstream regulators of BMP pathway in hMSCs, irrespective of osteogenic factor addition (55). One recent study demonstrated a synergistic effect of nanoscale titanium surface geometry and immobilized BMP-2 on stem cell osteogenic differentiation (56). Therefore, a combination of biomimetic surface topography and biochemical cues could be considered for development of highly osteoinductive scaffolds to improve stem cell osteogenesis.

Conclusions

In this review, we discuss various biomimetic polymer scaffolds developed to improve the efficacy of stem cell-mediated bone tissue engineering. These scaffolds show enhanced osteoinductive potential and are effective and functional in both *in vitro* and *in vivo* experiments on osteogenic differentiation and bone regeneration with stem cells. A mechanistic study of the beneficial effects of these scaffolds on stem cell-mediated osteogenesis would provide valuable information to aid in design of the next generation of highly osteoinductive scaffolds capable of maximizing stem cell-mediated bone formation.

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Potential conflict of interest

The authors have no conflicting financial interest.

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