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Biomimetic Scaffolds for Osteogenesis

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Abstract

Skeletal regenerative medicine emerged as a field of investigation to address large osseous deficiencies secondary to congenital, traumatic, and post-oncologic conditions. Although autologous bone grafts have been the gold standard for reconstruction of skeletal defects, donor site morbidity remains a significant limitation. To address these limitations, contemporary bone tissue engineering research aims to target delivery of osteogenic cells and growth factors in a defined three dimensional space using scaffolding material. Using bone as a template, biomimetic strategies in scaffold engineering unite organic and inorganic components in an optimal configuration to both support osteoinduction as well as osteoconduction. This article reviews the various structural and functional considerations behind the development of effective biomimetic scaffolds for osteogenesis and highlights strategies for enhancing osteogenesis.

Keywords

Osteogenesis; scaffolds; biomimetic; bone tissue engineering

Introduction

Large bony defects secondary to traumatic, congenital, and post-oncologic causes remain a clinical challenge. Although autologous vascularized or non-vascularized bone grafts are currently the standard for replacing osseous defects, bone harvest is known to cause significant donor site morbidity[1–3]. Bone tissue engineering offers a promising alternative. The field of bone tissue engineering aims to create bone graft substitutes that confer the benefits of bone autografts without the associated donor site morbidity. These biomaterials

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should mimic native bone in terms of their mechanical properties as well as their osteoinductive and osteoconductive characteristics.[4,5] Potential alternatives to autogenous bone grafting include bone grafting from cadaveric sources, inorganic materials, and growth factor supplementation. However, each of these reconstructive modalities has limitations. Specifically, allografts are associated with a known risk of infection transmission as well as the possibility for immunoreaction.[6,7] Inorganic implants for osteogenesis are frequently derived from hydroxyapatite, the main inorganic component of bone, and are challenging to use because of their brittleness and slow degradation rates.[8] Growth factors including BMP-2 and BMP-7 continue to be of interest in bone tissue engineering. However, barriers associated with the use of these agents include substantial cost and an unfavorable side effect profile, including the risk of heterotopic ossification and decreased maxillary growth following treatment.[9–12] Additionally, the use of BMP-2 and other similar proteins has traditionally been limited by their short half-life, which prevents the controlled and sustained release of these agents into the site of injury.

Biomimetic strategies to generate bone currently utilize three basic components: cells that can undergo osteogenic differentiation, scaffolding material, and additional growth factors to help induce osteogenesis[12–13]. These scaffolds have variable osteogenic properties depending on their material composition, porosity, and the incorporation of osteoblasts or mesenchymal stem cells into the scaffold prior to implantation.[9,12] The function of these scaffolds is to augment bone regeneration via osteoinduction of the seeded progenitor cells as well as osteoconduction. The function of the extra-cellular matrix (ECM) in natural tissues is to support bone regeneration through cell attachment, proliferation, and differentiation, all of which are essential to the process of osteogenesis.[8] The ideal osteogenic scaffold should therefore serve as an osteoconductive moiety, mimicking the natural ECM of bone as much as possible.[8,14] Specifically, scaffolds should emulate the nano-scale surface topography and biochemistry of natural bone ECM to facilitate favorable cell binding and differentiation.[15] Scaffolds may also serve as carriers for bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs) and transforming growth factors (TGFs), all of which help induce the transformation of host precursor cells into bone matrix producing cells.[14] The objectives of this review are to describe the structural and functional considerations associated with the development of effective biomimetic osteogenic scaffolds and to provide future directions of study for the development of an ideal osteogenic scaffold.

Material Choice and Functional Considerations

Depending on the type of material, scaffolds may mimic the organic and nonorganic properties of the normal extracellular matrix of bone.[12] Differences in the properties and type of scaffold material affect the quality of osteogenesis, and the optimization of scaffold material is essential to the development of clinically relevant engineered bone. The majority of scaffolds currently in use are derived from synthetic polymers based on calcium phosphate or calcium sulfate or naturally derived polymers such as chitosan and collagen.[16]

Ideal graft substitutes should be resorbed or replaced once new bone has formed and they are no longer needed. Long-term presence of a scaffold could potentially hamper bone formation and limit radiologic assessment of bone healing. Inorganic scaffolds such as hydroxyapatites serve as an osteoconductive matrix but have variable solubility and resorption profiles as limitations.[17–19] Such materials have been noted to have more brittle mechanical properties as well.[8] In comparing different types of inorganic materials, beta-tricalcium phosphates (beta-TCPs) are more rapidly reabsorbed than hydroxyapatite.[20]

Naturally derived scaffolds such as those based on collagen have also been used. Kruger et al., found that when compared to poly (L-lactide-co-glycolide) (PLGA) scaffolds, type I collagen allowed for more long-term mineralization. Early timepoints of 4 and 7 days revealed increased osteogenic and angiogenic markers in mesenchymal stem cells seeded on a PLGA scaffold, while long-term mineralization endpoints at 8 weeks favored the collagen scaffolds. However, without an inorganic component, such collagen scaffolds have been found to lack structural strength and demonstrate significant contraction during mineralization.[21–23]

Combinations of collagen and mineral content have shown particular promise in osteoconduction and bone healing.[24–27] Combining the organic and inorganic components of the extracellular matrix using a novel nanoparticulate mineralized collagen glycosaminoglycan resulted in a highly osteogenic and structurally stable scaffold for both primary rabbit bone marrow stromal cells and primary human mesenchymal stem cells.[23, 28–30]

Poly-(E-caprolactone) (PCL) is a biodegradable hydrophobic synthetic polymer with semi-crystalline properties, and has been studied extensively as a component of various osteogenic scaffolds. PCL is commonly used for the development of scaffolds in both bone and cartilage tissue engineering.[31–33] Requicha et al. concluded that eight weeks of treatment with a double-layered scaffold comprised of starch and PCL (“SPCL”) as well as SPCL functionalized with silanol groups (SPCL-Si) significantly induced new bone formation when compared to treatment with commercial collagen membrane as well as empty control defects.[34] More recent studies have explored methods of improving PCL’s mechanical profile, osteoconductive, and osteoinductive properties through the addition of functional groups. Of note, Baykan et al. have examined the osteogenic potential of rat bone marrow mesenchymal stem cells on a biomimetic three-dimensional construct comprised of a PCL/β-tricalcium phosphate composite scaffold and conclude that the addition of β-TCP to a PCL scaffold results in a hybrid scaffold which is osteoinductive and osteoconductive, as well as structurally sound.[31] Further, the authors demonstrate that an *in vivo* application of this porous composite scaffold resulted in infiltration with tissue and deposition of a calcium-rich matrix during osteogenesis, as well as induction of neovascularization at the subcutaneous site.

Other recent studies have dealt with the use of novel scaffold materials, including those based on graphene oxide. Liu et al. sought to determine the suitability of graphene oxide-gelatin (“GO-gel”) composites for bone regeneration.[35] The authors conclude that GO-gel composites are capable of supporting cell attachment and proliferation as well as providing an environment conducive to osteogenic differentiation of MC3T3-E1 cells and mineralization.

Other studies have focused on the development of scaffolds capable of mimicking the osteogenic niche of trabecular bone. Minardi et al. recently performed a series of experiments using a magnesium-doped HA (MHA)/type I collagen scaffold fabricated through a biologically-inspired mineralization process and designed to mimic human trabecular bone.[36] Following the evaluation of scaffold microstructure by SEM, hMSCs were added to the scaffold and their tendency towards osteogenesis was assessed by quantification of alkaline phosphatase (ALP). The authors' work with this innovative MHA/collagen scaffold - capable of mimicking the osteogenic niche at the chemical, physical, and morphological levels - led them to conclude that a high level of mimicry by the scaffold to the structure and material composition of the natural osteogenic niche translates to faster and more efficient osteoinduction *in vitro* and *in vivo*. Despite the multitude of scaffolds that have been studied, no studies to date have established an optimal carrier for the induction of osteogenesis in hMSCs. Thus, scaffold optimization continues to be an active area of research.

Porosity

The permeability of osteogenic scaffolds is a crucial consideration as it determines the rate of cell migration, as well as the diffusion of nutrients through the scaffold. Permeability is ultimately related to porosity, pore size, and distribution of pores.[8,38,39] Walsh, et. al. compared three chemically similar beta-TCP scaffolds in granular form in an *in vivo* rabbit tibial defect model and found that the materials differed in resorption time, likely related to differences in porosity and particle geometry.[20] Gandhimathi et al. generated a porous poly(L-lactic acid)-co-poly-(E-caprolactone)/silk fibroin/ ascorbic acid/ tetracycline hydrochloride (PLACL/SF/AA/TC) and nanohydroxyapatite (n-HA) nanofibrous scaffold and characterized this scaffold in terms of its porosity and mechanical properties.[40] This novel scaffold was shown to be highly porous (87–94%) and to also have good potential for the osteogenic differentiation of MSCs. The authors emphasize that the high porosity of their scaffold together with its looseness at the periphery likely facilitates cell infiltration and provides a favorable environment for proliferation and mineralization of MSCs. In this study, the authors also conclude that greater amounts of structural space as a result of internal, interconnecting porous structures augment the exchange of nutrients and metabolic wastes in a fashion similar to that of the matrix of natural bone.

Coating of Porous Scaffolds

Coating of porous scaffolds has been explored as a possible adjunct for the enhancement of cell attachment, proliferation, and osteogenic differentiation within osteogenic scaffolds. Recent studies have demonstrated that the polydopamine-assisted coating of porous, titanium-based, Ti6A14V scaffolds with hydroxyapatite (HA) promoted adhesion, proliferation, and differentiation of MC3T3-E1 cells compared with bare control scaffolds.[41] Furthermore, these coated scaffolds had greater osteointegration and osteogenesis in vivo compared with bare pTi scaffolds. The authors offer these "biofunctionalized" porous titanium-based scaffolds as a promising bone substitute. Similarly, Ren et al. determined that the use of a novel cell sheet engineering technique allowed for the fabrication of a biomimetic induced membrane with an inner pre-vascularized layer and an outer osteogenic layer.[42] This synthetic membrane demonstrated quick vascularization, functional anastomosis properties, and improved osteogenic potential *in vivo*.

Addition of Growth Factors

Recent studies have investigated the effect of embedding nanofiber scaffolds with various growth factors, with the primary aim of developing an effective technique by which to deliver these agents to the site of injury in a controlled and sustained fashion. Li et al. developed a novel nanoparticle-embedded electrospun nanofiber scaffold for the controlled dual delivery of both BMP-2 and dexamethasone.[6] The authors maintained the bioactivity of BMP-2 by utilizing bovine serum antigen (BSA) as a nano-carrier. They concluded that this dual-drug-loaded nanofiber scaffold is capable of promoting significant osteogenesis both *in vitro* and *in vivo* in a rat calvarial defect model. They hypothesized that while dexamethasone promotes earlier calcified bone regeneration, the sustained release of BMP-2 may establish a long-term beneficial effect for bone regeneration.

In a recent study, HJ Lee et al. developed multi-functional biomimetic tissue-engineered scaffolds that could control spatial distribution of stem cells and that could release multiple growth factors with a controlled dose and rate of delivery.[43] Electrospinning and photolithography were used to develop this novel scaffold from PCL, gelatin fibers, and poly(ethylene glycol) (PEG) hydrogel. The authors found that when this novel scaffold was seeded with hMSCs, these cells selectively adhered within the "fiber-region" because of the non-adhesiveness of the PEG hydrogel. The addition of this hydrogel therefore allowed for spatial positioning of hMSCs within the scaffold to within a micrometer of gel placement. The authors also utilized the same principle to ensure the sequential release of both bFGF and BMP-2; bFGF was quickly released from its attachment site on the nanofibers while BMP-2, which preferentially bound the PEG gel, was released more gradually. Their *in vivo* studies indicate that this spatiotemporal control of stem cell attachment and growth factor release leads to stronger osteogenic commitment when compared to scaffolds without growth factors or scaffolds with single administration of either bFGF or BMP-2 under the same conditions.

Further additives designed to enhance cell attachment, proliferation, and osteogenic differentiation have recently been studied. Kasten et al. have demonstrated elevated ALP activity when using β-TCP based scaffolds treated with platelet-rich plasma (PRP) and seeded with human bone marrow hMSCs when compared to a control carrier scaffold treated with PRP without hMSCs.[44,45] These authors have concluded that the addition of PRP to scaffolds leads to higher cell loading efficiency of hMSCs on calcium and HA-based constructs, as well as improved cell proliferation. Together, these studies indicate that the addition of PRP to osteogenic scaffolds may enhance the therapeutic benefit of these constructs for bone augmentation.

Growth-Factor Independent Osteogenic Induction of hMSCs

Bioactive factors including BMP-2 are associated with significant cost and side-effect profiles. These issues warrant the investigation of alternative, clinically accessible methods of stimulating osteogenesis. Sun et al. have studied citric acid-based polymer/hydroxyapatite composites (CABP-HAs), a recently developed class of biomimetic composites.[46] In these studies, CABP-HA disc-shaped scaffolds were tested and compared to autologous bone grafts, poly(1,8-octanediol citrate)-click-HA (POC-Click-HA) scaffolds, as well as empty defects. The authors utilized 4 mm rat calvarial defects and demonstrated that these highly-porous, disc-shaped, citric acid-based polymer scaffolds promoted significant levels of osteogenesis and angiogenesis in an intramembranous bone regeneration model. Of note, the scaffolds used in this study were bare, and did not contain growth factors or implanted cells.

Similarly, we have previously utilized a novel nano-particulate mineralized collagen glycosaminoglycan scaffold (MC-GAG) to demonstrate growth factor independent osteogenic induction of hMSCs.[9] This work is significant as it may ultimately lead to methods of osteogenesis that minimize or eliminate reliance on artificial implants and growth factors. Osteogenic induction of hMSCs was measured on both a MC-GAG and a nonmineralized scaffold (Col-GAG). Mineralization of hMSCs on MC-GAG scaffolds turned out to be independent of addition of BMP-2. The canonical BMP receptor Smad (Smad 1/5) was constitutively phosphorylated in MC-GAG scaffolds, whereas phosphorylated Smad appeared to be dependent on BMP for the Col-GAG scaffolds. Also mineralization of hMSCs in the Col-GAG scaffolds occurred at the periphery whereas MC-GAG scaffolds showed good consistent mineralization throughout the scaffold. The scaffolds were relatively similar in porosity, but previous studies showed that these scaffolds differ in terms of presence of nanoparticulate calcium phosphate particles and elastic moduli which may help explain differences in mineralization.[47–48]

Implantation of scaffolds with cells

In addition to enhancing the osteoconduction of scaffolds through design of the scaffold and the application of growth factors, there is significant interest in the use of scaffolds seeded with cells capable of differentiating into bone. Pre-seeding scaffolds with cells such as mesenchymal stem cells offer a strategy for improving bone differentiation and ingrowth compared to empty scaffolds.[49] Bone-marrow derived stem cells (BMSCs) have been particularly widely studied, are relatively easily harvested, and have been shown to differentiate successfully into bone.[50] BMSCs have been successfully used in clinical applications including spinal fusion, segmental bone defects, and craniotomy defects.[51–53] The osteogenic potential of BMSC was found to be higher than that of osteoblasts in a bovine *in vitro* model.[54] Importantly, BMSCs loaded on scaffolds implanted into an osteochondral defect resulted in both osteogenesis and chondrogenesis.[55] However, BMSCs may have less osteogenic potential than dentoalveolar cells and periosteal cells.[56] Adipose-derived stem cells (ASCs) also appear to be a viable and promising alternative to BMSCs. Osteodifferentiated ASCs seeded on to a Type I collagen matrix were able to form abundant bone when implanted into the hind-limbs of severe combined immunodeficient mice.[57] ASCs seeded onto a starch and polycaprolatone scaffold were also able to form new bone tissue in an *in vivo* murine model.[58] Induced pluripotent stem cells (iPSCs) have been more recently studied. Tang et al. showed that MSCs derived from iPSCs were able to show good viability and osteogenic differentiation.[59] The optimal type of cell for scaffold seeding is still under investigation.

Conclusion

Bone tissue engineering remains an area of significant clinical interest. Factors that affect the timing and quality of osteogenesis include scaffolding material as well as addition of growth factors and cells to favor osteogenic differentiation. Future studies will further elucidate ways to optimize biomimetic methods of bone formation.

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