REVIEW ARTICLE

Stem cells, growth factors and scaffolds in craniofacial regenerative medicine

Viktor Tollemar a,b,c, Zach J. Collier a,b, Maryam K. Mohammed a,b, Michael J. Lee b, Guillermo A. Ameer d,e, Russell R. Reid c,*

a The University of Chicago Pritzker School of Medicine, Chicago, IL 60637, USA
b Department of Orthopedic Surgery and Rehabilitation Medicine, The University of Chicago Medical Center, Chicago, IL 60637, USA
c Laboratory of Craniofacial Biology and Development, Section of Plastic and Reconstructive Surgery, Department of Surgery, The University of Chicago Medicine, Chicago, IL 60637, USA
d Department of Surgery, Feinberg School of Medicine, Chicago, IL 60611, USA
e Biomedical Engineering Department, Northwestern University, Evanston, IL 60208, USA

Received 31 July 2015; accepted 22 September 2015
Available online 17 October 2015

KEYWORDS
Bone regeneration; Craniofacial defects; Osteogenesis; Regenerative medicine; Scaffolds; Tissue engineering

Abstract Current reconstructive approaches to large craniofacial skeletal defects are often complicated and challenging. Critical-sized defects are unable to heal via natural regenerative processes and require surgical intervention, traditionally involving autologous bone (mainly in the form of nonvascularized grafts) or alloplasts. Autologous bone grafts remain the gold standard of care in spite of the associated risk of donor site morbidity. Tissue engineering approaches represent a promising alternative that would serve to facilitate bone regeneration even in large craniofacial skeletal defects. This strategy has been tested in a myriad of iterations by utilizing a variety of osteoconductive scaffold materials, osteoblastic stem cells, as well as osteoinductive growth factors and small molecules. One of the major challenges facing tissue engineers is creating a scaffold fulfilling the properties necessary for controlled bone regeneration. These properties include osteoconductivity, osteoinduction, biocompatibility, biodegradability, vascularization, and progenitor cell retention. This review will provide an overview of how optimization of the aforementioned scaffold parameters facilitates bone regenerative capabilities as well as a discussion of common osteoconductive scaffold materials.

Copyright © 2015, Chongqing Medical University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
**Introduction**

Large craniofacial skeletal defects secondary to trauma, congenital condition, or cancer resection pose serious challenges to reconstructive surgeons. Extensive defects which prevent spontaneous re-osseification are termed ‘critical-sized’ and often require complex reconstructive approaches (Fig. 1A). Repair of these defects has traditionally required autologous bone grafts from a variety of sources, including cranium, tibia, rib, and iliac crest (Fig. 1B). These procedures, although they have seen success clinically and are currently the gold standard of care, necessitate a second surgical site with a significant risk of morbidity. In particular, undesirable sequelae at the donor site include infection, bleeding, pain, swelling, unanticipated fractures, and injury to adjacent critical structures. Additionally, autologous bone graft procedures have been complicated by unpredictable graft resorption rates, limited supply of autologous bone, and rapid bone remodeling in young children.

Alternatives in the alloplast category, including demineralized bone matrix, bone ceramics, porous polyethylene implants, and various other polymers, have seen variable success. However, they generally carry a greater risk of infection than autologous bone grafts and are more likely to fail over time. Permanent methods of rigid fixation utilizing metals or metal alloys suffer similar limitations in addition to integrating poorly with the surrounding tissue. Because craniofacial reconstructive surgeries are often performed on children (Fig. 1) who require repair capable of accommodating natural growth and development, permanent rigid fixation is not the most favorable alternative.

Biocompatible implants that augment natural bone-regenerative capabilities currently represent the most promising and versatile approach to repairing critical-sized craniofacial defects. This tissue engineering-based strategy generally involves three key elements: osteoconductive scaffolding, stem cells, and growth factors (Fig. 2). These three elements allow osteoblastic and endothelial progenitor cell differentiation, bone formation, and integration with surrounding bone tissue even in large defects. Osteoblastic stem cells within an osteoconductive scaffold provide the possibility of a tailored three-dimensional space for bone growth. Osteoblastic differentiation can be induced by a variety of osteoinductive growth factors both in vivo and in vitro. Finally, efficacious bone regeneration requires integration with surrounding tissue, including vascularization, fusion of the implant with autologous bone without fibrous tissue at the bone-implant interface, and eventual complete replacement of the scaffold with new bone.

The goal of achieving these prerequisites has challenged tissue engineers to choose the optimum combination of cell types, scaffold properties, and growth factors. The process is inherently complex and multidisciplinary due to requisite collaboration between molecular biology, materials science, surgery, and mechanical engineering. This review will explore current progress toward achieving reliable repair of craniofacial defects using osteoconductive scaffold and osteogenic stem cell-based tissue engineering.

**Stem cells used for bone regeneration**

Irrespective of craniofacial bone defect size or complexity, healing is fundamentally dependent on the presence of osteogenic and vasculogenic precursor cells in surrounding tissues. These precursors migrate to the injury site and differentiate into osteoblasts and endothelial cells, promoting bone formation and vascularization. In recent years, clinical reports have suggested that stem cell supplementation may work synergistically with this natural progenitor cell migration and differentiation to produce the best results in healing critical-sized bone defects.

Several stem cell types have been used both in vitro and in vivo to produce new bone (Fig. 3). Bone marrow-derived mesenchymal stromal cells (BMSCs) are increasingly being applied to craniofacial defect repair, and several studies have substantiated their effectiveness as osteoblastic precursors in critical-sized defect reconstruction. A recent phase I/II clinical trial determined that CD90+ osteoblastic BMSCs and neovascularization-inducing CD14+ monocytes and macrophages seeded onto a β-tricalcium phosphate (β-TCP) scaffold provided a viable treatment for patients with severe maxillary bone deficiency. When compared with scaffold alone, the progenitor cell-seeded scaffold treatment showed a higher proportion of regenerated viable, highly vascularized, and mineralized bone in addition to a lower proportion of residual β-TCP particles four months postoperatively. Mesenchymal stem cells derived from umbilical cord blood have also been used successfully, in conjunction with poly-lactic co-glycolic acid (PLGA) implants, to heal critical-sized alveolar cleft defects in a swine model. Investigators reported no inflammation and better bone quality than autologous bone graft from the iliac crest by CT volumetric and histological analysis. However, despite its success, the use of BMSCs is limited by finite supply and the morbidity associated with procurement procedures.

Adipose-derived stem cells (ADSCs) represent a promising alternative to BMSCs in that they are more plentiful, less painful to harvest, and easily expandable. ADSCs have showed similar osteogenicity to BMSCs, with certain subpopulations demonstrating enhanced tendency toward osteoblast differentiation and others successfully induced through gene therapy. The necessity for invasive procedures during harvesting still constrains ease of access to ADSCs and the scope of their clinical significance.

Urine-derived stem cells (USCs), which can be obtained from voided urine and require no invasive procedures, have recently garnered a great deal of attention in the bone tissue engineering community as a promising, but still poorly studied, alternative stem cell source. Research regarding USCs is still in its infancy, but recent studies by Guan et al have demonstrated their applicability to bone regeneration.

USCs are biologically similar to ADSCs and are capable of osteogenic differentiation in vitro. Furthermore, USCs have successfully differentiated into osteoblasts via calcium silicate ion induction of the Wnt/β-catenin signaling pathway. They have also been shown to be compatible with both calcium sulfate/PLGA composite and β-TCP scaffolds.
Neovascularization is a critical component of bone tissue engineering, and can be facilitated by incorporation of endothelial progenitor cells (EPCs) in scaffold design. EPCs have been shown to enable neovascularization in response to ischemia. This ischemic response is seen in the context of critical-sized craniofacial defects, and EPCs have been used in combination with MSCs and a thermoresponsive porous nano-calcium sulfate/alginate scaffold to repair calvarial defects in rats. EPCs are also compatible with β-TCP scaffolds, in which they have been shown to contribute directly to neovascularogenesis through endothelial cell differentiation and recruitment of additional host EPCs. Exogenous EPCs have also been shown to release pro-angiogenic factors such as vascular endothelial growth factor (VEGF).

Osteoinductive factors

A critical component of osteoblastic progenitor cell differentiation and subsequent bone formation are osteoinductive growth factors (Table 1). Many growth factors are known to enhance bone regeneration, including transforming growth factor β (TGF-β), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and platelet derived growth factor (PDGF). Several bone
morphogenic proteins (BMPs), members of the TGF-β family, have been used clinically to induce bone regeneration in critical-sized craniofacial defects as well as alveolar ridge and sinus augmentation. They bind receptors on multiple stem cell types and induce osteoblastic differentiation through the Smad protein signaling pathway. BMPs, particularly BMP-2 and BMP-7, have been studied extensively in bone healing and produce superior fusion rates with fewer complications than autologous bone grafts. Infuse/C226 Bone Graft (Medtronic and Wyeth) and Osigraft/C226 (Stryker Biotech) are two FDA-approved collagen-based scaffolds containing recombinant BMP-2 and BMP-7, respectively. The clinical success of these products demonstrates the importance of growth factors in osteogenesis and underscores the potential of growth factor-infused scaffolds.

Other osteoinductive BMPs include BMP-4, 6, and 9, and previous evidence suggests that BMP-9, a relatively poorly characterized growth factor, is the most potent BMP in promoting in vitro and in vivo osteogenic differentiation of mesenchymal stem cells. Despite such auspicious results, relatively high dose requirements, cases of ectopic bone formation, and paradoxical increase in bone resorption — particularly observed with BMP-2 — have tarnished some of BMPs’ initial promise. Efforts are ongoing to combine synergistic growth factors and carrier molecules to lower the necessary BMP dose and control its release.

Growth factor incorporation into scaffolds may be accomplished in a number of ways, each of which confers unique properties. Soaking a scaffold in growth factor-containing solution results in a loose association with the structural material and, therefore, facilitates quick release of the desired stimulatory molecules. Conversely, growth factors may be incorporated into and even covalently linked to the scaffold microstructure for extended release. Cells modified to express and secrete osteoinductive growth factors may also be seeded in the scaffold, achieving a similar effect. The necessary cell modifications typically involve gene therapy accomplished either by viral or nonviral transduction. Viral transduction is the most effective means of gene transfer and is generally carried out using retroviruses, adenoviruses, or adeno-associated viruses. Gene transfer can also be accomplished via direct uptake of gene-containing plasmids from solution or as a conjugate with a nucleus-bound biomolecule.

Issues with growth factor-enriched scaffolds are generally associated with mismatched release profiles — the release of growth factor is often dictated by passive diffusion or degradation rate, and does not appropriately parallel the rate of bone regeneration and healing. It has been shown that covalent linkage of the growth factor to the scaffold may slow and improve its release profile to
more closely approximate cellular demands. For example, covalently incorporated VEGF in a fibrin scaffold results in a more tightly controlled release and, subsequently, a more organized vascularization in comparison to scaffold with unlinked VEGF. One risk inherent in covalently incorporated growth factors is altering established mechanical, osteoconductive, or other properties of the scaffold material. Despite this, it has been used in animal models to successfully repair mandibular, zygomatic, and calvarial bone defects.

As a supplement to BMPs or other osteoinductive growth factor proteins, small molecules that help induce osteoblast differentiation have been used. Small molecules are generally more cost-effective, easier to synthesize and handle, and diffuse rapidly. Statins, as well as several immunosuppressants, are small molecules that have demonstrated capability to induce osteoblastic differentiation and bone formation. Phenamil, an irreversible amiloride analogue, is another small molecule that has been shown to induce osteogenesis in dental pulp cells and BMSCs through robust activation of the BMP signaling pathway. Most recently, phenamil has demonstrated synergistic effects with BMP-2 by inducing osteogenic differentiation of ADSCs in calvarial defect repair.

**Characteristics of an optimal scaffold**

**Osteoconduction**

In designing scaffolds for bone regeneration, there are several key properties that tissue engineers consider. First is the capacity to deliver exogenous osteoblastic and epithelial progenitor cells to the defect site and/or to facilitate recruitment of host progenitor cells that aid in bone generation and tissue integration. Osteoconduction refers to the ability of the scaffold to not only act as a carrier for these progenitor cells but also to provide a viable template for bone growth. Osteoconductive materials that provide a supportive microenvironment in which exogenous and endogenous progenitor cells can differentiate and produce vascularized bone are a key part of scaffold design.

**Osteoinduction**

In smaller fractures, natural regenerative healing occurs via recruitment of mesenchymal stem cells from adjacent tissues and bone marrow to the site of injury, where they are induced to differentiate into osteoblasts and deposit new bone to bridge the fracture. Differentiation of these migratory progenitor cells is accomplished via mechanical, biochemical, and biophysical factors in a process called osteoinduction. Osteoinductive scaffold designs seek to emulate this natural phenomenon through biochemical structure, progenitor cell adhesion properties, and delivery of growth factors.

**Biocompatibility**

Biocompatibility is an essential attribute of any scaffold implant, and in order to be clinically successful, it must not elicit a damaging inflammatory response. In the context of

| Growth factor | Osteoblastic differentiation | Osteoblast proliferation | Neovasculogenesis |
|---------------|-----------------------------|--------------------------|-------------------|
| TGF-B         | Promoting                   | Promoting                |                   |
| FGF           |                             | Promoting                | Promoting/Inducing|
| VEGF          |                             |                          |                   |
| PDGF          | Promoting                   | Promoting early; Inhibiting late |                   |
| BMP-2         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-4         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-6         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-7         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-9         | Inducing                    | Promoting early; Inhibiting late |                   |

*Only PDGF-AA has been shown to promote osteoblastic differentiation in MSCs.*

Natural fracture healing is characterized by the formation of a cartilaginous callus, which undergoes mineralization, resorption, and replacement by new bone. It is this role of the cartilaginous callus as an osteoconductive template that current scaffolds seek to emulate. However, whereas physiologic bone healing is limited to small defects, scaffolds enhance these processes to bridge large segmental defects. Collagen and hydroxyapatite, the primary organic and mineral components of bone, respectively, are prototype osteoconductive materials and will be discussed later in this review.

The concept of mimicking native bone ECM, which serves as a collogenous framework for osteoblasts and a reservoir for growth factors, has played a significant role in scaffold design. Interplay between the scaffold and progenitor cells should closely mimic natural cell surface receptor and ECM interactions. These interactions are critical in bone regeneration processes such as osteoblast adhesion, proliferation, migration, differentiation, and matrix deposition. The importance of biophysical cell/scaffold interactions on cell function has been underscored by studies demonstrating significant differences in cell adhesion and differentiation behavior with changes in scaffold elasticity and surface microstructure.

### Table 1 Osteoinductive growth factors. Growth factors that can be used in bone tissue engineering and their general contribution to osteogenesis.

| Growth factor | Osteoblastic differentiation | Osteoblast proliferation | Neovasculogenesis |
|---------------|-----------------------------|--------------------------|-------------------|
| TGF-B         | Promoting                   | Promoting                |                   |
| FGF           |                             | Promoting                | Promoting/Inducing|
| VEGF          |                             |                          |                   |
| PDGF          | Promoting                   | Promoting early; Inhibiting late |                   |
| BMP-2         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-4         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-6         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-7         | Inducing                    | Promoting early; Inhibiting late |                   |
| BMP-9         | Inducing                    | Promoting early; Inhibiting late |                   |

*Only PDGF-AA has been shown to promote osteoblastic differentiation in MSCs.*
biodegradable scaffolds, the most common way for unwanted inflammatory processes to occur is by production of reactive oxygen species (ROS). Accumulation of degradation products may generate toxic levels of ROS. Approaches to minimizing the inflammatory response include incorporation of biomimicking materials as well as conjugate antioxidants in the scaffold itself. Utilizing scaffolds that can be delivered through minimally invasive techniques, such as injectable hydrogels or thermoresponsive scaffolds, is also an important tactic to reduce inflammation.

**Biodegradability**

Osteoconductive scaffolds should act only as a temporary framework for bone regeneration. Temporality is critically important, as the ideal scaffold is not meant to be a permanent prosthesis, but rather a provisional support for osteoblastic differentiation, bone regeneration, and vascularization until fully functional tissue has replaced the scaffold and the defect is healed. Full resorption of the original scaffold is necessary for uninterrupted bone remodeling and physiologic responses to mechanical stimuli. Unmatched rates of scaffold material resorption and bone formation may result in incomplete bone regeneration or obstructed remodeling and tissue integration. Therefore, degradability of the scaffold into biocompatible byproducts is an essential property that is governed by scaffold chemical composition, micro- and macrostructure, and numerous host factors. Clinical factors affecting bone regeneration and scaffold degradation rates, including patient co-morbidities and defect anatomy, must be considered in selecting graft substitutes for repairing craniofacial defects.

**Vascularization**

An extensive variety of scaffolds and stem cell therapy approaches to healing craniofacial defects have been proposed and tested, but successful treatment ultimately depends on integration with surrounding tissue. That success hinges on two key factors — the ability to recruit local osteoblastic and endothelial progenitor cells to the site of injury and the existence of functioning vasculature near the defect. Vasculogenesis, or formation of new blood vessels through differentiation of recruited endothelial progenitor cells (EPCs), is a normal response to traumatic injury and is largely mediated by vascular endothelial growth factor (VEGF). Downstream effects of VEGF culminate in proliferation of circulating EPCs, which initiate vasculogenesis at the defect site. Vasculogenesis and angiogenesis, collectively known as neovascularization, are necessary prerequisites for osteogenesis, and it has been shown that bone regenerative capabilities are directly linked to circulating EPC levels.

However, effective delivery of these EPCs is complicated by the vascular deficiency that often exists in the context of critical-sized craniofacial and other bone defects. In order to promote vascularization despite these challenges, scaffolds can be enriched with both growth factors and endothelial progenitor cells. Several strategies have been attempted, including direct integration of neovasculogenic growth factors and cytokines, incorporating cells capable of secreting these growth factors, featuring adhesion proteins conducive to endothelial cell attachment and blood vessel formation, and seeding with endothelial progenitor cells. Multipotent bone marrow stromal cells enriched for mesenchymal and endothelial phenotypes have also demonstrated capacity for highly vascularized bone generation in mandibular defect repair.

The importance of vascular supply in bone reconstruction is well recognized. Osteoprogenitor cells associate with endothelial cells, which supply not only oxygen and nutrients but also growth factors necessary for osteoblastic differentiation. For this reason, neo-vascularization is an essential step in promoting sustained bone regeneration. Accommodating for endothelial progenitor cell invasion and attachment, delivery of pro-angiogenic factors, and blood vessel formation necessitates a porous scaffold structure. It is thought that 150–500 µm is a sufficient pore diameter to support neo-vascularization and blood vessel invasion. However, porosity often relates inversely with material strength. The idea that reduced porosity and higher density confers greater mechanical strength while increased porosity facilitates growth factor delivery, cell migration, and vascularization has been a key principle of scaffold design. As a result, the ideal scaffold strikes a balance between the two competing properties.

Head and neck cancer treatments involving bone resection and radiation therapy also pose a significant challenge for reconstructive surgeons due to the debilitating nature of radiation toxicity on bone regeneration. Radiation therapy severely complicates bone development, remodeling, and fracture healing secondary to progenitor cell loss and compromised vasculature. These complicating factors require a combination of neo-vascularogenic progenitor cells and growth factors to ensure proper vascularization.

**Biomaterials for osteoconductive scaffold construction**

Although autologous bone grafts remain the gold standard for repairing critical-sized craniofacial defects, their use is cost-prohibitive, requires a second surgical site, is associated with significant donor site morbidity, and is limited by the finite supply of autologous bone. The use of biocompatible scaffolds in healing these defects may provide a more cost-effective and less complicated alternative to autologous bone grafts. Scaffolds provide an osteoconductive and osteoinductive extracellular matrix analog to facilitate cellular migration, proliferation, adhesion, differentiation, and generation of new bone. A variety of materials for this purpose have been studied, including ceramics, natural and synthetic polymers, various composite materials, silicon-based bioglass, and metals (Table 2).
Demineralized bone matrix

Demineralized bone matrix (DBM) is produced by acid extraction of allogenic bone, a process that removes the inorganic mineral component of bone and leaves a type I collagen framework. Demineralization also exposes osteoinductive growth factors, including BMPs, making DBM more osteoinductive than complete bone grafts. DBM is currently available as powder, granules, gel, putty, and more osteoinductive than complete bone grafts. DBM is considered as an alternative to bone and long shelf life, calcium phosphate scaffolds have considerable promise as an alternative to bone grafts. Because of such findings, DBM alone is not considered a promising scaffold material. However, recent efforts using poly(lactic acid) (PLA)/DBM composite scaffolds for bone engineering have proven to be more effective.

Ceramics

Some of the most promising initial scaffolds closely mimic the chemistry and structure of native extracellular matrix in bone. Foremost among these are calcium phosphate ceramics, including hydroxyapatite (HA), β-TCP, and biphasic calcium phosphate. Due to their biocompatibility, safety, reliability, availability, ease of sterilization, and long shelf life, calcium phosphate scaffolds have considerable promise as an alternative to bone grafts.

Hydroxyapatite bioceramics confer a high degree of osteoconductivity but are brittle and resorbed at a rate much slower than desired, often taking several years. This is in contrast to tricalcium phosphate (TCP) scaffolds, which have been reported to fully resorb within 12 weeks. By altering calcium-to-phosphate ratios, internal pore architecture, and other parameters of these TCP scaffolds, engineers have been able to control resorption rates and improve osteogenicity. Furthermore, HA-TCP composite scaffolds have demonstrated both osteoconductivity and favorable resorption rates. Similarly, it has been shown that HA/collagen composite implants are characterized by improved stiffness and osteointegration in comparison to collagen alone in critical-sized rat calvarial defects. An injectable collagen/calcium phosphate hydrogel has also exhibited efficient umbilical cord-derived mesenchymal stem cell (UCMSC) seeding and ability to support osteoblastic differentiation and osteogenesis.

Although conferring essential osteoconductive, porous, and resorption properties, ceramic scaffolds are relatively brittle and do not have the strength optimally desired. To that end, more recent experiments have found that incorporating hydroxyapatite nanoparticles into more structurally competent polymer scaffolds has resulted in a more favorable combination of strength, protein loading, cell adhesion and migration, and osteogenic properties. In addition, a scaffold comprised of calcium phosphate ceramic tiles set within a titanium framework has recently been described in the context of complex craniofacial defect repair.

Calcium carbonate is another potential ceramic material for osteoconductive scaffold fabrication. It has better natural biodegradation properties than calcium phosphate, and may prove useful in pediatric craniofacial reconstruction, where highly active skeletal remodeling necessitates rapid scaffold resorption. As of yet, this material has most significantly been used to repair burr holes from hematoma-related neurosurgery cases. Two studies have tested alveolar bone regenerative capabilities of calcium carbonate scaffolds and concluded that its mechanism of supporting bone growth is primarily through space-provision rather than previously hypothesized osteoconductive properties. Since then, little research has been done to further characterize bone tissue engineering applications for calcium carbonate.

Polymers

Natural and synthetic polymers are often used as scaffold materials for bone tissue engineering because of a well-balanced combination of properties, including biodegradability, biocompatibility, porosity, and ease of handling. Naturally-derived materials, such as collagen and fibrin proteins, or chitin-derived chitosan polysaccharide, are also an option for bone tissue engineering. Such materials may confer greater cell adhesion and functional support properties than synthetic materials, but in most cases, this is offset by several disadvantages. Natural polymers often offer less control over mechanical properties, sometimes exhibit immunogenicity, and frequently exist in finite supply; therefore, they are difficult and expensive to obtain. Synthetic polymers,

---

**Table 2** Biomaterials for bone tissue engineering. Commonly used biomaterials for bone regeneration in craniofacial defect repair.

| Osteoconductive biomaterials for scaffold construction | Demineralized bone matrix (DBM) |
|-----------------------------------------------------|--------------------------------|
| Allogenic bone derivative                           | Demineralized bone matrix (DBM) |
| Ceramics                                            |                                |
| Hydroxyapatite (HA)                                 |                                |
| Tricalcium phosphate (TCP)                          |                                |
| Biphasic calcium phosphate                          |                                |
| Calcium carbonate                                   |                                |
| Polymers                                            |                                |
| Poly(lactic acid) (PLA)                              |                                |
| Poly(glycolic acid) (PGA)                           |                                |
| Poly(lactic-co-glycolic acid) (PLGA)                 |                                |
| Poly(propylene fumarate) (PPF)                      |                                |
| Polycaprolactone (PCL)                              |                                |
| Polyamide (PA)                                      |                                |
| Chitosan                                            |                                |
| Metals                                              |                                |
| Titanium                                            |                                |
| Magnesium Alloy                                     |                                |
| Zinc (doping)                                       |                                |
| Bioglass                                            |                                |
| Silicon                                             |                                |
| Calcium-silicate (CS)                               |                                |
| Thermoresponsive                                    |                                |
| N-isopropylacrylamide (NIPAA)                       |                                |
| Polypolyethylene glycol citrate-co-                 |                                |
| N-isopropylacrylamide (PPCN)                        |                                |
however, do not suffer from these shortcomings and have been a more important source of biomaterials for osteoconductive scaffold construction.\(^{162}\)

Synthetic polymers can be produced on a large scale using reproducible and tunable methods, providing fine control over mechanical and physical properties. They have a well-documented history of clinical application in craniofacial bone reconstruction, especially in children.\(^{163}\) Synthetic polymers like poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and various iterations of combined poly(lactic-co-glycolic acid) (PLGA) have been used for a range of clinical applications, including critical-sized craniofacial defect repair.\(^{57,164}\)

PLA is an FDA-approved synthetic biomaterial that has several properties conducive to bone tissue engineering, including controllable biodegradation rate, biocompatibility, and good mechanical strength.\(^{165}\) It has been applied clinically to fabrication of resorbable sutures, as a drug delivery scaffold, and as resorbable bone fixation devices in fracture healing. However, its application as a scaffold biomaterial for craniofacial bone regeneration is limited by poor osteoinductive properties.\(^{145}\) PGA is another FDA-approved synthetic biomaterial with a variety of tissue engineering applications, including regeneration of cartilage, bone, tendon, muscle, and skin.\(^{166–168}\) Despite such adaptability, its mechanical properties are not ideal for the precision bone reconstruction necessary for craniofacial defect repair because of its softness and inability to maintain shape. PGA and PLA alone are not suitable bone tissue engineering scaffold materials, but their respective softness and low osteoconductivity have been partially addressed by combining them to form a PLGA composite scaffold.\(^{169}\) PLGA has been shown to have a controllable degradation rate (through varying composition of its constituent homopolymers) in addition to supporting osteoblast attachment, growth, and differentiation both in vitro and in vivo.\(^{162,170–173}\) Nevertheless, PLGA’s mechanical properties and osteoconductivity are suboptimal for bone tissue engineering, and it is most often used as part of a composite material with ceramics, bioglass, or other more osteoconductive materials.\(^{173,174}\)

Poly(propylene fumarate) (PPF) is a synthetic, unsaturated, linear polyester polymer that is biodegradable, biocompatible, osteoconductive, injectable, and sufficiently strong for craniofacial bone engineering.\(^{175–185}\) It generally requires a small monomer accelerating agent, such as N-vinylpyrrolidone, in order to crosslink as an injectable polymer.\(^{186}\) A two-phase PPF cement incorporating cross-linked microparticles to increase strength and lower setting temperature has been developed. This PPF-based system has improved injectability, setting temperature, and setting time over clinically available polymethyl methacrylate (PMMA) bone cement and is believed to be suitable for application in craniofacial bone regeneration.\(^{175}\) PPF has also been used as a co-polymer with polycaprolactone (PCL) as a scaffold for osteoblastic differentiation and maturation in vitro.\(^{187}\) PCL is a non-aromatic polyester that is highly flexible and has a controllable biodegradation rate owed to alterable substituent molecular weight.\(^{188–190}\) Similarly, the PPF-PCL co-polymer setting time, setting temperature, mechanical strength, and other physical properties can be tuned through variation of substituent molecular weight as well as relative proportion of PPF and PCL.\(^{186,187}\) PPF-PCL’s chemical structure also allows for HA incorporation, which aids osteoblast progenitor cell adhesion and proliferation.\(^{187}\)

Polyamide (PA) is a synthetic polymeric collagen analog that provides excellent strength as well as biocompatibility. Those properties have made PA a promising partner for HA or other bioceramics in osteoconductive composite scaffolds. As a BMP-7-transduced MSC-laden composite with HA nanoparticles, PA has been successfully used to repair mandibular defects in rabbits.\(^{191}\)

### Metals

Currently, metals such as titanium are used clinically in craniofacial reconstruction. However, as inert alloplasts, they do not integrate with surrounding tissue and do not stimulate new bone formation.\(^{13}\) Metals that degrade in a physiological setting have been proposed in order to solve this problem and promote more long-term success. Biodegradable metals, such as magnesium alloys, have generally been shown to possess mechanical properties mimicking that of natural bone while retaining the critical ability to resorb over time.\(^{64,192}\) Mg–Ca, pure Fe, Fe–Mn alloys, and Fe foam have all been tested as osteoconductive scaffold materials for bone tissue engineering.\(^{193–204}\) In particular, Mg and its alloys have been shown to support osteoblastic differentiation of progenitor cells and are degraded in vivo to Mg hydroxide and hydrogen gas.\(^{15}\) Given the importance of porosity for progenitor cell migration and neovascularization, porous Mg scaffolds have been investigated and can be fabricated with preserved mechanical properties.\(^{164,205,206}\) Their strength, ductility, biodegradability, and osteoconductive properties make Mg alloys, and potentially other metals, possible alternatives to polymer or ceramic scaffolds.\(^{164}\)

Incorporating metal nanoparticles into polymer scaffold materials has also been an ongoing effort to produce higher strength composite scaffolds that retain their osteoinductivity and osteoconductivity.\(^{144,155,207}\) Addition of other trace impurities, such as zinc oxide, iron, and silicon dioxide, has been shown to confer a greater degree of control in degradation rates, density, mechanical strength, and biocompatibility.\(^{105}\) The addition of zinc and silicon has boosted both expression of type I collagen and extracellular signaling promoting angiogenesis as well as osteoblast differentiation.\(^{208,209}\)

### Bioglass

There are two major groups of glass-based osteogenic scaffolds: glass-ceramic and glass-polymer porous composites.\(^{144}\) It has been demonstrated that silicon found in glass enhances angiogenesis as well as gene expression regulating osteogenesis and growth factor production in osteoblasts.\(^{13}\) Several studies have confirmed that silicate-based scaffolds are capable of stimulating osteogenesis.\(^{210–212}\) Accordingly, silicon has been successfully incorporated into bioceramics in order to augment bioactivity and osteostimulatory effects.\(^{211,213–216}\)
For example, silicon/HA scaffolds have also shown increased bone ingrowth over HA alone, but these hybrids are limited by low mechanical load strength. Alternatives include calcium silicate (CS)-containing scaffolds, which are able to stimulate osteogenic differentiation of several adult stem cell lines, including BMSCs, and have pro-angiogenic properties. Importantly, these scaffolds are able to have these effects without the addition of exogenous growth factors. Osteogenic and angiogenic growth factors have previously been utilized in bone tissue engineering, but the prospect of a single scaffold capable of inducing both osteogenesis and angiogenesis without exogenous growth factors has exciting implications.

Injectable biomaterials

Injectable biomaterials provide two major advantages over traditional solid scaffolds; they can be delivered through minimally invasive means, and they spontaneously mold to the shape of even the most complicated defects. This has important implications for reducing inflammatory side effects and subsequent scar formation stemming from invasive surgery and imprecise scaffold fit. Injectable biomaterials have been tested in the context of tissue engineering and may be appropriate for facilitating osteogenesis in craniofacial defects. In particular, thermoresponsive biomaterials have been shown to predictably undergo liquid-to-solid phase change at appropriate physiological temperatures and may be a potent delivery mechanism for osteogenic growth factors and progenitor cells.

N-isopropylacrylamide (NIPAA) is a particularly well studied thermoresponsive biomaterial, but it is limited by issues including toxicity, nondegradability, and hydrophobicity-driven syneresis with subsequent release of compounds or lysis of cells entrapped within the scaffold. Many of these limitations may be overcome with incorporation of poly(polyethylene glycol citrate) acrylate (PPCac) to form a poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN) polymer. This material not only preserves the thermoresponsive properties of NIPAA but also possesses higher protein loading efficiency, supports three-dimensional cell proliferation, retains viable cells for at least 72 days, and has intrinsic antioxidant properties.

Hydrogels comprise another important class of osteoconductive scaffolds that can be delivered through noninvasive means. They are water-absorbing matrices composed of cross-linked hydrophilic polymers that are well suited to harboring growth factors and viable stem cells. As a result, hydrogels are ideal for stem cell and biofactor delivery that promote bone tissue regeneration. For example, a composite hydrogel incorporating BMP-2 and synergistic chitosan (deacetylated chitin) has demonstrated controlled release of BMP-2 with minimal burst phase and shows remarkable bone regenerative capability.

Other injectable scaffolds include hydroxyapatite or calcium sulfate pastes, but are complicated by syneresis and contraction, as well as brittleness following setting. Using a combination of these and other materials in injectable composites helps overcome many of the individual materials’ limitations and enhances osteoconductivity. For example, PLGA microspheres coated with HA form a colloidal gel that can be seeded with osteoblastic progenitor cells and successfully support osteogenesis in vivo. Furthermore, PLGA-HA microsphere gel is an effective delivery vehicle for the anti-osteoporotic drug alendronate, demonstrating a sustained drug release profile and minimal burst phase. If this can be replicated with osteoinductive small molecules or growth factors, it would greatly enhance the osteogenic potential of PLGA-HA as a biomaterial for bone tissue regeneration. Another composite microgel scaffold composed of chitin, polycaprolactone, and HA has been investigated with ADSCs and has produced promising results for application in bone tissue engineering. As with other composite scaffolds, relative proportions of each component can be tuned to provide optimal degradation rate, viscoelastic and mechanical properties, cell adhesion properties, and osteoconductivity.

Osteoinductive molecular structure

In addition to the composition of the scaffold, the molecular structure is also a design priority for optimizing osteoconductive and osteoinductive properties. It has been suggested that an optimal approach for bone regeneration should closely mimic that of natural healing, and the design of an osteoinductive scaffold should reflect the basic multicellular unit of corticocancellous bone. This basic structure consists of a long cylindrical unit in line with the bone’s long axis and is composed of osteoclasts on the leading end and osteoblasts laying down new bone on the lagging end. Designing scaffolds to initiate this bone remodeling step without the need to first deposit a temporary bone matrix is a novel idea pursued by some investigators. This strategy would utilize osteoinductive geometric cues within the scaffold to initiate bone formation without the need for exogenous osteogenic molecular signals.

Conclusions and future directions

Thorough understanding of the physiology and molecular pathways involved in bone formation and remodeling is a prerequisite for making advances in craniofacial bone tissue engineering. Innovations in material science and molecular biology have allowed tissue engineers to augment physiologic bone healing and make bone regeneration via scaffold/stem cell therapy a clinical possibility. Combining biomaterials, often with competing properties, to fabricate optimized scaffolds for use in craniofacial skeletal
regeneration is representative of current research trends and the most promising strategy for tissue engineers and craniofacial surgeons. New advances unlocking the osteogenic potential of several stem cell types, as well as the discovery of more readily available stem cell sources (e.g., urine-derived stem cells), are also providing exciting prospects for craniofacial bone regeneration.

Despite such advances in tissue engineering, craniofacial bone reconstruction is often complicated by scarring, osteomyelitis, osteonecrosis, or previous radiation damage. The combination of stem cells, growth factors, small molecules, and scaffold materials used in reparative bone tissue engineering will largely be guided by these and other complicating factors. Still, relatively little research explores the behavior of tissue engineering approaches in the context of extensive medical comorbidities or compromised wound healing capability. Craniofacial skeletal repair via tissue engineering remains the most promising alternative to autologous bone grafts, and numerous modalities involving a variety of stem cells, growth factors, and osteoconductive scaffold materials have been tested and met with success in animal models. In the future, strategies and materials must be refined to achieve more reliable outcomes and to address the various challenges posed by real clinical scenarios in which craniofacial reconstruction is appropriate.

Conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

The reported work was funded in part by the Chicago Biomedical Consortium with support from the Searle Funds at The Chicago Community Trust (RRR, GA), and a NIH/NIDDK Career Development Award (#1K08 DE020140-01; RRR). VT was a recipient of the Pritzker Summer Research Fellowship funded through a National Institutes of Health (NIH) T-35 training grant (NIDDK). ZC was a recipient of the Pritzker Research Fellowship. MKM was a recipient of Howard Hughes Medical Institute Medical Research Fellowship.

References

1. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop. 1986;205:299–308.
2. Van Aalst JA, Eppeley BL, Hathaway RR, Sadove AM. Surgical technique for primary alveolar bone grafting. J Craniofac Surg. 2005;16:706–711.
3. Engstrand T, Kihlström L, Neovius E, et al. Development of a bioactive implant for repair and potential healing of cranial defects. J Neurosurg. 2014;120:273–277.
4. Misch CM. Autogenous bone: is it still the gold standard? Implant Dent. 2010;19:361.
5. Banwart JC, Asher MA, Hassanein RS. Iliac crest bone graft harvest donor site morbidity. A statistical evaluation. Spine. 1995;20:1055–1060.
6. Mangano FG, Zecca PA, van Noort R, et al. Custom-made computer-aided-design/computer-aided-manufacturing biphasic calcium-phosphate scaffold for augmentation of an atrophic mandibular anterior ridge. Case Rep Dent. 2015;2015:e941265.
7. Rosenthal AH, Buchman SR. Volume maintenance of inlay bone grafts in the craniofacial skeleton. Plast Reconstr Surg. 2003;112:802–811.
8. Acarturk TO, Hollinger JO. Commercially available demineralized bone matrix compositions to regenerate calvarial critical-sized bone defects. Plast Reconstr Surg. 2006;118:862–873.
9. Smartt JM, Karmacharya J, Gannon FH, et al. Repair of the immature and mature craniofacial skeleton with a carbonated calcium phosphate cement: assessment of biocompatibility, osteoconductivity, and remodeling capacity. Plast Reconstr Surg. 2005;115:1642–1650.
10. Gosain AK, Persing JA. Biomaterials in the face: benefits and risks. J Craniofac Surg. 1999;10:404–414.
11. Sargent LA, Fulkis KD. Reconstruction of internal orbital fractures with vitallium mesh. Plast Reconstr Surg. 1991;88:31–38.
12. Hurvitz KA, Kobayashi M, Evans GRD. Current options in head and neck reconstruction. Plast Reconstr Surg. 2006;118:122e–133e.
13. Tevlin R, McArdle A, Atashroo D, et al. Biomaterials for craniofacial bone engineering. J Dent Res. 2014;93:1187–1195.
14. Fishero BA, Kohli N, Das A, Christophel JJ, Cui Q. Current concepts of bone tissue engineering for craniofacial bone defect repair. Craniomaxillofacial Trauma Reconstr. 2015;8:23–30.
15. Fu Y, Deng S, Wang J, et al. Potential replication of induced pluripotent stem cells for craniofacial reconstruction. Curr Stem Cell Res Ther. 2014;9:205–214.
16. Zaidi N, Nixon AJ. Stem cell therapy in bone repair and regeneration. Ann N Y Acad Sci. 2007;1117:62–72.
17. Albrektsson T, Johansson C. Osteoinduction, osteoconductivity and osseointegration. Eur Spine J Off Publ Eur Spine Soc Eur Spinal Deform Soc Eur Spor Sect Cerv Spine Res Soc. 2001;10(suppl 2):S96–S101.
18. Zaky SH, Cancetta D. Engineering craniofacial structures: facing the challenge. J Dent Res. 2009;88:1077–1091.
19. Kunert-Keil C, Scholz F, Gedrange T, Gredes T. Comparative study of biphasic calcium phosphate with beta-tricalcium phosphate in rat cranial defects—a molecular-biological and histological study. Ann Anat Anz. 2015;199:79–84.
20. Zhao M, Song B, Pu J, et al. Electrical signals control wound healing through phosphatidylinositol-3-OH kinase-gamma and PTEN. Nature. 2006;442:457–460.
21. McAllister BS, Haghighat K. Bone augmentation techniques. J Periodontol. 2007;78:377–396.
22. McAllister BS, de Bruijn JD, Koole R, van Blitterswijk CA. Cell-based bone tissue engineering in jaw defects. Biomaterials. 2008;29:3053–3061.
23. Gimbel M, Ashley RK, Sidodia M, et al. Repair of alveolar cleft defects: reduced morbidity with bone marrow stem cells in a resorbable matrix. J Craniofac Surg. 2007;18:895–901.
24. McAllister BS, Haghighat K, Gonschor A. Histologic evaluation of a stem cell-based sinus-augmentation procedure. J Periodontol. 2009;80:679–686.
25. Sándor GK, Numminen J, Wolff J, et al. Adipose stem cells used to reconstruct 13 cases with cranio-maxillofacial hard-tissue defects. Stem Cells Transl Med. 2014;3:530–540.
26. Mendonça JJ, Juiz-Lopez P. Regenerative facial reconstruction of terminal stage osteoradionecrosis and other advanced craniofacial diseases with adult cultured stem and progenitor cells. Plast Reconstr Surg. 2010;126:1699–1709.
27. Zamiri B, Shahidi S, Esalminejad MB, et al. Reconstruction of human mandibular continuity defects with allogenic scaffold
and autologous marrow mesenchymal stem cells. J Craniofac Surg. 2013;24:1292–1297.

28. Yamada Y, Nakamura S, Ito K, et al. Injectable bone tissue engineering using expanded mesenchymal stem cells. Stem Cells Dayt Ohio. 2013;31:572–580.

29. Behnia H, Khojasteh A, Soleimani M, Tehranchi A, Atashi A. Repair of alveolar cleft defect with mesenchymal stem cells and platelet derived growth factors: a preliminary report. J Cranio-Maxillo-Fac Surg. Off Publ Eur Assoc Cranio-Maxillo-Fac Surg. 2012;40:2–7.

30. Rickert D, Sauerbier S, Nagursky H, Menne D, Vissink A, Raghoebear GM. Maxillary sinus floor elevation with bovine bone mineral combined with either autogenous bone or autogenous stem cells: a prospective randomized clinical trial. Clin Oral Implants Res. 2011;22:251–258.

31. ShayestehYS, Khojasteh A, Soleimani M, Alkhashi M, Khoshzaban A, Ahmadbeigi N. Sinus augmentation using human mesenchymal stem cells loaded into a beta-tricalcium phosphate/hydroxyapatite scaffold. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008;106:203–209.

32. Ueda M, Yamada Y, Ozawa R, Okazaki Y. Clinical case reports and miR-148b. J Orthop Res Off Publ Orthop Res Soc. 2009;27:1074–1081.

33. Li A, Xia X, Yeh J, et al. PDGF-AA promotes osteogenic differentiation and migration of mesenchymal stem cell by down-regulating PDGFRα and derepressing BMP-Smad1/5/8 signaling. PLoS One. 2015;10:e0118823.

34. Cheng MT, Liu C, Hyun JS, et al. CD90 (Thy-1)-Positive selection enhances osteogenic capacity of human adipose-derived stromal cells. Tissue Eng Part A. 2012;19:989–997.

35. Kaigler D, Avila-Ortiz G, Travan S, et al. Bone engineering of osteoblastic progenitor cells in combination with enriched CD90+ cells in women with osteopenia. Tissue Eng Part A. 2015;21:898–908.

36. Chung MT, Liu C, Hyun JS, et al. CD90 (Thy-1)-Positive selection enhances osteogenic capacity of human adipose-derived stromal cells. Tissue Eng Part A. 2012;19:989–997.

37. Lam RM, Lee YJ, Sun Y, et al. In vitro and in vivo evaluation of bone regeneration using recombinant adenoviral vector carrying the human BMP-2 gene. J Craniomaxillofac Surg. 2014;42:1794–1806.

38. Eldesoqi K, Seebach C, Nguyen Ngoc C, et al. High calcium bioglass enhances differentiation and survival of endothelial progenitor cells, inducing early vascularization in critical size bone defects. PLoS One. 2013;8:e79058.
62. Helm GA, Alden TD, Beres EJ, et al. Use of bone morphogenic protein-9 gene therapy to induce spinal arthrodesis in the rodent. J Neurosurg. 2000;92(2 suppl):191–196.

63. Lee AR, Wilkins AC, Leather C, Brenton AG. Translational energy spectra for single-electron capture by O2 as in He, Ne, and Ar. Phys Rev A. 1994;50:1149–1154.

64. Partridge K, Yang X, Clarke NMP, et al. Adenoviral BMP-2 gene transfer in mesenchymal stem cells: in vitro and in vivo bone formation on biodegradable polymer scaffolds. Biochem Physiol Res Commun. 2002;292:144–152.

65. Varady P, Li JZ, Tang N, et al. Regulation of osteogenic differentiation of mesenchymal stem cells. Acad Radiol. 2002;9:632–637.

66. Lamplot JD, Qin J, Nan G, et al. BMP9 signaling in stem cell differentiation and osteogenesis. Am J Stem Cells. 2013;2:1–21.

67. Luu HH, Song W-X, Luo X, et al. Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells. J Orthop Res Off Publ Orthop Res Soc. 2007;25:665–677.

68. Deng Z-L, Sharff KA, Tang N, et al. Regulation of osteogenic differentiation during skeletal development. Front Biosci J Virtual Libr. 2008;13:2001–2021.

69. Ducy P, Karsenty G. The family of bone morphogenetic proteins. Kidney Int. 2000;57:2207–2214.

70. Kang Q, Song W-X, Luo Q, et al. A comprehensive analysis of the dual roles of BMPs in regulating adipogenic and osteogenic differentiation of mesenchymal progenitor cells. Stem Cells Dev. 2009;18:545–559.

71. Luo J, Sun MH, Kang Q, et al. Gene therapy for bone regeneration. Curr Gene Ther. 2005;5:167–179.

72. Reddi AH. Role of morphogenetic proteins in skeletal tissue engineering and regeneration. Nat Biotechnol. 1998;16:247–252.

73. Shi Y, Massagué J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. Cell. 2003;113:685–700.

74. Tang N, Song W-X, Luo J, Haydon RC, He T-C. Osteosarcoma development and stem cell differentiation. Clin Orthop. 2008;466:2114–2130.

75. Wagner ER, He B-C, Chen L, et al. Therapeutic implications of PPAR gamma in human osteosarcoma. PPAR Res. 2010;2010:965427.

76. Cahill KS, Chi JH, Day A, Claus EB. Prevalence, complications, and hospital charges associated with use of bone morphogenetic protein-2 in transforaminal lumbar interbody fusion. J Neurosurg Spine. 2010;12:40–46.

77. Aghaloo T, Jiang X, Soo C, et al. A study of the role of neri-1 gene modified goat bone marrow stromal cells in promoting new bone formation. MOL Ther J Am Soc Gene Ther. 2007;15:1872–1880.

78. Chen N-F, Smith ZA, Larysyens, Anand N. Adverse swelling associated with use of rh-BMP-2 in anterior cervical discectomy and fusion: a case study. Spine J Off J North Am Spine Soc. 2007;7:235–239.

79. Perri B, Cooper M, Lauryssen C, Anand N. Adverse swelling associated with use of rh-BMP-2 in anterior cervical discectomy and fusion: a case study. Spine J Off J North Am Spine Soc. 2007;7:235–239.

80. Issa JPM, Bentley MVLB, lyomasa MM, Sebald W, De Albuquerque RF. Sustained release carriers used to delivery bone morphogenetic proteins in the bone healing process. Anat Histol Embryol. 2008;37:181–187.

81. Cao L, Wang J, Hou J, Xing W, Liu C. Vascularization and bone regeneration in a critical sized defect using 2-H, 6-D-sulfated chitosan nanoparticles incorporating BMP-2. Biomaterials. 2014;35:684–698.

82. Amini AR, Laurencin CT, Nukavarapu SP. Bone tissue engineering: recent advances and challenges. Crit Rev Biomed Eng. 2012;40:363–408.

83. Scheller EL, Krebsbach PH. Gene therapy: design and prospects for craniofacial regeneration. J Dent Res. 2009;88:585–596.

84. Scheller EL, Villa-Diaz LG, Krebsbach PH. Gene therapy: implications for craniofacial regeneration. J Craniofac Surg. 2012;23:333–337.

85. Zisch AH, Lutolf MP, Ehrbar M, et al. Cell-damaged release of VEGF from synthetic, biointeractive cell-ingrowth matrices for vascularized tissue growth. FASEB J. 2003;17:2260–2262.

86. Ehrbar M, Djonov VG, Schnell C, et al. Cell-damaged liberation of VEGF121 from fibrin implants induces local and controlled blood vessel growth. Circ Res. 2004;94:1124–1132.

87. Fong KD, Nacamuli RP, Song HM, Warren SM, Lorenz HP, Longaker MT. New strategies for craniofacial repair and replacement: a brief review. J Craniofac Surg. 2003;14:333–339.

88. Laurencin CT, Ashe KM, Henry N, Kan HM, Lo KW-H. Delivery of small molecules for bone regenerative engineering: preclinical studies and potential clinical applications. Drug Discov Today. 2014;19:794–800.

89. Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. Science. 1999;286:1946–1949.

90. Zhou Y, Ni Y, Liu Y, Zeng B, Xu Y, Ge W. The role of simvastatin in the osteogenesis of injectable tissue-engineered bone based on human adipose-derived stromal cells and platelet-rich plasma. Biomaterials. 2010;31:5325–5335.

91. Dai W, Dong J, Fang T, Uemura T. Stimulation of osteogenic activity in mesenchymal stem cells by FK506. J Biomed Mater Res A. 2008;86:235–243.

92. Isomoto S, Hattori K, Ohgushi H, Nakajima H, Tanaka Y, Takakura Y. Rapamycin as an inhibitor of osteogenic differentiation in bone marrow-derived mesenchymal stem cells. J Orthop Sci Off J Jpn Orthop Assoc. 2007;12:83–88.

93. Park KW, Waki H, Kim W-K, et al. The small molecule phenamil induces osteoblast differentiation and mineralization. Mol Cell Biol. 2009;29:3905–3914.

94. Lo KW-H, Ulery BD, Kan HM, Ashe KM, Laurencin CT. Evaluating the feasibility of utilizing the small molecule phenamil as a novel biofactor for bone regenerative engineering. J Tissue Eng Regen Med. 2014;8:728–736.

95. Kim J-G, Son KM, Park HC, Zhu T, Kwon JH, Yang H-C. Stimulating effects of quercetin and phenamil on differentiation of human dental pulp cells. J Craniofac Surg. 2014;25:665–676.

96. Lo KW-H, Kan HM, Laurencin CT. Short-term administration of statins results in decreased osteogenic activity in mesenchymal stem cells. J Orthop Sci Off J Jpn Orthop Assoc. 2007;12:83–88.

97. Fan J, Im CS, Cui Z-K, et al. Delivery of phenamil enhances BMP-2-induced osteogenic differentiation of adipose-derived stem cells and bone formation in calvarial defects. Tissue Eng Part A. 2015;21:2053–2065.

98. Marseill R, Einhorn TA. The biology of fracture healing. Injury. 2011;42:551–555.

99. Tuli SM, Singh AD. The osteoninductive property of decalcified bone matrix: a review. J Biomech. 2008;41:3333–3337.

100. Bucholz RW, Carlton A, Holmes RE. Hydroxyapatite and tricalcium phosphate bone graft substitutes. Orthop Clin North Am. 1987;18:323–334.

101. Gentili C, Cancedda R. Cartilage and bone extracellular matrix. Curr Pharm Des. 2009;15:1334–1348.

102. Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. Cell. 2006;126:677–689.
120. Habibovic P, de’Aquino R, Cusella-De Angelis MG, et al. Scaffold’s surface geometry significantly affects human stem cell bone tissue engineering. *J Cell Physiol.* 2008;214:166–172.

121. Ward B, Brown S, Krebsbach P. Bioengineering strategies for regeneration of craniofacial bone: a review of emerging technologies. *Oral Dis.* 2010;16:709–716.

122. Wang XX, Allen RJ, Tutela JP, et al. Progenitor cell mobilization enhances bone healing by means of improved neoangiogenesis and osteogenesis. *Plast Reconstr Surg.* 2011;128:395–405.

123. Nomia M, Miyake H, Sugita Y, Fujisawa M, Soker S. Role of growth factors and endothelial cells in therapeutic angiogenesis and tissue engineering. *Curr Stem Cell Res Ther.* 2006;1:333–343.

124. Lovett M, Lee K, Edwards A, Kaplan DL. Vascularization strategies for tissue engineering. *Tissue Eng Part B Rev.* 2009;15:351–370.

125. Yang J, Zhou W, Zheng W, et al. Effects of myocardial transplantation of marrow mesenchymal stem cells transfected with vascular endothelial growth factor for the improvement of heart function and angiogenesis after myocardial infarction. *Cardiology.* 2007;107:17–29.

126. Mark von der K, Park J, Bauer S, Schmuki P. Nanoscale engineering of biomimetic surfaces: cues from the extracellular matrix. *Cell Tissue Res.* 2009;339:131–153.

127. Conconi MT, Ghezzo F, Dettin M, et al. Effects on in vitro and in vivo angiogenesis induced by small peptides carrying adhesion sequences. *J Pept Sci.* 2010;16:349–357.

128. Kaigler D, Pagni G, Park C-H, Tarle SA, Bartel RL, Giannobile WV. Radioprotective and osteogenic potential of bone repair cells for craniofacial regeneration. *Tissue Eng Part A.* 2010;16:2809–2820.

129. Warnke PH, Springer ING, Wiltfang J, et al. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet.* 2004;364:766–770.

130. McDowell F. Plastic surgery in the twentieth century. *Ann Plast Surg.* 1978;1:217–224.

131. Kusumbe AP, Ramasamy SK, Adams RH. Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. *Nature.* 2014;507:323–328.

132. Hollister SJ. Porous scaffold design for tissue engineering. *Nat Mater.* 2005;4:518–524.

133. Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. *J Bone Jt Surg Am.* 2004;86-A:1541–1558.

134. Adachi T, Osako Y, Tanaka M, Hojo M, Hollister SJ. Framework for optimal design of porous scaffold microstructure by computational simulation of bone regeneration. *Biomaterials.* 2006;27:3964–3972.

135. Jones AC, Arns CH, Sheppard AP, Milthorpe BK. Assessment of bone ingrowth into porous biomaterials using MICRO-CT. *Biomaterials.* 2007;28:2491–2504.

136. Mao JJ, Giannobile WV, Helms JA, et al. Craniofacial tissue engineering of biomimetic surfaces: cues from the extracellular matrix. *Cell Tissue Res.* 2006;77A:889–909.

137. Mitchell MJ, Logan PM. Radiation-induced changes in bone. *Int J Radiat Oncol Biol Phys.* 1998;45:1125–1136.

138. Spear MA, Dupuy DE, Park JJ, Halpern EF, Spiro JJ. Tolerance of autologous and allogeneic bone grafts to therapeutic radiation in humans. *Int J Radiat Oncol Biol Phys.* 1999;45:1275–1280.

139. Okunieff P, Mester M, Wang J, et al. In vivo radioprotective effects of angiogenic growth factors on the small bowel of C3H mice. *Radiat Res.* 1998;150:204–211.

140. Okunieff P, Wang X, Rubin P, Finkelstein JN, Constine LS, Ding I. Radiation-induced changes in bone perfusion and angiogenesis. *Int J Radiat Oncol Biol Phys.* 1998;42:885–889.

141. Nussenbaum B, Rutherford RB, Krebsbach PH. Bone regeneration in cranial defects previously treated with radiation. *Laryngoscope.* 2005;115:1170–1177.
Ceramic and non-ceramic hydroxyapatite as a bone graft material: a brief review—Springer.

Mantripragada VP, Lecka-Czernik B, Ebraheim NA, Jayasuriya AC. An overview of recent advances in designing orthopedic and craniofacial implants. J Biomed Mater Res. 2013;101:3349–3364.

Preparation of porous PLA/DBM composite biomaterials and experimental research of repair rabbit radius segmental bone defect—Springer.

B N, H N, A KM, A BH. Demineralized dentin matrix and bone graft: a review of literature. J West Soc Periodontal Abstr. 2013;62:35–38.

Plum AW, Tatum SA. A comparison between autograft and bone cement, and demineralized bone matrix in cranioplasty. Laryngoscope. 2015;125:1322–1332.

Schwartz C, Liss P, Jacqueme B, Lecestre P, Frayssinet P. Biphasic synthetic bone substitute use in orthopedic and trauma surgery: clinical, radiological and histological results. J Mater Sci Mater Med. 1999;10:821–825.

Nihouannen DL, Duval L, Lecomte A, et al. Interactions of total bone marrow cells with increasing quantities of macroporous calcium phosphate ceramic granules. J Mater Sci Mater Med. 2007;18:1983–1990.

Walsh WR, Vizese F, Michael D, et al. Ji-TCP bone graft substitutes in a bilateral rabbit tibial defect model. Biomaterials. 2008;29:266–271.

Cancedda R, Giannoni P, Mastrogiacomo M. A tissue engineering approach to bone repair in large animal models and in clinical practice. Biomaterials. 2007;28:4242–4250.

Papadimitropoulos A, Mastrogiacomo M, Peyrin F, et al. Kinetics of in vivo bone deposition by bone marrow stromal cells within a resorbable porous calcium phosphate scaffold: an X-ray computed microtomography study. Biotechnol Bioeng. 2007;98:271–281.

Friedman CD, Costantino PD, Takagi S, Chow LC. Bone source hydroxyapatite cement: a novel biomaterial for craniofacial skeletal tissue engineering and reconstruction. J Biomed Mater Res, 1998;43:428–432.

Thein-Han W, Xu HHK. Collagen-calcium phosphate cement scaffolds seeded with umbilical cord stem cells for bone tissue engineering. Tissue Eng Part A. 2011;17:2943–2954.

Kim S-S, Ahn K-M, Park MS, Lee J-H, Choi CY, Kim B-S. A macroporous calcium phosphate ceramic granules. J Biomed Mater Res A. 2008;80:206–215.

Costantino PD, Hiltzik D, Govindaraj S, Moche J. Bone healing and bone substitutes. Facial Plast Surg Surg. 2002;18:13–26.

Wikesjö UME, Lim WH, Razi SS, et al. Periodontal repair and regeneration using polyglycolic acid sheet insertion repair and regeneration using polyglycolic acid sheet in the rabbit rotator cuff injury model. Am J Sports Med. 2008;36:1298–1309.

Sedrakyan S, Zhou ZY, Perlin L, Leach K, Mooney D, Kim TH. Tissue engineering of a small hand phalanx with a porous casted polylactic acid–polycyclic acid copolymer. Tissue Eng. 2006;12:2675–2683.

Mooney DJ, Mazzoni CL, Breuer C, et al. Stabilized polycyclic acid fibre-based tubes for tissue engineering. Biomaterials. 1996;17:115–124.

Ishaug SL, Crane GM, Miller MJ, Yasko AW, Yaszemski MJ, Mikos AG. Bone formation by three-dimensional stromal osteoblast culture in biodegradable polymer scaffolds. J Biomed Mater Res. 1997;36:17–28.

Ishaug-Riley SL, Crane GM, Gurlék A, et al. Student research award in the doctoral degree candidate category, society for biomaterials 23rd annual meeting, New Orleans, LA, April 30–May 4, 1997: ectopic bone formation by marrow stromal osteoblast transplantation using polylactic-co-glycolic acid foams implanted into the rat mesentery. J Biomed Mater Res. 1997;36:1–8.

Ca V, Jp V. Bone and cartilage reconstruction with tissue engineering approaches. Otolaryngol Clin North Am. 1994;27:263–276.

Gentile P, Chiono V, Carmagnola I, Hatton PV. An overview of polylactic-co-glycolic acid (PLGA)-based biomaterials for bone tissue engineering. J Mol Sci. 2014;15:3640–3659.

Pan Z, Ding J. Polylactic-co-glycolic acid) porous scaffolds for tissue engineering and regenerative medicine. Interface Focus. 2012;2:366–377.

Henslee AM, Gwak D-H, Mikos AG, Kasper FK. Development of a biodegradable bone cement for craniofacial applications. J Biomed Mater Res A. 2012;100A:2252–2259.

Hedberg EL, Kroese-Deutman HC, Shih CK, et al. In vivo degradation of porus poly(propylene fumarate)/poly(DL-lactic-co-glycolic acid) composite scaffolds. Biomaterials. 2005;26:4616–4623.

Mistry AS, Pham QP, Schouten C, et al. In vivo bone biocompatibility and degradation of porous fumarate-based polymer/alumoxane nanocomposites for bone tissue engineering. J Biomed Mater Res A. 2010;92:451–462.

Timmer MD, Ambrose CG, Mikos AG. In vitro degradation of polymeric networks of poly(propylene fumarate) and the crosslinking macromer poly(propylene fumarate)-diacylate. Biomaterials. 2003;24:571–577.

Peter SJ, Miller ST, Zhu G, Yasko AW, Mikos AG. In vivo degradation of a poly(propylene fumarate)/β-tricalcium phosphate injectable composite scaffold. J Biomed Mater Res. 1998;41:1–7.

Fisher JP, Vehof JWM, Dean D, et al. Soft and hard tissue response to photocrosslinked poly(propylene fumarate) scaffolds in a rabbit model. J Biomed Mater Res. 2002;59:547–556.

Kim K, Dean D, Mikos AG, Fisher JP. Effect of initial cell seeding density on early osteogenic signal expression of rat bone marrow stromal cells cultured on cross-linked...
poly(propylene fumarate) disks. Biomacromolecules. 2009; 10:1810–1817.

182. Fisher JP, Holland TA, Dean D, Engel PS, Mikos AG. Synthesis and properties of photocross-linked poly(propylene fumarate) scaffolds. J Biomater Sci Polym Ed. 2001;12:673–687.

183. Chu T-MG, Warden SJ, Turner CH, Stewart RL. Segmental bone regeneration using a load-bearing biodegradable carrier of bone morphogenetic protein-2. Biomaterials. 2007;28: 459–467.

184. Henslee AM, Spicer PP, Yoon DM, et al. Biodegradable composite scaffolds incorporating an intramedullary rod and delivering bone morphogenetic protein-2 for stabilization and bone regeneration in segmental long bone defects. Acta Biomater. 2011;7:3627–3637.

185. Peter SJ, Kim P, Yasko AW, Yaszenski MJ, Mikos AG. Cross-linking characteristics of an injectable poly(propylene fumarate)/β-tricalcium phosphate paste and mechanical properties of the crosslinked composite for use as a biodegradable bone cement. J Biomed Mater Res. 1999;44:314–321.

186. Yan J, Li J, Runge MB, et al. Cross-linking characteristics and mechanical properties of an injectable biomaterial composed of polypropylene fumarate and polycaprolactone co-polymer. J Biomater Sci Polym Ed. 2011;22:489–504.

187. Becker J, Lu L, Runge MB, Zeng H, Yaszenski MJ, Dadsetan M. Nanocomposite bone scaffolds based on biodegradable polymers and hydroxyapatite. J Biomed Mater Res A. 2015;103: 2549–2557.

188. Ng KW, Hutmacher DW, Schantz J-T, et al. Evaluation of ultra-thin poly(ε-caprolactone) films for tissue-engineered skin. Tissue Eng. 2001;7:441–455.

189. Elfick APD. Poly(ε-caprolactone) as a potential material for a temporary joint spacer. Biomaterials. 2002;23:4463–4467.

190. Kweon H, Yoo MK, Park IK, et al. A novel degradable poly-caprolactone networks for tissue engineering. Biomaterials. 2003;24:801–808.

191. Li J, Li Y, Ma S, Gao Y, Zuo Y, Hu J. Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nanohydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. J Biomed Mater Res A. 2010;95A: 973–981.

192. Staiger MP, Pietak AM, Huadmaj J, Dias G. Magnesium and its alloys as orthopedic biomaterials: a review. Biomaterials. 2006; 27:1728–1734.

193. Di Mario C, Griffiths H, Goktekin O, et al. Drug-eluting bioabsorbable magnesium stent. J Interv Cardiol. 2004;17: 391–395.

194. Peeters P, Bosiers M, Verbiest J, Deloose K, Heublein B. Long-term biocompatibility of a bioabsorbable magnesium stent in patients with critical limb ischemia. J Endovasc Ther. 2005;12:1–5.

195. Witte F, Kaese V, Haferkamp H, et al. In vivo corrosion of four magnesium alloys and the associated bone response. Biomaterials. 2005;26:3557–3563.

196. Waksman R, Pakala R, Kuchulakanti PK, et al. Safety and efficacy of bioabsorbable magnesium alloy stents in porcine coronary arteries. Catheter Cardiovasc Interv. 2006;68: 607–617.

197. Händz AC, Sologubenko AS, Uggowitzer PJ. Design strategy for microalloyed ultra-ductile magnesium alloys for biomedical application. Mater Sci Forum. 2009;619:77–82.

198. Zhang E, Yang L. Microstructure, mechanical properties and bio-corrosion properties of Mg–Zn–Mn–Ca alloy for biomedical application. Mater Sci Eng A. 2008;497:111–118.

199. Li Z, Gu X, Lou S, Zheng Y. The development of binary Mg–Ca alloys for use as biodegradable materials within bone. Biomaterials. 2008;29:1329–1344.

200. Peuster M, Wohlein P, Brügmann M, et al. A novel approach to temporary stenting: degradable cardiovascular stents produced from corrodbile metal—results 6–18 months after implantation into New Zealand white rabbits. Heart. 2001;86: 563–569.

201. Peuster M, Hesse C, Schloo T, Fink C, Beerbaum P, von Schnakenburg C. Long-term biocompatibility of a corrodbile peripheral iron stent in the porcine descending aorta. Biomaterials. 2006;27:4955–4962.

202. Hermawan H, Alamdari H, Mantovani D, Dubé D. Iron-manganese: new class of metallic degradable biomaterials prepared by powder metallurgy. Powder Metall. 2008;51: 38–45.

203. Schinhammer M, Hänzi AC, Löffler JF, Uggowitzer PJ. Design strategy for biodegradable Fe-based alloys for medical applications. Acta Biomater. 2010;6:1705–1713.

204. Farack J, Wolf-Brandstetter C, Glorius S, et al. The effect of perfusion culture on proliferation and differentiation of human mesenchymal stem cells on biocorrosible bone replacement material. Mater Sci Eng B. 2011;176: 1767–1772.

205. Zhuang H, Han Y, Feng A. Preparation, mechanical properties and in vitro biodegradation of porous magnesium scaffolds. Mater Sci Eng C. 2008;28:1462–1466.

206. Wen CE, Yamada Y, Shimojima K, Chino Y, Hosokawa H, Mabuchi M. Compressibility of porous magnesium foam: dependency on porosity and pore size. Mater Lett. 2004;58: 357–360.

207. Tran N, Webster TJ. Increased osteoblast functions in the presence of hydroxyapatite-coated iron oxide nanoparticles. Acta Biomater. 2011;7:1298–1306.

208. Ricci JL, Clark EA, Murrkly A, Smay JE. Three-dimensional printing of bone repair and replacement materials: impact on craniofacial surgery. J Craniofac Surg. 2012;23:304–308.

209. Shee MY, Chang HC, Ding SJ. Effects of altering the Si/Ca molar ratio of a calcium silicate cement on in vitro cell attachment. Int Endod J. 2012;45:337–345.

210. Ni S, Chang J, Chou L. A novel bioactive porous CaSiO3 scaffold for bone tissue engineering. J Biomed Mater Res A. 2006; 76A:196–205.

211. Wang C, Lin K, Chang J, Sun J. Osteogenesis and angiogenesis induced by porous β-CaSiO3/PDLGA composite scaffold via activation of AMPK/ERK1/2 and PI3K/Akt pathways. Biomaterials. 2013;34:64–77.

212. Sun J, Wei L, Liu X, et al. Influences of ionic dissolution products of dicalcium silicate coating on osteoblastic proliferation, differentiation and gene expression. Acta Biomater. 2009;5:1284–1293.

213. Gomes S, Renaudin G, Mesbah A, et al. Thorough analysis of silicon substitution in biphasic calcium phosphate bio-ceramics: a multi-technique study. Acta Biomater. 2010;6: 3264–3274.

214. Reid JW, Tuck L, Sayar M, Fargo K, Hendry JA. Synthesis and characterization of single-phase silicon-substituted α-tricalcium phosphate. Biomaterials. 2006;27:2916–2925.

215. Li H, Xue K, Liu J, Liu K, Chang J. Silicate bioceramics and in vitro biodegradation of porous magnesium scaffolds. J Biomed Mater Res A. 2009;5:1284–1293.

216. Wu C, Zhang Y, Fan W, et al. CaSiO3 microstructure modulating the in vitro and in vivo bioactivity of poly(lactide-glycolide) microspheres. J Biomed Mater Res A. 2011;98A: 122–131.

217. Li H, Zhai W, Chang J. Effects of wollastonite on proliferation and differentiation of human bone marrow-derived stromal cells in PHBV/wollastonite composite scaffolds. J Biomater Appl. 2009;24:231–246.

218. Li H, Chang J. Fabrication and characterization of bioactive wollastonite/PHBV composite scaffolds. Biomaterials. 2004;25: 5473–5480.
| Page | Reference |
|------|-----------|
| 220 | Ding S-J, Shie M-Y, Hoshiba T, Kawazoe N, Chen G, Chang H-C. Osteogenic differentiation and immune response of human bone-marrow-derived mesenchymal stem cells on injectable calcium-silicate-based bone grafts. *Tissue Eng Part A*. 2010;16: 2343–2354. |
| 221 | Li H, Chang J. Stimulation of proangiogenesis by calcium silicate bioactive ceramic. *Acta Biomater*. 2013;9:5379–5389. |
| 222 | Zhai W, Lu H, Wu C, et al. Stimulatory effects of the ionic products from Ca–Mg–Si bioceramics on both osteogenesis and angiogenesis in vitro. *Acta Biomater*. 2013;9: 8004–8014. |
| 223 | Wu C, Chang J. A review of bioactive silicate ceramics. *Biomater Med Bristol Engl*. 2013;8:032001. |
| 224 | Li H, Chang J. Preparation and characterization of bioactive and biodegradable wollastonite/poly(ε-caprolactone) composite scaffolds. *J Mater Sci Mater Med*. 2004;15:1089–1095. |
| 225 | Li H, Chang J. pH-compensation effect of bioactive inorganic fillers on the degradation of PLGA. *Compos Sci Technol*. 2005; 65:2226–2232. |
| 226 | Van Vlierberghe S, Dubrueil P, Schacht E. Biopolymer-based hydrogels as scaffolds for tissue engineering applications: a review. *Biomacromolecules*. 2011;12:1387–1408. |
| 227 | Ni P, Ding Q, Fan M, et al. Injectable thermosensitive PEG–PLA–PEG hydrogel/acellular bone matrix composite for bone regeneration in cranial defects. *Biomaterials*. 2014;35:236–248. |
| 228 | He C, Kim SW, Lee DS. In situ gelling stimulsi-sensitive block copolymer hydrogels for drug delivery. *J Control Release*. 2008;127:189–207. |
| 229 | Kavanagh CA, Roche YA, Gallagher WM, Dawson KA, Keenan AK. Local drug delivery in restenosis injury: thermoresponsive co-polymers as potential drug delivery systems. *Pharmacol Ther*. 2004;102:1–15. |
| 230 | Ganta S, Devalapally H, Shahiwala A, Amiji M. A review of approaches to craniofacial defect repair. 2014;35:236–248. |
| 231 | Brun-Graeppi AKAS, Richard C, Bessodes M, Scherman D, Merren O-W. Cell microcarriers and microcapsules of stimuli-responsive polymers. *J Control Release*. 2011;149:209–224. |
| 232 | Cellesi F. Thermoresponsive hydrogels for cellular delivery. *Ther Deliv*. 2012;3:1395–1407. |
| 233 | Klouda L, Mikos AG. Thermoresponsive hydrogels in biomedical applications. *Eur J Pharm Biopharm*. 2008;68:34–45. |
| 234 | Jasionowski M, Krzyminski K, Chrisler W, Markille LM, Morris J, Gutowska A. Thermally-reversible gel for 3-D cell culture of chondrocytes. *J Mater Sci Mater Med*. 2004;15:575–582. |
| 235 | Wu C, Zhou S. Light scattering study of spherical poly(ε-caprolactone) microgels. *J Macromol Sci Part B*. 1997; 36:345–355. |
| 236 | Otake K, Inomata H, Konno M, Saito S. Thermal analysis of the volume phase transition with N-isopropylacrylamide gels. *Macromolecules*. 1990;23:283–289. |
| 237 | Hacker MC, Klouda L, Ma BB, Kretlow JD, Mikos AG. Synthesis and characterization of injectable, thermally and chemically gelable, amphiphilic poly(ε-isopropylacrylamide)-based macromers. *Biomacromolecules*. 2008;9:1558–1570. |
| 238 | Lau TT, Wang D-A. Stromal cell-derived factor-1 (SDF-1): homing factor for engineered regenerative medicine. *Expert Opin Biol Ther*. 2011;11:189–197. |
| 239 | Van Lith R, Gregory EK, Yang J, Kibbe MR, Ameer GA. Engineering biodegradable polyester elastomers with antioxidant properties to attenuate oxidative stress in tissues. *Biomaterials*. 2014;35:8113–8122. |
| 240 | Diniz IM, Chen C, Xu X, et al. Pluronic F-127 hydrogel as a promising scaffold for encapsulation of dental-derived mesenchymal stem cells. *J Mater Sci Mater Med*. 2015;26:153. |
| 241 | Hennink WE, van Nostrum CF. Novel crosslinking methods to design hydrogels. *Adv Drug Deliv Rev*. 2002;54:13–36. |
| 242 | Lee S-H, Shin H. Matrices and scaffolds for delivery of bioactive molecules in bone and cartilage tissue engineering. *Adv Drug Deliv Rev*. 2007;59:339–359. |
| 243 | Shah AM, Jung H, Skirboll S. Materials used in cranioplasty: a history and analysis. *Neurosurg Focus*. 2014;36:E19. |
| 244 | Kretlow JD, Young S, Klouda L, Wong M, Mikos AG. Injectable biomaterials for regenerating complex craniofacial tissues. *Adv Mater*. 2009;21:3368–3393. |
| 245 | Fu S, Ni P, Wang B, et al. In vivo biocompatibility and osteogenesis of electrospin poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone)/nano-hydroxyapatite composite scaffold. *Biomaterials*. 2012;33:8363–8371. |
| 246 | Fu S, Ni P, Wang B, et al. Injectable and thermo-sensitive PEG–PCL–PEG copolymer/collagen/n-HA hydrogel composite for guided bone regeneration. *Biomaterials*. 2012;33:4801–4809. |
| 247 | Wang Q, Gu Z, Jamal S, Detamore MS, Berkland C. Hybrid hydroxyapatite nanoparticle colloidal gels are injectable fillers for bone tissue engineering. *Tissue Eng Part A*. 2013;19: 2586–2593. |
| 248 | Kang S-W, Yang HS, Seo S-W, Han DK, Kim B-S. Apatite-coated poly(ε-caprolactone-co-glycolic acid) microspheres as an injectable scaffold for bone tissue engineering. *J Biomed Mater Res A*. 2008;85A:747–756. |
| 249 | Shi X, Wang Y, Ren L, Gong Y, Wang D-A. Enhancing alendronate release from a novel PLGA/hydroxyapatite microspheric system for bone repairing applications. *Pharm Res*. 2008;26: 422–430. |
| 250 | Woodruff MA, Hutmacher DW. The return of a forgotten polymer—polycaprolactone in the 21st century. *Prog Polym Sci*. 2010;35:1217–1256. |
| 251 | Zakaria SM, Sharif Zein SH, Othman MR, Yang F, Jansen JA. Nanophase hydroxyapatite as a biomaterial in advanced hard tissue engineering: a review. *Tissue Eng Part B Rev*. 2013;19: 431–441. |
| 252 | Ripamonti U, Roden LC, Ferretti C, Klar RM. Biomimetic matrices self-initiating the induction of bone formation. *J Craniofac Surg*. 2011;22:1859–1870. |
| 253 | Wu Q, Shao H, Darwin ED, et al. Extracellular calcium increases CXCR4 expression on bone marrow-derived cells and enhances pro-angiogenesis therapy. *J Cell Mol Med*. 2009;13:3764–3773. |