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REVIEW ARTICLE

Stem cells, growth factors and scaffolds in craniofacial regenerative medicine

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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 osteoconduction, 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.

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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-ossification are termed 'critical-sized' and often require complex reconstructive approaches (Fig. 1A).[1] Repair of these defects has traditionally required autologous bone grafts from a variety of sources, including cranium, tibia, rib, and iliac crest (Fig. 1B).[2,3] 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.[4–6] 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.[2,3,7]

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.[8–12] Permanent methods of rigid fixation utilizing metals or metal alloys suffer similar limitations in addition to integrating poorly with the surrounding tissue.[13] 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.[14] 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.[15] 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*.[16] 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.[17–19]

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.[20] 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.[21] These precursors migrate to the injury site and differentiate into osteoblasts and endothelial cells, promoting bone formation and vascularization.[22] 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.[22–31]

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.[32–34] 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.[35,36] 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.[35] 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.[37] However, despite its success, the use of BMSCs is limited by finite supply and the morbidity associated with procurement procedures.[38]

Adipose-derived stem cells (ADSCs) represent a promising alternative to BMSCs in that they are more plentiful, less painful to harvest, and easily expandable.[39] ADSCs have showed similar osteogenicity to BMSCs, with certain subpopulations demonstrating enhanced tendency toward osteoblast differentiation and others successfully induced through gene therapy.[34,40] 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.[38,41–43] USCs are biologically similar to ADSCs and are capable of osteogenic differentiation *in vitro*.[43] Furthermore, USCs have successfully differentiated into osteoblasts via calcium silicate ion induction of the Wnt/β-catenin signaling pathway.[38] They have also been shown to be compatible with both calcium sulfate/PLGA composite and β-TCP scaffolds.[38,42]

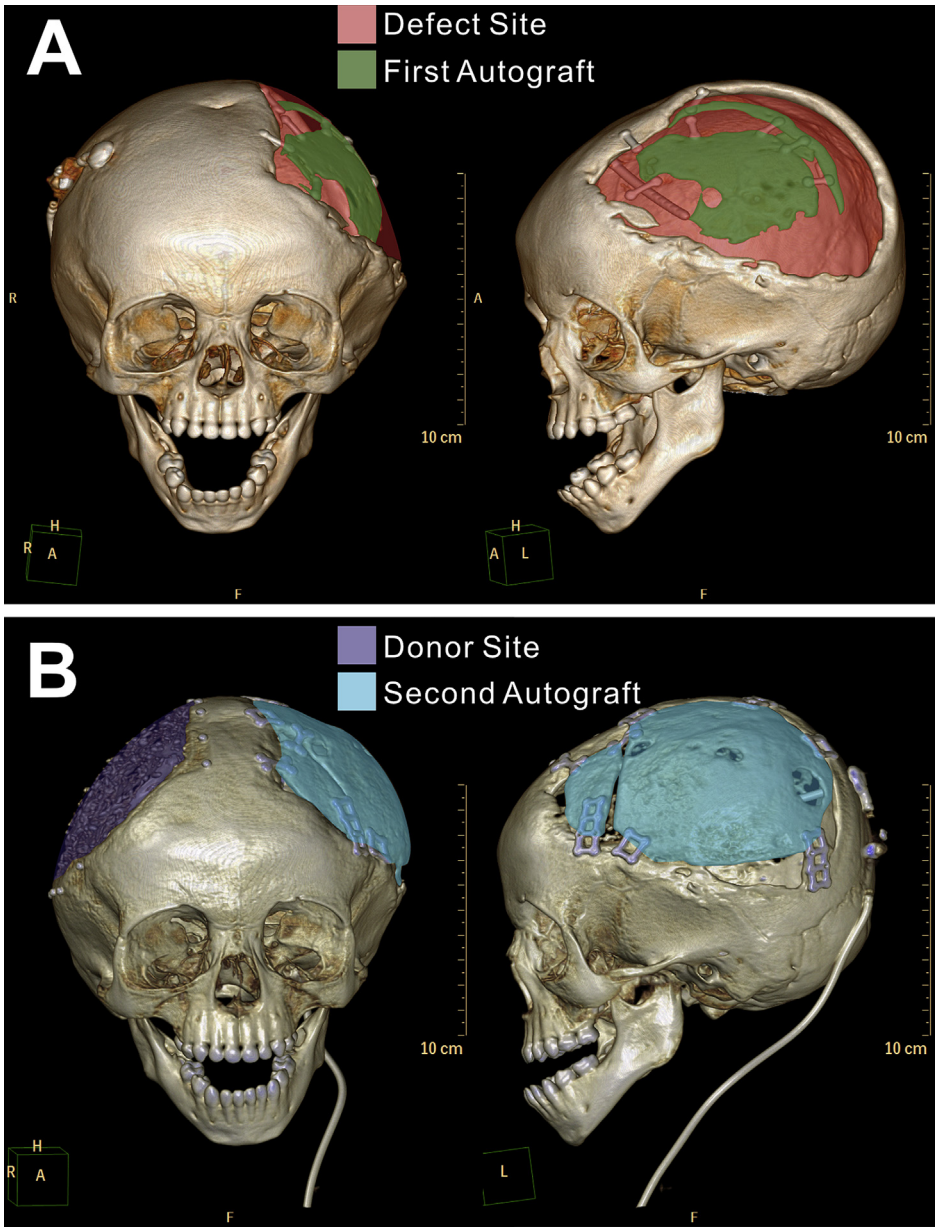

Fig. 1 Case example of a pediatric craniofacial defect. A) Depicted is a large craniofacial skeletal defect resulting from resorption of an autogenous bone graft following emergency craniectomy and delayed replacement of the bone. B) Reconstruction was accomplished through a second autograft involving full-thickness resection of large portions of the frontal and right parietal bones. The donor site was repaired using demineralized bone matrix and particulate bone graft. The use of these CT images follows the guidelines of the University of Chicago Institutional Review Board.

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.[44,45] 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.[45] EPCs are also compatible with β-TCP scaffolds, in which they have been shown to contribute directly to neovasculogenesis 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).[46]

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).[47–50] Several bone

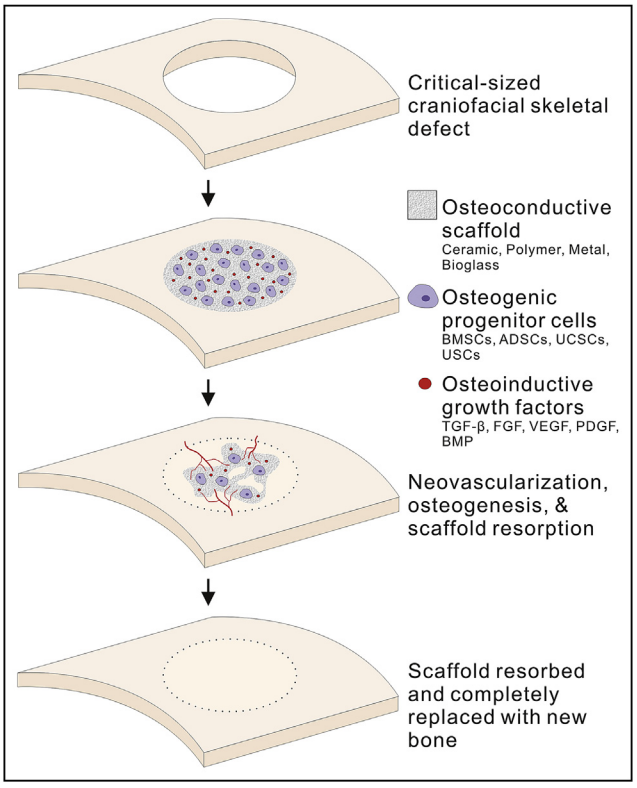

Fig. 2 Tissue engineering paradigm for craniofacial defect repair. Illustration depicting ideal modality for craniofacial defect repair. The strategy involves growth factor-induced osteoblastic differentiation and bone formation within an osteoconductive and biodegradable scaffold.

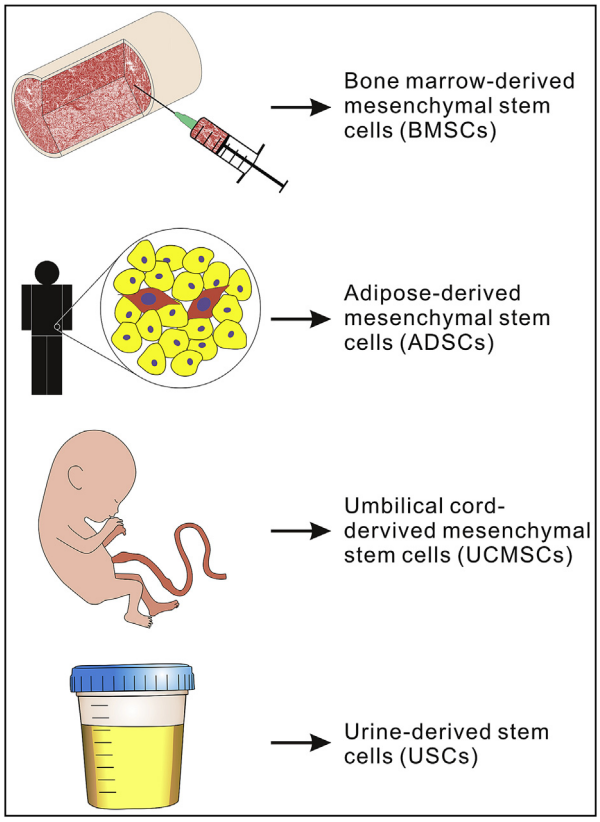

Fig. 3 Osteoblastic stem cell sources. The potential sources of mesenchymal stem cells (MSCs) that can be used for bone tissue engineering and regeneration. The recently described urine-derived stem cells (USCs) may represent one of the most promising and convenient sources of MSCs for tissue engineering and regenerative medicine.

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 augumentation.[51–53] They bind receptors on multiple stem cell types and induce osteoblastic differentiation through the Smad protein signaling pathway.[14] 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.[54–65] Infuse® Bone Graft (Medtronic and Wyeth) and Osigraft® (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.[66–75] 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.[76–79] Efforts are ongoing to combine synergistic growth factors and carrier molecules to lower the necessary BMP dose and control its release.[80,81]

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.[82] 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.[83,84] Gene transfer can also be accomplished via direct uptake of gene-containing plasmids from solution or as a conjugate with a nucleus-bound biomolecule.[84]

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.[82] It has been shown that covalent linkage of the growth factor to the scaffold may slow and improve its release profile to

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	
VEGF			Promoting/Inducing
PDGF	Promoting[a]	Promoting	Promoting
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	

[a] Only PDGF-AA has been shown to promote osteoblastic differentiation in MSCs.

more closely approximate cellular demands.[85] 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.[86] 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.[14,87]

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.[88] Statins, as well as several immunosuppressants, are small molecules that have demonstrated capability to induce osteoblastic differentiation and bone formation.[89–92] 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.[93–96] Most recently, phenamil has demonstrated synergistic effects with BMP-2 by inducing osteogenic differentiation of ADSCs in calvarial defect repair.[97]

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.[17] 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.

Natural fracture healing is characterized by the formation of a cartilaginous callus, which undergoes mineralization, resorption, and replacement by new bone.[98] 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.[99] Collagen and hydroxyapatite, the primary organic and mineral components of bone, respectively, are prototype osteoconductive materials and will be discussed later in this review.[100]

The concept of mimicking native bone ECM, which serves as a collagenous framework for osteoblasts and a reservoir for growth factors, has played a significant role in scaffold design.[101] Interplay between the scaffold and progenitor cells should closely mimic natural cell surface receptor and ECM interactions.[18] These interactions are critical in bone regeneration processes such as osteoblast adhesion, proliferation, migration, differentiation, and matrix deposition.[18] 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.[102,103]

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.[98,104] Differentiation of these migratory progenitor cells is accomplished via mechanical, biochemical, and biophysical factors in a process called osteoinduction.[104] Osteoinductive scaffold designs seek to emulate this natural phenomenon through biochemical structure, progenitor cell adhesion properties, and delivery of growth factors.[44,105,106]

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

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.[107–111] Approaches to minimizing the inflammatory response include incorporation of biomimicking materials as well as conjugate antioxidants in the scaffold itself.[112–115] 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.[116,117]

Biodegradability

Osteoconductive scaffolds should act only as a temporary framework for bone regeneration.[18] Temporality is critically important, as the ideal scaffold is not meant to be a permanent prosthetic, 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.[18] Full resorption of the original scaffold is necessary for uninterrupted bone remodeling and physiologic responses to mechanical stimuli.[19] Unmatched rates of scaffold material resorption and bone formation may result in incomplete bone regeneration or obstructed remodeling and tissue integration.[118–120] 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.[19,121] 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.[19]

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.[13,45] 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).[16,122] Downstream effects of VEGF culminate in proliferation of circulating EPCs, which initiate vasculogenesis at the defect site.[16] 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.[122]

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.[45] 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.[46,123–127] Multipotent bone marrow stromal cells enriched for mesenchymal and endothelial phenotypes have also demonstrated capacity for highly vascularized bone generation in mandibular defect repair.[128]

The importance of vascular supply in bone reconstruction is well recognized.[129,130] Osteoprogenitor cells associate with endothelial cells, which supply not only oxygen and nutrients but also growth factors necessary for osteoblastic differentiation.[131] For this reason, neovascularization is an essential step in promoting sustained bone regeneration. Accommodating for endothelial progenitor cell invasion and attachment, delivery of proangiogenic factors, and blood vessel formation necessitates a porous scaffold structure.[132] It is thought that 150–500 μm is a sufficient pore diameter to support neovascularization and blood vessel invasion.[133] 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.[18,134,135] As a result, the ideal scaffold strikes a balance between the two competing properties.[18]

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.[13,136] Radiation therapy severely complicates bone development, remodeling, and fracture healing secondary to progenitor cell loss and compromised vasculature.[137–140] These complicating factors require a combination of neovasculogenic progenitor cells and growth factors to ensure proper vascularization.[141,142]

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.[3,4,143] The use of biocompatible scaffolds in healing these defects may provide a more cost-effective and less complicated alternative to autologous bone grafts.[121] Scaffolds provide an osteoconductive and osteoinductive extracellular matrix analog to facilitate cellular migration, proliferation, adhesion, differentiation, and generation of new bone.[105,121] 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).[13,121,144]

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

Osteoconductive biomaterials for scaffold construction	
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)
	Poly(polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN)

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.[145] 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 paste, but an intrinsic limitation of all DBM types is poor mechanical strength and porosity.[146] A recent retrospective study investigating craniofacial defect reconstruction outcomes using bone cement, autologous bone grafts, and DBM revealed the highest rate of residual defect using DBM.[147] 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.[145]

Ceramics

Some of the most promising initial scaffolds closely mimic the chemistry and structure of native extracellular matrix in bone.[13] Foremost among these are calcium phosphate ceramics, including hydroxyapatite (HA), β-TCP, and biphasic calcium phosphate.[13] 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.[148,149]

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.[18,150] 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.[4–6] Furthermore, HA-TCP composite scaffolds have demonstrated both osteoconductivity and favorable resorption rates.[151,152] 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.[153] 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.[154]

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.[155] 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.[3]

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.[14,156] As of yet, this material has most significantly been used to repair burr holes from hematoma-related neurosurgery cases.[156] 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.[157,158] 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.[159–161] Naturally-derived materials, such as collagen and fibrin proteins, or chitin-derived chitosan polysaccharide, are also an option for bone tissue engineering.[117,162] 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,

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.[37,164]

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 osteoinductivity 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 tissue 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.[164,192] Mg-rare earth element compounds, 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.[195] 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.[13] 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.[38,215,217–221] Importantly, these scaffolds are able to have these effects without the addition of exogenous growth factors.[217,218] 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.[215,222,223] Silicate bioglass as well as some ceramic scaffolds have been shown to posses this dual-inductive attribute.[211,215,221]

As previously discussed, composite scaffolds combining materials with different desirable properties are a step toward the ideal. Silicate composite scaffolds have been tested, and varying the relative proportion of each component affords some degree of control over mechanical properties, hydrophobicity, and degradation.[217,218,224,225]

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.[117,226,227] 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.[228–233]

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.[234–237] 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.[116] 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.[116,238,239]

Hydrogels comprise another important class of osteoconductive scaffolds that can be delivered through noninvasive means.[13,240,241] They are water-absorbing matrices composed of cross-linked hydrophilic polymers that are well suited to harboring growth factors and viable stem cells.[241,242] As a result, hydrogels are ideal for stem cell and biofactor delivery that promote bone tissue regeneration.[240–242] 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.[81]

Other injectable scaffolds include hydroxyapatite or calcium sulfate pastes, but are complicated by syneresis and contraction, as well as brittleness following setting.[243,244] Using a combination of these and other materials in injectable composites helps overcome many of the individual materials' limitations and enhances osteoconductivity.[245,246] 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*.[247,248] 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.[249] 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.[117] 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.[117,250,251]

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.[252] 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.[252] This strategy would utilize osteoinductive geometric cues within the scaffold to initiate bone formation without the need for exogenous osteogenic molecular signals.[252,253]

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.

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