Available online at www.sciencedirect.com

ScienceDirect

British Journal of Oral and Maxillofacial Surgery 55 (2017) 875–891

BRITISH Journal of Oral and Maxillofacial Surgery

www.bjoms.com

Review

Three-dimensional macroporous materials for tissue engineering of craniofacial bone

Akhilesh Kumar Shakya [a], Umadevi Kandalam [b,*]

[a] *Department of Chemical Engineering, Texas Tech University, Lubbock, TX, USA*
[b] *Department of Paediatric Dentistry, Nova Southeastern University, Fort Lauderdale, Florida*

Accepted 18 September 2017
Available online 19 October 2017

Abstract

Repair of critical-size defects caused by trauma, removal of a tumour, or congenital abnormalities is a challenge in the craniomaxillofacial region because of the limitations associated with treatment. We have reviewed research papers and updated information relevant to the various types of macroporous scaffolds. We have included papers on several biomaterials and their use in various craniofacial defects such as mandibular, calvarial, and others, as well as the latest technological developments such as 3-dimensional printed scaffolds. We selected all papers about scaffolds, stem cells, and growth factors for review. Initial selection was by review of titles and abstracts, and the full texts of potentially suitable articles were then assessed. Methods of tissue engineering for repair of critical-size defects in the craniofacial bones seem to be viable options for surgical treatment in the future. Macroporous scaffolds with interconnected pores are of great value in regeneration of bone in the craniofacial region. In recent years, various natural or synthetic materials, or both, have been developed, on which macroporous scaffolds can be based. In this review we present a review on the various types of three-dimensional macroporous scaffolds that have been developed in recent years, and evaluate their potential for regeneration of craniofacial bone.

Keywords: Craniofacial bone regeneration; Macroporous materials; Hydrogels; Cryogels; Composite; Bioactive foams

Introduction

The treatment of bone lost from the craniofacial region as a result of trauma, resection of tumours, or congenital deformities, is challenging. The cranium is a complex structure made up of bone, cartilage, soft tissues, nerves, and vasculature, so impairment may have an appreciable effect on both function and aesthetics. The goal of reconstruction is to restore form and function to facial aesthetics, and to enable the patient to achieve a reasonable quality of life with early oral functional rehabilitation. In this review we provide a brief description of current surgical techniques and give updates about 3-dimensional macroporous scaffolds for reconstruction of bone in the craniomaxillofacial region.

Metals

Common metals used in craniofacial applications are steel, chrome, and molybdenum, but titanium is the most widely used, as it is biocompatible and resistant to corrosion. Its modulus of elasticity is more like that of bone than any other metals. Currently it is being used in the reconstruction of mandibular bone, calvarial defects, and for osteosynthesis.[1–3] Miniplates and microplates made from titanium alloys have important advantages because of their biocompatibility and stability.[2] In recent years, porous metals (those with a pore size of >100 μm) have been developed with intriguing characteristics that enable them to heal bone with good

* Corresponding author.
E-mail address: kandalam@nova.edu (U. Kandalam).

http://dx.doi.org/10.1016/j.bjoms.2017.09.007

osseointegration.[4] The advantages of these materials are their biocompatibility, ability to osseointegrate, and mechanical stability. However, their limitations include inherent lack of biological recognition.

Resorbable systems

Resorbable systems have the advantage over metals that they overcome the problems associated with metal implants such as a second operation for their removal, and they are technically easy to use and cost effective. Materials such as polylactic acid (PLA) and polyglycolic acid (PGA) are the most commonly-used materials. Compared with metal implants, resorbable materials have less tensile strength, but it depends on the type of fracture, and in non-load-bearing areas the resorbable systems work well. A copolymer miniplate and microplate system made of poly-L-lactic polyglycolic polymer has been shown to have more initial stability than metal plates for facial fractures.[5] In another study, bioabsorbable mesh and screws were used to fix various reconstructions for craniofacial trauma, and healing was effective in patients with frontal fractures and zygomaticomaxillary complex fractures. However, the fact that the resorbed material may cause a foreign body response with accumulation of macrophages and granulocytes is a major disadvantage.[6]

Bone substitutes

Autografts
Of many surgical techniques used to reconstruct lost bone, autologous bone tissue remains the gold standard for critical-size bony defects,[7] though allografts and xenografts are options.[8] The use of an autograft has limitations, including donor site morbidity, limited availability of tissue, an additional operation, and prolonged healing time.

Allografts
Allografted bone is harvested from human donors, mainly cadavers. The disadvantages include infection and immune resistance.

Xenografts
Bovine bone is a commonly-used material for a xenograft. However, the immunogenicity and transmission of infectious diseases are serious concerns.

Tissue engineering scaffold
The advent of tissue engineering techniques offers a promising choice for the reconstruction of critical-size craniofacial defects.[9,10] In bone tissue engineering, scaffolds act as the delivery vehicles of cells and growth factors. Autologous cells (from the patient's own tissue) are expanded and seeded on to the scaffolds either in vitro or in situ, and delivered to the appropriate anatomical site (Fig. 1).[11] The 3-dimensional scaffold serves as a temporary extracellular matrix (ECM) to provide mechanical support within an environment conducive to the growth of cells and regeneration of tissue.[12] However, the design of scaffolds used in the craniofacial region is extremely complicated. The scaffold must fit into a complex 3-dimensional anatomical defect, and should be temporarily load-bearing until the tissue forms. Scaffolds must also be porous enough to deliver biofactors, and sustain the mechanical forces until the regenerated tissue can bear them. There are therefore many considerations, including porosity, biocompatibility, degradability, surface morphology,[13] and mechanical strength, to be taken into account when designing a scaffold for the craniofacial region.[14,15]

Bone possesses a porous structure ranging from 20 to 400 μm, which is necessary for bone cells to penetrate, adhere, grow, and proliferate to form new bone.[16] To regenerate bony tissue and correct defects efficiently, scaffolds should mimic the hierarchical structure of bone. Three-dimensional porous scaffolds consisting of an interconnected macroporous network with diameters of at least 100 μm are required to facilitate the growth of cells, vascularisation, production of an ECM, and the removal of waste material.[17,18] Scaffolds synthesised by conventional methods, or with solvent leaching or gas foaming, have a fixed pore size that can be modulated by adding some stimulatory responsive pyrogens to generate tenability.[19,20]

In recent years a wide range of macroporous scaffold materials such as cryogels, injectable hydrogels, bioactive foams, and biocomposite materials have been developed for bony regeneration (Table 1). Materials used for tissue fabrication of scaffolds range from polymers such as self-assembled peptides,[21] arginylglycyl aspartic acid (RGD) peptides, proteins, and elastomers;[22] ceramics such as calcium phosphates[23] and bioactive glasses;[24] to metallic materials such as titanium oxide (Fig. 2).[25] Some of the macroporous scaffolds have been tested in clinical trials for the reconstruction of craniomaxillofacial bones (Table 2). In this review we provide an update on various types of macroporous scaffolds that have been developed during the last few years, and describe their current use and potential applications in the regeneration of craniofacial bone.

Hydrogels

Hydrogels are a specific class of biomaterials that have been used in various ways for tissue engineering. The hydrophilic polymers of these gels form 3-dimensional networks through crosslinking, either by covalent bonds or through physical intramolecular and intermolecular attractions. Hydrogels swell rapidly in contact with water, form structurally similar macromolecular-based components in the body,[26–28] and have excellent biocompatibility with minimal inflammatory responses.[29] Their inherent hydrated architecture allows the transport of soluble factors, nutrients, and waste.[30] During the process of synthesis, the pores in hydrogels can be formed

Table 1
Preclinical studies in craniofacial bone tissue engineering.

Nature of scaffold	Seeded cells/growth factors	Craniofacial site	Animal model	Year	Remarks
Fibroin scaffold[141]	Human amniotic fluid, dental pulp stem cells	Critical size cranial defect	Rat	2012	Fibroin scaffold induced mature bone formation and defect correction
Injectable nano calcium sulphate/alginate[142]	Mesencymal stem cells (MSC), endothelial progenitor cells, (EPC), bone morphogenetic protein (BMP2)	Critical size calvarial bone defect	Rat	2013	BMP2 gene modification of MSC and EPC induced new bone formation
Titanium enriched hydroxyapatite-gelatin scaffold[116]	Multipotent adult progenitor cells	Critical size calvarial bone defect	Rat	2013	Titanium- enriched polymeric scaffold induced the osteointegration and new bone formation
Bioactive implant[143]	Human adipose-derived stromal cells (ADSC)	Critical size mandibular defects	Rat	2013	ADSC was capable for defect construction
Macroporous calcium phosphate scaffold[132]	Human embryonic stem cells	Cranial defect	Rat	2014	Scaffold with stem cells enhanced the new bone and blood vessels formation
Polyethylene glycol-polycaprolactone-polyethylene glycol/hydroxyapatite (PCL/HA) composite scaffold[128]	Porcine bone marrow stem cells	Temporal bone	Pig	2014	PCL/HA scaffold induced better bone regeneration than the PCL alone
Calcium phosphate cement scaffolds containing porogen, fibres, and microbeads[112]	Recombinant human BMP2, Vascular endothelial growth factor	Critical size cranial defects	Rat	2014	Scaffold containing porogen had faster regeneration of cranial defect
Absorbable collagen sponges[144]	Recombinant human BMP2	Critical size mandible defect	Rat	2014	More ectopic bone formation observed in collagen based scaffold than the control scaffolds
Hydroxyethylmethacrylate (HEMA)-lactate-dextran cryogels[145]	Bone marrow- derived mesenchymal stem cells	Cranial defect	Rat	2014	Higher blood vessel density observed in scaffold-stem cells scaffold than the scaffold only group
Polyvinyl alcohol-tetraethylorthosilicate-alginate-calcium oxide (PTAC) biocomposite cryogel[146]	–	Cranial defect	Rat	2014	Cryogel scaffold shown greater regeneration of bone defects in comparison to non scaffold treated group
Polycaprolactone scaffold[135]	Dental follicle stem cells	Craniofacial defects	Rat	2015	Bone regeneration was observed in group with scaffold-stem cells transplanted
β-tricalcium phosphate (β-TCP)[147]	Bone marrow- derived stem cells	Critical size defects in mandibular region	Rabbit	2015	β-TCP with stem cells shown better and rapid bone formation than the controls
Extracellular matrix (ECM) based scaffold[148]	Human dental pulp cells	Calvarial defects	Rat	2015	Superior new bone formation occurred in ECM based scaffold than the controls
Collagen gel scaffolds[149]	Dental pulp stem cells	Critical size calvarial defect	Rat	2016	High fibrous and mineralised tissue volume observed in scaffold and stem cells treated group

Table 1 (*Continued*)

Nature of scaffold	Seeded cells/growth factors	Craniofacial site	Animal model	Year	Remarks
Nano-hyaluronic acid/collagen/polylactic acid scaffold[150]	Human alveolar bone-derived stem cells	Critical size mandibular bone defect	Rabbit	2016	Total bone formation was higher with scaffold-stem cells than the scaffold alone
Collagen matrix[134]	Human deciduous dental pulp stem cells	Maxillary alveolar defects	Rat	2016	Dental stem cells seeded on scaffold indicates a promising model for human maxillary alveolar bone defects
3-Dimensional printed scaffold[136]	Multipotent mesenchymal stromal cells (MSC)	Mandibular reconstruction	Rabbit	2017	3-Dimensional printed scaffold with stem cells indicates the promising construct for mandibular regeneration
Calcium phosphate scaffold[130]	Endothelial cells with human bone marrow MSC, human umbilical cord MSC, human-induced pluripotent stem cell-derived MSC, human embryonic stem cell derived-MSC	Cranial defects	Rat	2017	Scaffold with stem cells construct improved angiogenesis and new bone formation

by the phase separation method.[31] The size and distribution of pores, as well as the interconnectivity between them, are important factors that govern the use of hydrogels in tissue engineering. Hydrogel scaffolds that are used in the engineering of bone tissue must be highly porous with open, interconnected geometry. The highly interconnected porosity is critical for the cells to distribute within the gel. Emerging techniques have shown that the microarchitectural features

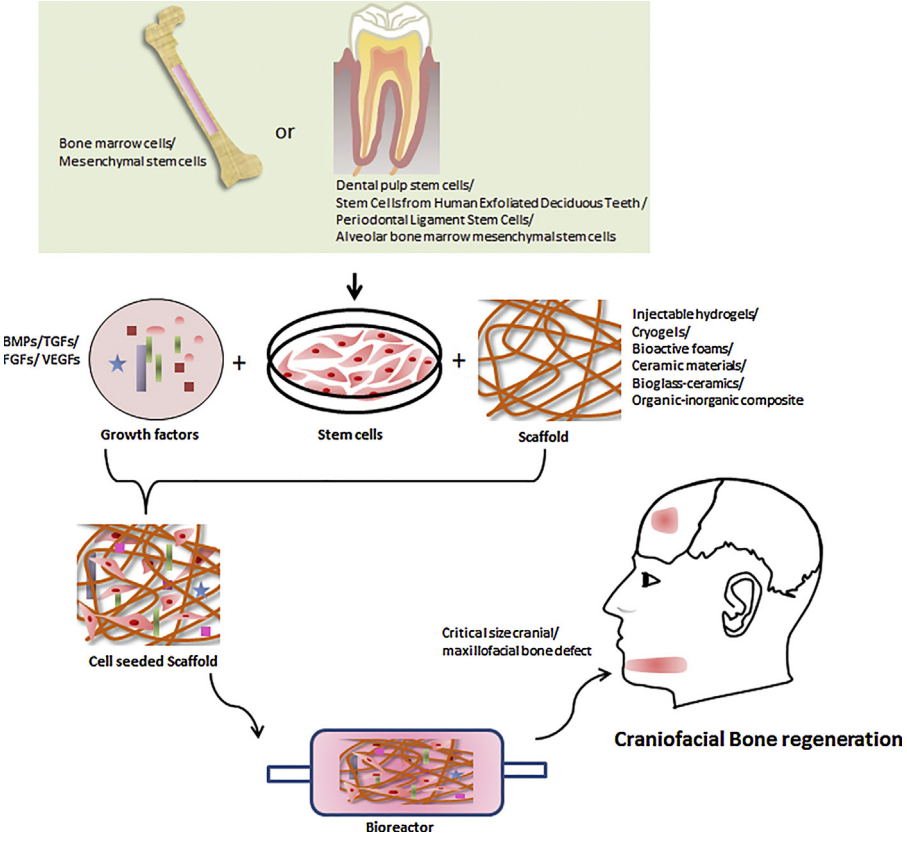

Fig. 1. Components of craniofacial bone regeneration. Bone regeneration requires a scaffold, source of osteogenic cells and growth factors which direct the growth of cells towards desirable phenotype.

Table 2
Details of clinical trials on 3-dimensional scaffold used in craniofacial bone tissue regeneration.

Type of scaffold	Type of cells seeded on scaffold	Craniofacial site	Clinical trials.gov Identifier	Country	Started year	Status	Remarks
Poly(lactide-co-glycolide) scaffold (PLGA)	–	Alveolar sockets	NCT00836797	Singapore	2007	Completed (2009)	No study results posted after completion
Beta-tricalcium phosphate	Bone marrow cells	Sinus floor bone	NCT00980278	USA	2010	Completed (2013)	Autologous bone repair cell treatment have shown augmentation of sinus floor bone
Cross-linked matrix of autologous plasma	Autologous bone mesenchymal stem cells	Maxillary bone cysts	NCT01389661	Spain	2011	Ongoing	No study results posted yet
Collagen and hydroxyapatite biomaterial	Mesenchymal stem cells	Alveolar bone	NCT01932164	Brazil	2013	Unknown*	New bone formation closing the alveolar cleft was observed
Bioactive glass (Sol–gel)	–	Alveolar bone	NCT01878084	Egypt	2013	Ongoing	No study results posted
Ceramic and palstic based resorbabale biomaterial	Mesenchymal stem cells	Cranial reconstruction	NCT01742260	Australia	2013	Ongoing	No study results posted
SilOss® (dicalcium phosphate anhydrous + hydroxyapatite + silica + zinc)	–	Chronic periodontitis	NCT02639572	India	2014	Completed (2014)	No study results posted
Gene-activated matrix (“Nucleostim”)	–	Maxillofacial defects	NCT02293031	Russia	2014	Ongoing	No study results posted yet
Periodontium injectable gel	Mesenchymal stem cells	Periodontal tissue	NCT00221130	Japan	2004	Completed (2005)	No study results posted
Collagen scaffold	Alveolar bone marrow mesenchymal stem cells	Periodontal defects	NCT02449005	United Kingdom	2014	Ongoing	No study results posted

* the recruitment status of the study is “unknown”. Status has not been updated since August 2013.

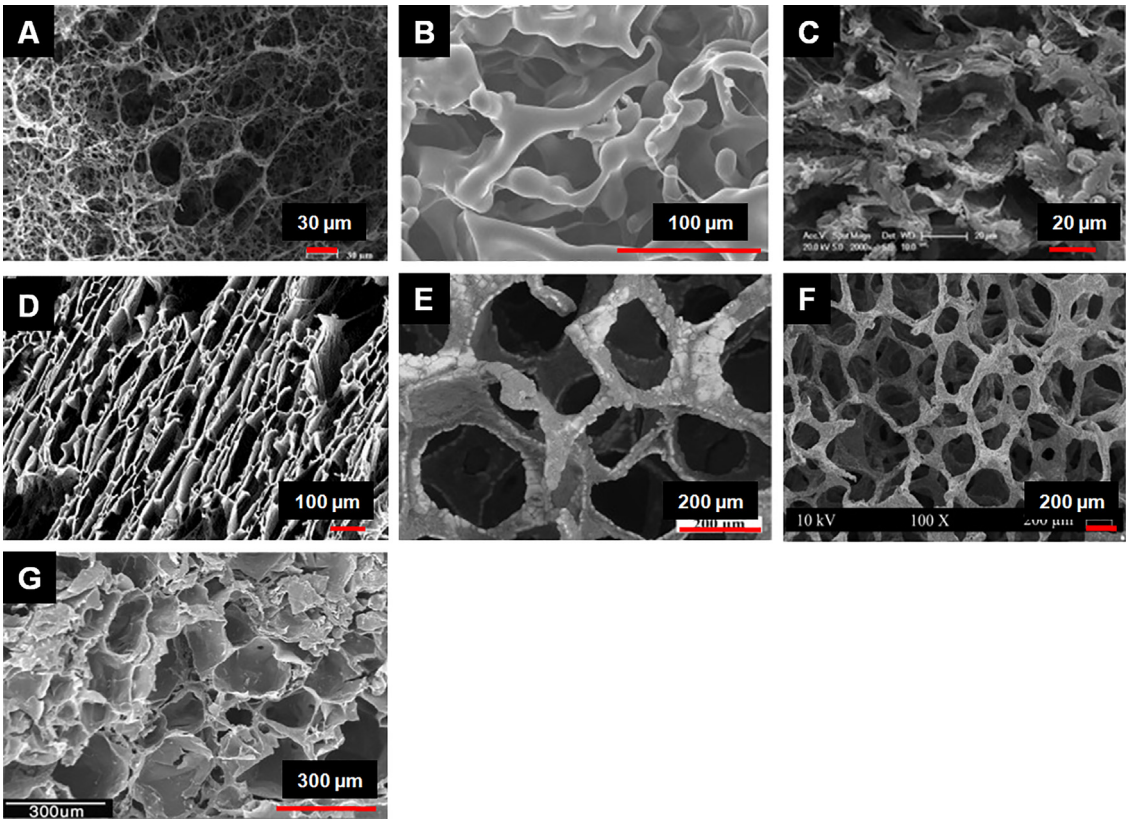

Fig. 2. Scanning electron microscopy images of different macroporous materials explored during bone tissue regeneration: A. Frozen silk fibroin hydrogels (reproduced with permission).[159] B. Photopolymerised chitosan derivative (EGAMA-CS)/polyethylene glycol dimethacrylate (PEGDA)/*N*,*N*-dimethylacrylamide (DMMA) injectable hydrogel (reproduced with permission).[160] C. PTA biocomposite cryogels (reproduced with permission).[161] D. Poly(L-lactide) PLLA freeze-dried foams (reproduced with permission).[162] E. Hydroxyapatite/poly(vinyl alcohol) nanocomposite coated porous TiO_2 ceramics scaffold, (reproduced with permission).[163] F. 45S5 Bioglass® -based glass–ceramic scaffolds (reproduced with permission).[164] G. Gelatin scaffold composite scaffolds with calcium phosphate content (reproduced with permission).[165]

in hydrogels (such as the size, porosity, and interconnectivity of the pores) could be regulated by varying the gelation conditions so that the results mimic the native tissue.[32,33] Many natural and synthetic hydrogels are used for the engineering of craniofacial bone. Naturally-derived materials include agarose, alginate, chitosan, collagen, fibrin, gelatine, and hyaluronic acid while synthetic materials include poly(ethyleneoxide) (PEO), poly(vinyl alcohol) (PVA), poly (acrylic acid) (PAA), poly(propylene furmarate-*co*-ethylene glycol) (P(PF-*co*-EG)), and polypeptides.

Of the natural polymers, alginate is a well-established biomaterial with various biomedical[34,35] and drug delivery uses[36,37] because of its low cost, biocompatibility, and simple gelation. Because alginate gels are biocompatible they are widely used in the repair of cartilage and bone tissues.[38–43] However, their biocompatibility depends on many factors such as the composition of alginates, their molecular weight, and the contaminants present in the gel.[14] In a previous study we reported that the survival of cells in the alginate gels depends on the type and composition of the alginate.[44] Alginates and chitosan composite scaffold were tested in a model of cranial defects in Sprague Dawley rats, and there was partial closure of the defect in all experimental groups at 12 weeks, with the most closures in the group treated with alginate-chitosan scaffolds and bone morphogenetic protein-2 (BMP-2).[45]

Collagen has received increasing attention because of its excellent biocompatibility, its degradation into physiological products, and the fact that it has suitable interaction with cells and other macromolecules. A bicomplex structure with collagen sponge and dental progenitor cells was used for four patients who had mandibular defects about 1.5 cm in size, and optimal bony regeneration was evident one year after grafting. This clinical study showed that a dental progenitor cell/collagen sponge biocomplex can restore bony defects completely in the human mandible.[46] Macroporous poly(ethylene glycol) di-acrylate hydrogel scaffold, with pores ranging from 100–600 µm in size, supported rapid cell uptake in human mesenchymal stem cells (MSC) which, when embedded in these hydrogels, resulted in increased mineralisation.[47]

Injectable hydrogels in the regeneration of bony tissue

Among various hydrogel scaffolds, injectable ones have gained considerable attention because they form a gel in

vivo,[48–52] and a major advantage of their use in bone tissue engineering is the gelation in situ of hydrogel materials from a liquid. This feature allows the hydrogels to fill defects of any size or shape. From a clinical perspective, the use of an injectable scaffold for local delivery of stem cells is attractive,[30,33,50–53] as it minimises discomfort for the patient, the risk of infection, formation of scars, and the cost of the treatment.[53,54] These materials also function as dynamic liquid support to carry the living cells, drugs, and growth factors that are required for tissue to regenerate. Many injectable scaffold materials have been tested for craniofacial bone reconstruction.[30,33]

Alginate as an injectable biomaterial that has been tested for craniofacial bone repair and regeneration. Alginate microcarriers that have been encapsulated in periodontal-ligament-derived stem cells, and gingival-derived stem cells, have been used to form bone in the calvarial region of mice.[55] Alginate microbeads loaded in calcium phosphate paste was tested for its injectability, and the results showed improved osteogenic differentiation in the presence of calcium phosphate and chitosan.[56] Yan et al used calcium alginate scaffolds combined with bone marrow stromal cells in a model of a parietal bone defect 20 mm in size in a sheep. Eighteen weeks after implantation, new bone had formed. An injectable paste of alginate, hydroxyapatite, and gelatin microspheres increased the proliferation of osteoblasts by the cells.[57]

Various other natural polymers such as gelatin and xanthan-based hydrogels were evaluated as injectable scaffolds for bone regeneration. Gelatin/xanthan gels with chitosan nanoparticles were used to encapsulate basic fibroblast growth protein (bFGF) and BMP-7 and used to grow and differentiate human osteoblasts seeded on to the scaffold. The cells in these hydrogel systems grew well and proliferated as a result of the sustained release of growth factors over a period. The mechanical strength of these scaffolds was similar to the strength of cancellous bone, and the gels possessed antibacterial properties and inhibited different strains of staphylococci, which are commonly found in infections of orthopaedic implants.[58]

In another study, the combination of natural and synthetic polymer-based injectable hydrogel gelatin-poly (ethylene glycol) tyramine encapsulated with the drug simvastatin resulted in mineralisation and osteogenic differentiation of osteoblasts (MC3T3-E1).[59] Chitosan-polyethyleneoxide based hydrogels incorporated with BMP-2 protein was evaluated for regeneration of calvarial bony defects in rats when scaffold was seeded with human-bone-marrow-derived stem cells (hBMSC). The sustained release of growth factors and signs of the formation of new bone were apparent four and eight weeks after implantation. Nevertheless, the degree of bony regeneration depended on the numbers of stem cells and the concentration of BMP-2 incorporated in the hydrogels.[60]

As well as the natural polymers, synthetic polymer-based injectable hydrogel systems have been used in bony regeneration. For instance, triblock polyethylene glycol-polycaprolactone-polyethylene glycol (PEG-PCL-PEG) polymer-based injectable hydrogels have been evaluated. Gels implanted at different time intervals (three, seven, and 14 weeks) in cranial defects of New Zealand rabbits caused bone to regenerate.[61]

Polymethylmethacrylate-(PMMA)-based hydrogels have long been used to secure orthopaedic implants into skeletal bone. PMMA is generally used in the form of monomers with an initiator mixture (which is slurry at room temperature) though polymerised when injected into the body.[62] In that series hydrophobic poly-(propylene fumarates) and polyanhydride-based injectable hydrogels were also investigated.[63–65] These polymers can become gelatinous easily inside the body in the presence of thermal or photo-initiators. Initiators can polymerise the monomers in a controllable way without causing damage to the surrounding tissues.[64] They are biodegradable, mechanically strong, and capable of supporting the growth of osteoblasts.[66] However, their uses are limited to the delivery of cells such as osteoprogenitor cells, because their cross-linking during photopolymerisation can affect the viability of the cells. To improve cell viability, therefore, hydrophilic and hydrophobic blocks have been incorporated into the polyfumarate-based polymers.[67] Polymethylmethacrylate, when used in combination with nanoparticles of hydroxyapatite, increased the biological functions of the osteoblasts.[68]

Self-assembly scaffolds are a class of injectable hydrogels that are formed by natural self-assembly, and they mimic nanofibrous polymer scaffolds from engineered self-assembling peptides.[21,69] These peptides can be designed to form a stable organised structure through spontaneous organisation of molecules caused by non-covalent interactions. The nanofibre structures of these peptides (<10 nm in diameter) are several times thinner than the cells, which permits them to surround the cells in a manner similar to a natural extracellular matrix. In this way they provide a cellular platform for the formation of bone. Many of these self- assembled scaffolds have been tested for the regeneration of craniofacial bone.[70] Commercially available self-assembled scaffold has been shown to facilitate the regeneration of bone in calvarial defects in mice,[71] and Yoshimi et al reported that the combination of Puramatrix™ (3D Matrix Inc, Japan), platelet-rich plasma, and canine MSC regenerated mature bone in eight weeks in the mandible of a dog.[72]

One of the important aspects of cell-based treatments that needs to be taken into consideration while designing the scaffold is its ability to adhere to cells within the material. Several techniques improve the adhesion of cells to hydrogels, including the incorporation of RGD peptides into the hydrogels. RGD is a known integrin recognition site within many cell attachment proteins, including laminin and fibrinogen. RGD peptides promote increased binding sites in osteogenic cells including MSC and many other types. The RGD peptides are a cell-binding domain for ECM, and allow cells to bind on hydrogel surfaces. Different 3-dimensional

hydrogel models based on RGD peptides have been developed, such as polyethylene glycol[73], acrylate, alginate,[74] and *N*-isopropylacrylamide hydrogels.[75] The RGD peptide in these hydrogels improved the binding to the cells and induced their proliferation. Incorporation of the RGD peptide also improved the mineralisation properties of the hydrogels.[76] In another study, temperature-responsive chitosan-based hydrogels were synthesised using β-glycerophosphate as a cross-linker, and strengthened by the inorganic component β-tricalcium phosphate. The hydrogels showed sol–gel phase transition at body temperature, and possessed enough mechanical properties to bear the stress of bony tissues. The inorganic content provides a unique physiochemical property to the scaffold, which is a favourable environment for the growth and proliferation of bone cells.[77] Different hydrogels composed of alginate, alginate/chitosan, and alginate/hyaluronic acid, were synthesised and cerium (III) ions were incorporated as the antimicrobial agent. All three hydrogels supported the growth of osteoblast-like (MG-63) cells well when they were implanted subcutaneously in rats.[78] In brief, hydrogels in situ are promising materials for craniofacial bone regeneration.

Although hydrogels have many interesting features, their limitations for regeneration of bone tissue are the lack of adequate mechanical strength, and poor mineralisation. Multifunctional, macroporous, composite scaffolds have been developed to satisfy these needs. For example, polysaccharide-based hydrogels of varying stiffness have been synthesised to achieve mechanical strength by using different amounts of cross-linkers such as Genipin or glutaraldehyde for regeneration of bone. By increasing the amounts of cross-linkers the gelation time is reduced, and the stiffness improved.[79]

A well-known way to improve hydrogels' mechanical strength as well as their ability to mineralise is to incorporate an inorganic component such as calcium or a silica-based compound. For instance, a chitin-hydrogel-containing nanosilica scaffold induced formation of a hydroxyapatite layer seven and 14 days after immersion in simulated body fluid.[80] The presence of silica-like inorganic components in hydrogel acts as a centre for the mineralisation process. Calcification is induced by dipping the hydrogels directly into a calcium salt solution such as calcium phosphate. For example, calcium-phosphate-polyacrylic-acid-based hydrogels are synthesised by cross-linking polyacrylic polymer in the presence of ammonium hydrogen phosphate, followed by immersing them in a calcium salt solution. The presence of calcium ions in the phosphate-polyacrylic-hydrogel induces mineralisation,[81] which is achieved by incorporation of specific enzymes such as alkaline phosphatase. Murine osteoblast-like cells MC3T3-E1 loaded on to gellan gum hydrogels that had been treated with alkaline phosphatase, showed mineralised tissue when cultured in medium that contained different concentrations of calcium and magnesium glycerophosphate. Binding and survival of MC3T3-E1 osteoblasts was seen to be greater in media that contained magnesium salts than in that containing only calcium salts.[82]

Another way of inducing mineralisation in hydrogel is through chemical modification of the hydrogel such as the incorporation of negatively-charged ions into a backbone of polymeric hydrogel. Negatively-charged groups such as phosphate and hydroxyl are commonly incorporated into hydrogel by copolymerisation between hydrogel-forming monomers and monomers that contain these charged groups.[83] For example, incorporation of phosphate-containing ethylene glycol methacrylate phosphate into polyethylene glycol hydrogel induced formation of a bone-like mineral phase.[84]

Cryogels

Cryogels are hydrogels that are synthesised at subzero temperatures to obtain good porosity and mechanical strength, and recently various cryogels have been tried for the tissue engineering of craniofacial bones (Table 3). They were synthesised at low temperatures by chemical or physical gelation of polymeric chains, or free radical polymerisation of monomeric precursors. At lower temperatures, polymeric/monomeric precursors are mixed in aqueous or organic solvents and immediately frozen, with the resultant formation of ice crystals surrounded by unfrozen solute particles. Polymerisation or gelation takes place around these ice crystals, and when the frozen system is unfrozen at room temperature the ice melts and leaves interconnected, micron-sized pores. The mass transfer resistance is negligible in these cryogels because of the microporosity and connectivity between the pores that allows the smooth circulation of nutrients to the growing cells (∼39–44 kPa).[85,86]

To improve mineralisation in cryogels, inorganic components such as silica glass are incorporated in them. For example, recently the in vivo potential for 3-dimensional polyvinyl alcohol-tetra-ethyl-orthosilicate-alginate-calcium oxide (PTAC) biocomposite cryogel was characterised for bone tissue engineering. These cryogels are new inorganic-organic composite cryogels that are synthesised at subzero temperatures.[87] In PTAC cryogels, tetra-ethyl-ortho-silicate (TEOS) with calcium oxide contributes the inorganic part while poly-vinyl-alcohol (PVA) and sodium alginate act as the organic component. TEOS, together with calcium oxide, is a bioactive glass that is essential for formation of the hydroxyapatite layer in the scaffold. PTAC cryogels are mechanically strong and can bear 0.67 MPa compressive strength, which is sufficient for bony defects in non-load bearing areas of cranial and maxillofacial bones.

The bony regeneration potential of PTAC cryogels in vivo was evaluated in the restoration of calvarial bone defects in rats.[88] PTAC implants formed calcium-phosphate-like crystals four weeks after implantation when they were implanted in bony defects in the calvarial region.[88] In a different study, chitosan-hydroxyapatite-based macroporous cryogel with good mechanical strength was synthesised by freeze

Table 3
Performance of macroporous cryogel scaffolds under in vitro conditions.

Cryogel scaffolds	Year	Remarks
2-hydroxyethyl methacrylate (HEMA)–lactate–dextran[151]	2008	Dynamic conditions (perfusion or compression, or both) considerably improved growth of osteoblast-like cell line (MG63) and extracellular matrix synthesis
Polylactide-co-glycolide cryogel[152]	2009	Bone marrow stromal cells survived in vivo and at least partially differentiated after implantation.
Polyvinyl alcohol–tetra–ethyl-orthosilicate–alginate–calcium oxide (PTAC) organic-inorganic composite cryogel[87]	2011	PTAC biocomposite cryogels biocompatible, biodegradable, and support growth of osteoblast cells
Gelatin–hyaluronic acid scaffolds[153]	2011	Porcine adipose-derived stem cells proliferated and differentiated well on 3-dimensional cryogel scaffold
Chitosan-hydroxyapatite-marine sponge (*Irciniafusca*) collagen[154]	2012	Composite scaffolds well supported for cell growth of MG-63 cells and their proliferation
Ceramic–polymer composite cryogel[155]	2012	Scaffold support attachment and growth of human mesenchymal stem cells
Collagen-nanohydroxyapatite[156]	2012	Better growth and proliferation of osteoblasts cells observed in collagen-nano-hydroxyapatite-biocomposite compared with pure collagen scaffold
Chitosan–hydroxyapatite macroporous scaffold[89]	2012	Scaffolds provided conductive environment for growth of human osteoblast cells and mineral formation
Gelatin cryogels crosslinked with oxidised dextran[157]	2013	Potential scaffold for non-load-bearing bone tissues such as craniofacial area
Gelatin-hydroxyapatite-based cryogels[158]	2017	Scaffold is biocompatible and supports cell growth considerably

drying, which increased the attachment and proliferation of osteoblasts.[89] Primary human osteoblasts seeded on a chitosan-hydroxyapatite scaffold proliferated and deposited minerals after a few days in culture medium.[89] Cryogels are therefore promising substitutes for regeneration of cancellous or calvarial bone, though they need to be evaluated for regeneration of segmental bone.

Bioactive foams

Bioactive foams are a new class of macroporous materials that show promising potential in the engineering of craniofacial bone. Their composition, porosity, and surface texture can be designed or controlled according to the defect in the damaged tissues. These bioactive composites can be synthesised by the sol-gel approach with a combination of organic and inorganic components. The composite foams with the Bioglass® porous structure form a bioactive and bioresorbable glass-based material. An organic polymer phase improves the mechanical behaviour of bioactive glass, and the use of surfactant during synthesis promotes the formation of foam, which contributes to the porosity in the scaffold. These features support cell proliferation and differentiation.[90,91]

Recently, various bioactive foams have been developed especially for bone tissue engineering – for example – biocomposite foam was synthesised by a combination of 50% bioactive glass and 50% polyvinyl alcohol using a sol–gel process in the presence of a surfactant.[92] Foaming produced by surfactant provides 68% of the interconnected porosity, which allow exchange of nutrients for growth of the cells. Foam can also bear 0.6 MPa of compressive strength, and in vitro supports the viability of osteoblastic cells.[92] During synthesis an osteogenic agent is incorporated to improve the mineralisation of the foam. When parathyroid hormone-related-protein-derived peptide (osteostatin) was incorporated into a silicon-doped mesoporous ceramic scaffold, it improved the osteogenic property in calavarial defects in rabbits.[93] In another study, osteostatin that had been immobilised covalently improved the proliferation of osteoblast MC3T3-E1 cells in vitro. Recently poly (DL-lactic acid)-based foam combined with Bioglass® particles was investigated for bone tissue regeneration, and the scaffold supported the growth of human adipose-derived stem cells well, and induced new bone formation when the scaffold was seeded with human adipose-derived stem cells implanted intraperitoneally in nude mice.[94] These studies suggest that bioactive foams have a potential for use in the reconstruction of craniofacial bone.

Ceramic materials

Of many ceramics materials, calcium phosphate has been widely investigated in bone regeneration in the form of cements, coatings, and 3-dimensional cell scaffolds because of its excellent osteoconductive properties. Ceramics are biocompatible, cost-effective, and easy to synthesise, which makes them a favoured choice in bony regeneration.[95,96] Porosity can be achieved in ceramics by using porogens, or changing the sintering temperature.[97,98]

Among the calcium phosphate ceramics, biphasic calcium phosphates are the most attractive ones currently being explored. They are formed by a combination of various concentrations of hydroxyapatite as a stable phase, and β-tricalcium phosphate (β-TCP) as a soluble phase. The bioactivity of these ceramics can be controlled by manipulating the hydroxyapatite:β-TCP ratios. Biphasic calcium phosphate in the form of particles may also be combined with a polymeric scaffold to increase its osteoconductive effect. For example, collagen scaffold coated with nanoparticles of biphasic calcium phosphate and fibroblast growth factor2 (FGF2) was used for regeneration of cranial bone 35 days after implantation in Wistar rats.[99] Biphasic calcium phosphate was also used to promote the vascularisation in a polymeric scaffold – for example – by β-TCP-induced formation of vessel-like microchannels in a collagen-based matrix.[100]

To improve ability of the ceramic scaffolds to mineralise, an antibody-mediated osseous regeneration strategy has also been developed. This strategy is based on immobilisation of antibodies on scaffolds that mediate new bone formation. For example, when murine anti-BMP-2 monoclonal antibodies were immobilised on four different biomaterials (titanium microbeads, alginate hydrogel, absorbable collagen sponge, and macroporus calcium phosphate), the titanium microbeads and calcium phosphate scaffolds resulted in strong bony regeneration when they were implanted in a critical-size calvarial defect in rats. A complex of anti-BMP-2 monoclonal antibodies with BMP-2 protein also induced considerable differentiation of C2C12 osteoblasts, as confirmed by expression of the RUNX2 gene and phosphorylation of the signalling protein Smad1.[101] In a recent study, ceramic-based materials and silk fibroin scaffolds were also evaluated for regeneration of cancellous bone. Silk fibroin scaffolds synthesised in different solvents have been shown to be biocompatible and have good cellular responses as verified by minimum infiltration of inflammatory cells in the tibia and humerus of sheep. Scaffolds were degraded using multinuclear foreign body giant cells and macrophages. Neovascularisation and cellular ingrowth were also homogeneous throughout the scaffold.[102]

Bioglass® ceramics

Bioglass® gained considerable attention as a material for the fabrication of scaffold for bone tissue engineering because of its excellent osteoconductive property. Bioglass® materials can integrate into the surrounding tissues and act as a substrate to which cells can attach. They also induce proliferation and differentiation of the cells. However, common problems associated with these materials are brittleness and weakness. To overcome these issues, Bioglass® materials are combined with polymers (Bioglass® composites). Among these, polymer ceramics-based composites are a good choice for bone tissue engineering because they are mechanically strong and have good osteoconductive properties.[103–109] For example, chitosan-polycaprolactone–Bioglass® bilayered composite was synthesised through foam replication and freeze-drying for osteochondral use.

This scaffold has considerable mechanical properties compared with uncoated scaffold.[110] Composite scaffolds of ceramics and hyaluronic acid-gelatin (Hya gel) functionalised with biphasic calcium phosphate ceramic had good compressive strength (2.8 MPa) with interconnected porosity. Hya gel scaffold seeded with BMSC showed excellent biocompatibility. The cell proliferation rate was considerably higher after three and seven days of culture compared with that of the control. Implantation of Hya gel scaffold in vivo with calcium phosphate began its degradation after the first three months. However, there was rapid bone formation with good mineralisation by three months after implantation.[111]

In a different study, a macroporous, calcium-phosphate-based scaffold that contained absorbable fibres, microbeads, and growth factor was evaluated for cancellous bone healing. The scaffold became porous after the use of mannitol-like substances, and nanofibres were incorporated to provide the mechanical strength in the scaffold. Scaffolds have mechanical strength similar to that of cancellous bone. Twice as much new bone was formed compared with conventional scaffolds.[112]

Phosphate glass-based ceramic scaffolds have also been evaluated as scaffolds for the treatment of various bony defects. They have good porosity, degradability, biocompatibility and mechanical strength to bear the load of bony tissue. Recently, the solubility and degradation of phosphate glass was studied under water, simulated body fluid, and TRIS-hydrochloride medium. Phosphate glass was dissolved over time, but a layer of hydroxyapatite was found in simulated body fluid and TRIS-hydrochloride medium, which confirmed the bioactivity of the glass scaffold. The porosity of the phosphate-glass-based ceramic scaffold was achieved by the use of polyethylene particles as porogens. Synthesised scaffolds possessed more than 80% porosity with 1.5 MPa compressive strength. In vitro, composite glass scaffold showed 76% degradation in four months when soaked in simulated body fluid. The gradual degradation, material strength, and porosity of these scaffolds mean that they have a potential use in craniofacial bone regeneration.[113]

Organic-inorganic composite scaffold

Composite systems that consist of organic and inorganic components have been explored as 3-dimensional scaffolds in bone tissue regeneration. In this series, composite scaffolds based on the natural polymers pullulan and dextran with hydroxyapatite nanocrystals were shown to have potential in bony regeneration. These scaffolds induced cellular aggregation and expressed bone-specific markers when seeded with human-bone-marrow MSC. A composite porous scaf-

fold seeded with BMP-2 and vascular endothelial growth factor 165 (VEGF165) induced mineralisation and formed dense tissue when implanted subcutaneously in mice and intramuscularly in goats. The capability of these scaffolds was evaluated in critical-size defects in clinical animal models at different sites such as the femoral condyles of rats and transverse mandibular defects in goats.[114]

In composite scaffolds vascularisation is a major obstacle, particularly when they are being used for regeneration of critical-size bone defects. In vitro pre-vascularisation is usually achieved by coculture of endothelial cells and osteogenically differentiating cells. Coculture of human-bone-marrow MSC and human progenitor-derived endothelial cells on polysaccharide scaffolds was shown to induce cellular interaction through multicellular aggregation of both types of cells. Subcutaneous implantation of this tissue-engineered construct in rats formed new osteoid-like structures.[115]

In another study, macroporous hydroxyapatite-gelatin nano-composite was polymerised and combined with titanium oxide. Scaffold was seeded with undifferentiated and osteogenically differentiated multipotent stem cells in aggregated form for regeneration of the calvarial bone in rats. This nanocomposite scaffold with titanium dioxide has the mechanical strength 13.8 (4.5) MPa, which mimics the calvarial bone strength of 24.5 (8.3) MPa. After 8–12 weeks implantation, there was better osteoconductivity in this scaffold than in the hydroxyapatite gel scaffold without titanium dioxide, and the scaffold based on polyethylene glycolic acid.[116]

Delivery of growth factor through 3-dimensional macroporous scaffolds

Growth factors are soluble, and secrete signalling polypeptides with the ability to instruct specific cellular responses. Those responses triggered by growth factor signalling can result in a wide range of cellular actions, including survival, migration, and differentiation. They exhibit their actions by short-range diffusion through the extracellular matrix, and act locally because of their short half-life. Because of their critical role in the regulation of cellular functions and their osteoinductive nature, a wide range of growth factors has been tested for regeneration of bony tissue. Major players in skeletal tissue engineering are members of the transforming growth factor beta (TGF-β) superfamily, notably the bone morphogenic proteins (BMP). Other growth factors are VEGF, fibroblast growth factor, and platelet-rich fibrin.

About 20 isoforms of BMP have been identified, among them BMP-2, BMP-4, BMP-7, and BMP-9, which are essentially used in bone tissue engineering.[117,118] While BMP-2 is most commonly used and is commercially available, rhBMP-2 and rhBMP-7 are currently used for lumbar fusion.[118]

Several biomaterials have also been investigated for BMP delivery for bone tissue engineering.[45,118,119] Collagen sponge as an effective carrier for BMP that has been proved to be of therapeutic use. Absorbable collagen sponge/rhBMP-2 has been associated with substantial formation of bone in the maxillary sinus of goats compared with no treatment or treatment with absorbable collagen sponge alone.[120] In rhesus monkeys, regeneration of alveolar ridges was seen in critical-size segmental defects created in the maxillofacial region when treated with rhBMP-2/absorbable collagen sponge.[121] The clinical study by Boyne et al of the repair of the maxillary sinus floor showed that the combination of BMP-2 with absorbable collagen sponge in the formation of bone is comparable with that of an autologous bone graft.[122] Arosarena and Collins compared the ability of BMP-2 and BMP-4 to form bone in the mandibles of rats,[117] and found similar volumes of new bone. However, the dose of BMP-4 used was higher that that of BMP-2. In a recent study of rhBMP-2 coated implants in the rabbit's sinus, the bone that formed around rhBMP-2 coated implants used with absorbable collagen sponge showed better augmentation than that of the implants coated with rhBMP-2 alone.[123] Taken together, these studies suggest that the efficacy of the growth factor depends on the carrier material.

While rhBMP hold great promise for spinal fusions, nonunion of fractures, and craniomaxillofacial defects, there are several concerns about protein stability and inadequate understanding of the optimal conditions for BMP-2 delivery that have limited its use. Many strategies have been developed to minimise the quantity of BMP-2 such as controlled delivery, combinational use with other growth factors, and use of genes.

The controlled release of recombinant BMP-2 with improved bone formation was achieved in MSC loaded with chitosan/alginate/hydroxyapatite scaffold (CAH/B2) that released BMP-2 into 8 mm critical-size calvarial defects in rats.[45] The histological results of this study showed that BMP-2 embedded-hydrogel, and gels that encapsulated BMP-2 and MSC, resulted in mature bone formation with vascular markers after four weeks of implantation in calvarial defects in Sprague Dawley rats.[45]

In another study, an injectable biopolymer of chitosan and inorganic phosphates seeded with MSC and BMP-2 was evaluated in a calvarial critical-size defect in rats. The results showed that the combination of the gel loaded with a low dose of BMP-2 (2 μg) and MSC improved bone formation compared with the group treated with gel alone or with gel and BMP-2 alone.[119] Combining it with a non-peptide compound, was found to be an effective way of reducing the dose of BMP-2. In this study a combination of a low dose of BMP-2 (0.5 μg) and the prostaglandin E2 receptor agonist EP4A (100 μg) was embedded in a nanofibre gel and healed the calvarial defects.

Tethering is one of the techniques used to entrap growth factors. Anti-BMP-2 monoclonal antibodies can trap ligands, and so provide the inductive signals for the osteogenic differentiation of progenitor cells. Coencapsulation of anti BMP-2 monoclonal antibody and MSC in alginate microspheres

was evaluated for regeneration in 5 mm calvarial defects, and showed considerable formation of bone by eight weeks after implantation.[124] Koh et al implanted virally-transduced murine B-lymphocyte kinase cells into critical-size defects created in mice, and found that the cells transduced with adenovirus (Ad)BMP-2/7 were more effective at healing craniofacial defects than the cells individually transduced with AdBMP-2 or AdBMP-7.[125]

VEGF is one of the growth factors that regulates vascular development. Osteogenesis and angiogenesis are closely correlated during the growth, remodelling, and repair of bone. It interacts with other growth factors such as BMP-2 and platelet-derived growth factor (PDGF) and regulates the function of stem cells and various activities in different extracellular matrices or engineered scaffolds. It is interesting that an extracellular matrix-like scaffold can serve as a reservoir for VEGF, and these scaffolds influence its loading ability, speed of release, and activities. While bolus delivery of VEGF failed to repair a critical size craniofacial defect in guided bone regeneration, VEGF loaded in hydrogels and released in a controlled manner successfully improved osteogenesis and angiogenesis.

Platelet-rich plasma containing PDGF has been widely studied in various reconstruction procedures. While platelet-rich plasma works efficiently for soft tissue regeneration, its function in bony regeneration has been controversial. Some research workers have reported that it improved bony regeneration, and there have been other reports that disagreed.[126] Its role in craniofacial bony regeneration needs to be explored further.

Stem cells in craniofacial tissue regeneration

Stem cells obtained from various sources such as embryonic stem cells, MSC, bone marrow stem cells, and those from dental pulp or olfactory bone have been tested with different kind of macroporous scaffolds for regeneration of critical-size bone defects in several animals (Table 2). MSC are the most commonly used stem cells, and they have shown good potential in craniofacial bony regeneration. For example, polylactic co-glycolic acid scaffold seeded with MSC have shown promising results on regeneration of porcine mandible, verified by radiographic analysis.[127]

In another study, a composite scaffold based on polyethylene glycol-polycaprolactone-polyethylene glycol/hydroxyapatite seeded with porcine-derived BMSC showed the regenerative capability to heal the temporal bone in pigs.[128] In many cases in vivo the biomaterial and the stem cells need to be critically evaluated, because the ability to form the bone depends on the type of cells as well as the type of biomaterial. Wittenburg et al evaluated the growth and differentiation of murine-derived BMSC and adipose stem cells isolated from green fluorescent protein transgenic animals grown directly on two types of hydroxyapatite ceramic scaffolds, BONITMatrix® (DOT, GmbH) and NanoBone® (Artoss, GmbH). Their results showed that the in vitro growth and differentiation of BMSC or adipose stem cells are distinctly influenced on the substratum. While NanoBone® supported the BMSC growth and differentiation, BONITMatrix® supported the proliferation and differentiation of adipose stem cells.[129]

The effect of coculture of stem cells on regeneration of cranial defects was also studied by using different kinds of stem cells. Human umbilical-vein-derived endothelial cells (hUVEC) cocultured with MSC derived from different origins and seeded on to a calcium phosphate cement scaffold showed good regeneration potential compared with the control group.[130] Similarly, BMSC cocultured with CD34+ cells from peripheral blood (an endothelial progenitor cell haemopoietic stem-cell-enriched source), promoted bone regeneration in critical-size calvarial defects in rabbits.[131]

Few studies have reported the use of embryonic stem cells for craniofacial bony regeneration.[132,133] For instance, human embryonic stem cells that have been seeded on to a macroporous calcium phosphate construct showed increased activity of alkaline phosphatase and expression of osteocalcin in vitro. In vivo a similarly-treated group showed more bone formation and density of blood vessels after 12 weeks of implantation compared with those given calcium phosphate construct alone as a control group in cranial defects in rats.[132] Stem cells from dental pulp and alveolar bone have also shown potential for regeneration in periodontal tissue. For example, human deciduous pulp stem cells seeded on collagen matrix have shown promising results in the reconstruction of maxillary alveolar defects in Wistar rats.[134] In another study, the regenerating potential of dental follicle stem cells was reported on a polycaprolactone scaffold to heal craniofacial defect in Sprague-Dawley rats.[135] Recently, BMSC and adipose-tissue-derived stem cells were also tried in the regeneration of osseous mandibular defects in rabbits.[136]

Three-dimensional printing

One of the major advancements in tissue engineering is 3-dimensional printing. The craniofacial region is complex, and to mimic any critical-size defect formed in this region is a challenge. To date, computer aided design (CAD) of models of the defect have made it easy to develop personalised treatment. Three-dimensional printing technology is one of them, which enables us to print a scaffold that replicates patient-specific anatomy. Three-dimensional technology leads to generation of a matrix scaffold with precision and accuracy, which effectively promotes the regeneration of functional tissue.[137]

The printing of a 3-dimensional scaffold is a layer-by-layer process: CAD, direct 3-dimensional printing, fused deposition modelling, stereolithography, and selective laser sintering. These techniques can produce 3-dimensional

scaffolds in a range from millimetre to nanometre in size. The advantages include the synthesis of the scaffold with complex shapes, which is capable of uniform cell distribution.[138] Recently polycaprolactone-hydroxylapatite (90:10 wt%) scaffolds were fabricated using 3-dimensional printing seamlessly in three phases for the regeneration of the periodontium.[139] Strontium oxide (SrO) and magnesium oxide (MgO)-doped microwave sintered a 3-dimensional printed tricalcium phosphate scaffold that showed excellent mechanical properties with improved in vivo osteogenesis in rabbits. The wound healing ability also increased in these scaffolds.[140] Three dimensional printing is an emerging technology that is being studied in many other fields.

Conclusion

The craniofacial skeleton is a complex 3-dimensional anatomical structure and to mimic such 3-dimensional complexity and design a scaffold to fit into an anatomical defect is a challenge. While various macroporous scaffolds were designed to fit into the anatomical defects of the craniofacial region, preformed scaffolds are difficult to fit into irregular defects. The injectable scaffold materials may combat these challenges as they can fill the irregular defects for the repair of irregular defects in non-load bearing areas. Investigations and more clinical studies are needed of these materials to promote the regeneration of bone in the craniofacial region.

Conflict of interest

We have no conflicts of interest.

Ethics statement/confirmation of patients' permission

Not applicable.

References

1. Barnard NA, Vaughan ED. Osteosynthetic titanium mini-plate fixation of composite radial forearm flaps in mandibular reconstruction. *J Craniomaxillofac Surg* 1991;**19**:243–8.
2. Hidalgo DA. Titanium miniplate fixation in free flap mandible reconstruction. *Ann Plast Surg* 1989;**23**:498–507.
3. Kim NK, Nam W, Kim HJ. Comparison of miniplates and biodegradable plates in reconstruction of the mandible with a fibular free flap. *Br J Oral Maxillofac Surg* 2015;**53**:223–9.
4. Paul SA, Karthik AK, Chacko R, et al. Audit on titanium reconstruction of mandibular defects for jaw lesions. *J Pharm Bioallied Sci* 2014;**6**(suppl 1):S39–43.
5. Eppley BL, Morales L, Wood R, et al. Resorbable PLLA-PGA plate and screw fixation in pediatric craniofacial surgery: clinical experience in 1883 patients. *Plast Reconstr Surg* 2004;**114**:850–7.
6. Bostman O, Partio E, Hirvensalo E, et al. Foreign-body reactions to polyglycolide screws. Observations in 24/216 malleolar fracture cases. *Acta Orthop Scand* 1992;**63**:173–6.
7. Voss P, Sauerbier S, Wiedmann-Al-Ahmad M, et al. Bone regeneration in sinus lifts: comparing tissue-engineered bone and iliac bone. *Br J Oral Maxillofac Surg* 2010;**48**:121–6.
8. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. *Clin Orthop Relat Res* 1986;**205**:299–308.
9. Zuk PA. Tissue engineering craniofacial defects with adult stem cells? Are we ready yet? *Pediatr Res* 2008;**63**:478–86.
10. Petrovic V, Zivkovic P, Petrovic D, et al. Craniofacial bone tissue engineering. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012;**114**:e1–9.
11. Putnam AJ, Mooney DJ. Tissue engineering using synthetic extracellular matrices. *Nat Med* 1996;**2**:824–6.
12. Hutmacher DW. Scaffold design and fabrication technologies for engineering tissues—state of the art and future perspectives. *J Biomater Sci Polym Ed* 2001;**12**:107–24.
13. Hinze MC, Wiedmann-Al-Ahmad M, Glaum R, et al. Bone engineering-vitalisation of alloplastic and allogenic bone grafts by human osteoblast-like cells. *Br J Oral Maxillofac Surg* 2010;**48**:369–73.
14. Tam SK, Dusseault J, Bilodeau S, et al. Factors influencing alginate gel biocompatibility. *J Biomed Mater Res A* 2011;**98**:40–52.
15. Tang D, Tare RS, Yang LY, et al. Biofabrication of bone tissue: approaches, challenges and translation for bone regeneration. *Biomaterials* 2016;**83**:363–82.
16. Vallet-Regi M, Ruiz-Hernandez E. Bioceramics: from bone regeneration to cancer nanomedicine. *Adv Mater* 2011;**23**:5177–218.
17. Hutmacher DW. Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 2000;**21**:2529–43.
18. Jones JR, Hench LL. Factors affecting the structure and properties of bioactive foam scaffolds for tissue engineering. *J Biomed Mater Res B Appl Biomater* 2004;**68**:36–44.
19. Han LH, Lai JH, Yu S, et al. Dynamic tissue engineering scaffolds with stimuli-responsive macroporosity formation. *Biomaterials* 2013;**34**:4251–8.
20. Sicchieri LG, Crippa GE, de Oliveira PT, et al. Pore size regulates cell and tissue interactions with PLGA-CaP scaffolds used for bone engineering. *J Tissue Eng Regen Med* 2012;**6**:155–62.
21. Zhang S. Fabrication of novel biomaterials through molecular self-assembly. *Nat Biotechnol* 2003;**21**:1171–8.
22. Chen QZ, Quinn JMW, Thouas GA, et al. Bone-like elastomer-toughened scaffolds with degradability kinetics matching healing rates of injured bone. *Adv Eng Mater* 2010;**12**:B642–8.
23. Samavedi S, Whittington AR, Goldstein AS. Calcium phosphate ceramics in bone tissue engineering: a review of properties and their influence on cell behavior. *Acta Biomater* 2013;**9**:8037–45.
24. Will J, Gerhardt LC, Boccaccini AR. Bioactive glass-based scaffolds for bone tissue engineering. *Adv Biochem Eng Biotechnol* 2011;**126**:195–226.
25. Gerhardt LC, Jell GM, Boccaccini AR. Titanium dioxide (TiO(2)) nanoparticles filled poly(D,L lactid acid) (PDLLA) matrix composites for bone tissue engineering. *J Mater Sci Mater Med* 2007;**18**:1287–98.
26. Wang C, Gong Y, Zhong Y, et al. The control of anchorage-dependent cell behavior within a hydrogel/microcarrier system in an osteogenic model. *Biomaterials* 2009;**30**:2259–69.
27. Lee KY, Alsberg E, Mooney DJ. Degradable and injectable poly(aldehyde guluronate) hydrogels for bone tissue engineering. *J Biomed Mater Res* 2001;**56**:228–33.
28. Benoit DS, Nuttelman CR, Collins SD, et al. Synthesis and characterization of a fluvastatin-releasing hydrogel delivery system to modulate hMSC differentiation and function for bone regeneration. *Biomaterials* 2006;**27**:6102–10.
29. Weiss P, Vinatier C, Sohier J, et al. Self-hardening hydrogel for bone tissue engineering. *Macromol Symp* 2008;**266**:30–5.
30. Lee KY, Mooney DJ. Hydrogels for tissue engineering. *Chem Rev* 2001;**101**:1869–79.
31. Hoffman AS. Hydrogels for biomedical applications. *Adv Drug Deliv Rev* 2002;**54**:3–12.

32. Shin M, Yoshimoto H, Vacanti JP. In vivo bone tissue engineering using mesenchymal stem cells on a novel electrospun nanofibrous scaffold. *Tissue Eng* 2004;**10**:33–41.
33. Park JB. The use of hydrogels in bone-tissue engineering. *Med Oral Patol Oral Cir Bucal* 2011;**16**:e115–8.
34. Gutowska A, Jeong B, Jasionowski M. Injectable gels for tissue engineering. *Anat Rec* 2001;**263**:342–9.
35. Hou QP, De Bank PA, Shakesheff KM. Injectable scaffolds for tissue regeneration. *J Mater Chem* 2004;**14**:1915–23.
36. Suzuki Y, Tanihara M, Suzuki K, et al. Alginate hydrogel linked with synthetic oligopeptide derived from BMP-2 allows ectopic osteoinduction in vivo. *J Biomed Mater Res* 2000;**50**:405–9.
37. Sotome S, Uemura T, Kikuchi M, et al. Synthesis and in vivo evaluation of a novel hydroxyapatite/collagen-alginate as a bone filler and a drug delivery carrier of bone morphogenetic protein. *Mater Sci Eng Biomim Supramol Syst* 2004;**24**:341–7.
38. Suzuki K, Suzuki Y, Tanihara M, et al. Reconstruction of rat peripheral nerve gap without sutures using freeze-dried alginate gel. *J Biomed Mater Res* 2000;**49**:528–33.
39. Smidsrod O, Skjak-Braek G. Alginate as immobilization matrix for cells. *Trends Biotechnol* 1990;**8**:71–8.
40. Marler JJ, Guha A, Rowley J, et al. Soft-tissue augmentation with injectable alginate and syngeneic fibroblasts. *Plast Reconstr Surg* 2000;**105**:2049–58.
41. Lin HR, Yeh YJ. Porous alginate/hydroxyapatite composite scaffolds for bone tissue engineering: preparation, characterization, and in vitro studies. *J Biomed Mater Res B Appl Biomater* 2004;**71**:52–65.
42. Fragonas E, Valente M, Pozzi-Mucelli M, et al. Articular cartilage repair in rabbits by using suspensions of allogenic chondrocytes in alginate. *Biomaterials* 2000;**21**:795–801.
43. Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering: part 1. Structure, gelation rate and mechanical properties. *Biomaterials* 2001;**22**:511–21.
44. Kandalam U, Cabel A, Omidian H, et al. Viability of human umbilical cord–derived mesenchymal stem cells in G-rich and M-rich alginates. *J Bioact Compat Pol* 2012;**27**:174–82.
45. He X, Liu Y, Yuan X, et al. Enhanced healing of rat calvarial defects with MSCs loaded on BMP-2 releasing chitosan/alginate/hydroxyapatite scaffolds. *PLoS One* 2014;**9**:e104061.
46. d'Aquino R, De Rosa A, Lanza V, et al. Human mandible bone defect repair by the grafting of dental pulp stem/progenitor cells and collagen sponge biocomplexes. *Eur Cell Mater* 2009;**18**:75–83.
47. Keskar V, Marion NW, Mao JJ, et al. In vitro evaluation of macroporous hydrogels to facilitate stem cell infiltration, growth, and mineralization. *Tissue Eng A* 2009;**15**:1695–707.
48. Martinez-Sanz E, Varghese OP, Kisiel M, et al. Minimally invasive mandibular bone augmentation using injectable hydrogels. *J Tissue Eng Regen Med* 2012;**6**(suppl 3):s15–23.
49. D'Este M, Eglin D. Hydrogels in calcium phosphate moldable and injectable bone substitutes: Sticky excipients or advanced 3-D carriers? *Acta Biomater* 2012;**9**:5421–30.
50. Rederstorff E, Weiss P, Sourice S, et al. An in vitro study of two GAG-like marine polysaccharides incorporated into injectable hydrogels for bone and cartilage tissue engineering. *Acta Biomater* 2011;**7**:2119–30.
51. Shin H, Quinten Ruhe P, Mikos AG, et al. In vivo bone and soft tissue response to injectable, biodegradable oligo(poly(ethylene glycol) fumarate) hydrogels. *Biomaterials* 2003;**24**:3201–11.
52. Piskounova S, Gedda L, Hulsart-Billstrom G, et al. Characterization of recombinant human bone morphogenetic protein-2 delivery from injectable hyaluronan-based hydrogels by means of (125) I-radiolabelling. *J Tissue Eng Regen Med* 2012;**8**:821–30.
53. Patel M, Fisher JP. Biomaterial scaffolds in pediatric tissue engineering. *Pediatr Res* 2008;**63**:497–501.
54. Liu X, Ma PX. Polymeric scaffolds for bone tissue engineering. *Ann Biomed Eng* 2004;**32**:477–86.
55. Moshaverinia A, Chen C, Xu X, et al. Bone regeneration potential of stem cells derived from periodontal ligament or gingival tissue sources encapsulated in RGD-modified alginate scaffold. *Tissue Eng A* 2014;**20**:611–21.
56. Weir MD, Xu HH. Osteoblastic induction on calcium phosphate cement-chitosan constructs for bone tissue engineering. *J Biomed Mater Res A* 2010;**94**:223–33.
57. Yan J, Miao Y, Tan H, et al. Injectable alginate/hydroxyapatite gel scaffold combined with gelatin microspheres for drug delivery and bone tissue engineering. *Mater Sci Eng C Mater Biol Appl* 2016;**63**:274–84.
58. Dyondi D, Webster TJ, Banerjee R. A nanoparticulate injectable hydrogel as a tissue engineering scaffold for multiple growth factor delivery for bone regeneration. *Int J Nanomedicine* 2013;**8**:47–59.
59. Park YS, David AE, Park KM, et al. Controlled release of simvastatin from in situ forming hydrogel triggers bone formation in MC3T3-E1 cells. *AAPS J* 2012;**15**:367–76.
60. Jo S, Kim S, Cho TH, et al. Effects of recombinant human bone morphogenic protein-2 and human bone marrow-derived stromal cells on in vivo bone regeneration of chitosan-poly(ethylene oxide) hydrogel. *J Biomed Mater Res A* 2012;**101**:892–901.
61. 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**:4801–9.
62. Jefferiss CD, Lee AJ, Ling RS. Thermal aspects of self-curing polymethylmethacrylate. *J Bone Joint Surg* 1975;**57B**:511–8.
63. Peter SJ, Kim P, Yasko AW, et al. Crosslinking characteristics of an injectable poly(propylene fumarate)/beta-tricalcium phosphate paste and mechanical properties of the crosslinked composite for use as a biodegradable bone cement. *J Biomed Mater Res* 1999;**44**:314–21.
64. Burdick JA, Peterson AJ, Anseth KS. Conversion and temperature profiles during the photoinitiated polymerization of thick orthopaedic biomaterials. *Biomaterials* 2001;**22**:1779–86.
65. Burkoth AK, Burdick J, Anseth KS. Surface and bulk modifications to photocrosslinked polyanhydrides to control degradation behavior. *J Biomed Mater Res* 2000;**51**:352–9.
66. Kim CW, Talac R, Lu L, et al. Characterization of porous injectable poly-(propylene fumarate)-based bone graft substitute. *J Biomed Mater Res A* 2008;**85**:1114–9.
67. Lee K, Silva EA, Mooney DJ. Growth factor delivery-based tissue engineering: general approaches and a review of recent developments. *J R Soc Interface* 2011;**8**:153–70.
68. Xing ZC, Han SJ, Shin YS, et al. Enhanced osteoblast responses to poly(methyl methacrylate)/hydroxyapatite electrospun nanocomposites for bone tissue engineering. *J Biomater Sci Polym Ed* 2013;**24**:61–76.
69. Hartgerink JD, Beniash E, Stupp SI. Self-assembly and mineralization of peptide-amphiphile nanofibers. *Science* 2001;**294**:1684–8.
70. Woo KM, Chen VJ, Jung HM, et al. Comparative evaluation of nanofibrous scaffolding for bone regeneration in critical-size calvarial defects. *Tissue Eng A* 2009;**15**:2155–62.
71. Misawa H, Kobayashi N, Soto-Gutierrez A, et al. PuraMatrix facilitates bone regeneration in bone defects of calvaria in mice. *Cell Transplant* 2006;**15**:903–10.
72. Yoshimi R, Yamada Y, Ito K, et al. Self-assembling peptide nanofiber scaffolds, platelet-rich plasma, and mesenchymal stem cells for injectable bone regeneration with tissue engineering. *J Craniofac Surg* 2009;**20**:1523–30.
73. Mann BK, Gobin AS, Tsai AT, et al. Smooth muscle cell growth in photopolymerized hydrogels with cell adhesive and proteolytically degradable domains: synthetic ECM analogs for tissue engineering. *Biomaterials* 2001;**22**:3045–51.
74. Rowley JA, Madlambayan G, Mooney DJ. Alginate hydrogels as synthetic extracellular matrix materials. *Biomaterials* 1999;**20**:45–53.
75. Stile RA, Healy KE. Thermo-responsive peptide-modified hydrogels for tissue regeneration. *Biomacromolecules* 2001;**2**:185–94.
76. Burdick JA, Anseth KS. Photoencapsulation of osteoblasts in injectable RGD-modified PEG hydrogels for bone tissue engineering. *Biomaterials* 2002;**23**:4315–23.

77. Dessi M, Borzacchiello A, Mohamed TH, et al. Novel biomimetic thermosensitive beta-tricalcium phosphate/chitosan-based hydrogels for bone tissue engineering. *J Biomed Mater Res A* 2013;**101**:2984–93.
78. Morais DS, Rodrigues MA, Lopes MA, et al. Biological evaluation of alginate-based hydrogels, with antimicrobial features by Ce(III) incorporation, as vehicles for a bone substitute. *J Mater Sci Mater Med* 2013;**24**:2145–55.
79. Pandit V, Zuidema JM, Venuto KN, et al. Evaluation of multifunctional polysaccharide hydrogels with varying stiffness for bone tissue engineering. *Tissue Eng A* 2013;**19**:2452–63.
80. Madhumathi K, Sudheesh Kumar PT, Kavya KC, et al. Novel chitin/nanosilica composite scaffolds for bone tissue engineering applications. *Int J Biol Macromol* 2009;**45**:289–92.
81. Furuichi K, Oaki Y, Ichimiya H, et al. Preparation of hierarchically organized calcium phosphate–organic polymer composites by calcification of hydrogel. *Sci Tech Adv Mater* 2006;**7**:219–25.
82. Douglas TE, Krawczyk G, Pamula E, et al. Generation of composites for bone tissue-engineering applications consisting of gellan gum hydrogels mineralized with calcium and magnesium phosphate phases by enzymatic means. *J Tissue Eng Regen Med* 2014;**10**:938–54.
83. Gkioni K, Leeuwenburgh SC, Douglas TE, et al. Mineralization of hydrogels for bone regeneration. *Tissue Eng Part B Rev* 2010;**16**:577–85.
84. Nuttelman CR, Benoit DS, Tripodi MC, et al. The effect of ethylene glycol methacrylate phosphate in PEG hydrogels on mineralization and viability of encapsulated hMSCs. *Biomaterials* 2006;**27**:1377–86.
85. Bhat S, Tripathi A, Kumar A. Supermacroprous chitosan-agarose-gelatin cryogels: in vitro characterization and in vivo assessment for cartilage tissue engineering. *J R Soc Interface* 2010;**8**:540–54.
86. Bhat S, Lidgren L, Kumar A. In vitro neo-cartilage formation on a three-dimensional composite polymeric cryogel matrix. *Macromol Biosci* 2013;**13**:827–37.
87. Mishra R, Kumar A. Inorganic/organic biocomposite cryogels for regeneration of bony tissues. *J Biomater Sci Polym Ed* 2011;**22**:2107–26.
88. Mishra R, Goel SK, Gupta KC, et al. Biocomposite cryogels as tissue-engineered biomaterials for regeneration of critical-sized cranial bone defects. *Tissue Eng A* 2013;**20**:751–62.
89. Zo SM, Singh D, Kumar A, et al. Chitosan–hydroxyapatite macroporous matrix for bone tissue engineering. *Curr Sci* 2012;**103**:1438–46.
90. Sepulveda P, Jones JR, Hench LL. Bioactive sol–gel foams for tissue repair. *J Biomed Mater Res* 2002;**59**:340–8.
91. Mishra R, Basu B, Kumar A. Physical and cytocompatibility properties of bioactive glass-polyvinyl alcohol-sodium alginate biocomposite foams prepared via sol–gel processing for trabecular bone regeneration. *J Mater Sci Mater Med* 2009;**20**:2493–500.
92. de Oliveira AA, Gomide V, Leite MF, et al. Effect of polyvinyl alcohol content and after synthesis neutralization on structure, mechanical properties and cytotoxicity of sol–gel derived hybrid foams. *Mater Res* 2009;**12**:1–10.
93. Trejo CG, Lozano D, Manzano M, et al. The osteoinductive properties of mesoporous silicate coated with osteostatin in a rabbit femur cavity defect model. *Biomaterials* 2010;**31**:8564–73.
94. Lu W, Ji K, Kirkham J, et al. Bone tissue engineering by using a combination of polymer/Bioglass composites with human adipose-derived stem cells. *Cell Tissue Res* 2014;**356**:97–107.
95. Khan Y, Yaszemski MJ, Mikos AG, et al. Tissue engineering of bone: material and matrix considerations. *J Bone Joint Surg* 2008;**90A**(suppl 1):36–42.
96. Le Nihouannen D, 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**:1983–90.
97. LeGeros RZ, Lin S, Rohanizadeh R, et al. Biphasic calcium phosphate bioceramics: preparation, properties and applications. *J Mater Sci Mater Med* 2003;**14**:201–9.
98. Lobo SE, Arinzeh TL. Biphasic calcium phosphate ceramics for bone regeneration and tissue engineering applications. *Materials* 2010;**3**:815–26.
99. Ibara A, Miyaji H, Fugetsu B, et al. Osteoconductivity and biodegradability of collagen scaffold coated with nano-β-TCP and fibroblast growth factor 2. *J Nanomater* 2013;**2013**:11.
100. Kang Y, Mochizuki N, Khademhosseini A, et al. Engineering a vascularized collagen-beta-tricalcium phosphate graft using an electrochemical approach. *Acta Biomater* 2015;**11**:449–58.
101. Ansari S, Moshaverinia A, Pi SH, et al. Functionalization of scaffolds with chimeric anti-BMP-2 monoclonal antibodies for osseous regeneration. *Biomaterials* 2013;**34**:10191–8.
102. Uebersax L, Apfel T, Nuss KM, et al. Biocompatibility and osteoconduction of macroporous silk fibroin implants in cortical defects in sheep. *Eur J Pharm Biopharm* 2013;**85**:107–18.
103. Blaker JJ, Maquet V, Jerome R, et al. Mechanical properties of highly porous PDLLA/Bioglass composite foams as scaffolds for bone tissue engineering. *Acta Biomater* 2005;**1**:643–52.
104. Hedberg EL, Shih CK, Lemoine JJ, et al. In vitro degradation of porous poly(propylene fumarate)/poly(DL-lactic-co-glycolic acid) composite scaffolds. *Biomaterials* 2005;**26**:3215–25.
105. Jiang G, Evans ME, Jones IA, et al. Preparation of poly(epsilon-caprolactone)/continuous bioglass fibre composite using monomer transfer moulding for bone implant. *Biomaterials* 2005;**26**:2281–8.
106. Kim HW, Knowles JC, Kim HE. Hydroxyapatite/poly(epsilon-caprolactone) composite coatings on hydroxyapatite porous bone scaffold for drug delivery. *Biomaterials* 2004;**25**:1279–87.
107. Lu HH, Tang A, Oh SC, et al. Compositional effects on the formation of a calcium phosphate layer and the response of osteoblast-like cells on polymer-bioactive glass composites. *Biomaterials* 2005;**26**:6323–34.
108. Niiranen H, Pyhalto T, Rokkanen P, et al. In vitro and in vivo behavior of self-reinforced bioabsorbable polymer and self-reinforced bioabsorbable polymer/bioactive glass composites. *J Biomed Mater Res A* 2004;**69**:699–708.
109. Zhang K, Wang Y, Hillmyer MA, et al. Processing and properties of porous poly(L-lactide)/bioactive glass composites. *Biomaterials* 2004;**25**:2489–500.
110. Yao Q, Nooeaid P, Detsch R, et al. Bioglass® /chitosan-polycaprolactone bilayered composite scaffolds intended for osteochondral tissue engineering. *J Biomed Mater Res A* 2014;**102**:4510–8.
111. Nguyen TB, Lee BT. A combination of biphasic calcium phosphate scaffold with hyaluronic acid-gelatin hydrogel as a new tool for bone regeneration. *Tissue Eng Part A* 2014;**20**:1993–2004.
112. Lee K, Weir MD, Lippens E, et al. Bone regeneration via novel macroporous CPC scaffolds in critical-sized cranial defects in rats. *Dent Mater* 2014;**30**:e199–207.
113. Bretcanu O, Baino F, Verne E, et al. Novel resorbable glass-ceramic scaffolds for hard tissue engineering: From the parent phosphate glass to its bone-like macroporous derivatives. *J Biomater Appl* 2013;**28**:1287–303.
114. Fricain JC, Schlaubitz S, Le Visage C, et al. A nano-hydroxyapatite–pullulan/dextran polysaccharide composite macroporous material for bone tissue engineering. *Biomaterials* 2013;**34**:2947–59.
115. Guerrero J, Catros S, Derkaoui SM, et al. Cell interactions between human progenitor-derived endothelial cells and human mesenchymal stem cells in a three-dimensional macroporous polysaccharide-based scaffold promote osteogenesis. *Acta Biomater* 2013;**9**:8200–13.
116. Ferreira JR, Padilla R, Urkasemsin G, et al. Titanium-enriched hydroxyapatite-gelatin scaffolds with osteogenically differentiated progenitor cell aggregates for calvaria bone regeneration. *Tissue Eng Part A* 2013;**19**:1803–16.
117. Arosarena O, Collins W. Comparison of BMP-2 and -4 for rat mandibular bone regeneration at various doses. *Orthod Craniofac Res* 2005;**8**:267–76.
118. Chrastil J, Low JB, Whang PG, et al. Complications associated with the use of the recombinant human bone morphogenetic proteins for

posterior interbody fusions of the lumbar spine. *Spine (Phila Pa 1976)* 2013;**38**:E1020–7.
119. Stephan SJ, Tholpady SS, Gross B, et al. Injectable tissue-engineered bone repair of a rat calvarial defect. *Laryngoscope* 2010;**120**:895–901.
120. Nevins M, Kirker-Head C, Nevins M, et al. Bone formation in the goat maxillary sinus induced by absorbable collagen sponge implants impregnated with recombinant human bone morphogenetic protein-2. *Int J Periodontics Restorative Dent* 1996;**16**:8–19.
121. Boyne PJ. Animal studies of application of rhBMP-2 in maxillofacial reconstruction. *Bone* 1996;**19**:83s–92s.
122. Boyne PJ, Lilly LC, Marx RE, et al. De novo bone induction by recombinant human bone morphogenetic protein-2 (rhBMP-2) in maxillary sinus floor augmentation. *J Oral Maxillofac Surg* 2005;**63**:1693–707.
123. Baek WS, Yoon SR, Lim HC, et al. Erratum re: Bone formation around rhBMP-2-coated implants in rabbit sinuses with or without absorbable collagen sponge grafting. *J Periodontal Implant Sci* 2016;**46**:360.
124. Moshaverinia A, Ansari S, Chen C, et al. Co-encapsulation of anti-BMP2 monoclonal antibody and mesenchymal stem cells in alginate microspheres for bone tissue engineering. *Biomaterials* 2013;**34**:6572–9.
125. Koh JT, Zhao Z, Wang Z, et al. Combinatorial gene therapy with BMP2/7 enhances cranial bone regeneration. *J Dent Res* 2008;**87**:845–9.
126. Wiltfang J, Kloss FR, Kessler P, et al. Effects of platelet-rich plasma on bone healing in combination with autogenous bone and bone substitutes in critical-size defects. An animal experiment. *Clin Oral Implants Res* 2004;**15**:187–93.
127. Abukawa H, Shin M, Williams WB, et al. Reconstruction of mandibular defects with autologous tissue-engineered bone. *J Oral Maxillofac Surg* 2004;**62**:601–6.
128. Liao HT, Chen YY, Lai YT, et al. The osteogenesis of bone marrow stem cells on mPEG-PCL-mPEG/hydroxyapatite composite scaffold via solid freeform fabrication. *Biomed Res Int* 2014;**2014**:321549.
129. Wittenburg G, Flade V, Garbe AI, et al. Scaffold preferences of mesenchymal stromal cells and adipose-derived stem cells from green fluorescent protein transgenic mice influence the tissue engineering of bone. *Br J Oral Maxillofac Surg* 2014;**52**:409–14.
130. Chen W, Liu X, Chen Q, et al. Angiogenic and osteogenic regeneration in rats via calcium phosphate scaffold and endothelial cell coculture with human bone marrow mesenchymal stem cells (MSCs), human umbilical cord MSCs, human induced pluripotent stem cell derived MSCs and human embryonic stem cell derived MSCs. *J Tissue Eng Regen Med* 2017, http://dx.doi.org/10.1002/term.2395.
131. Li G, Wang X, Cao J, et al. Coculture of peripheral blood CD34+ cell and mesenchymal stem cell sheets increase the formation of bone in calvarial critical-size defects in rabbits. *Br J Oral Maxillofac Surg* 2014;**52**:134–9.
132. Liu X, Wang P, Chen W, et al. Human embryonic stem cells and macroporous calcium phosphate construct for bone regeneration in cranial defects in rats. *Acta Biomater* 2014;**10**:4484–93.
133. Harkness L, Mahmood A, Ditzel N, et al. Selective isolation and differentiation of a stromal population of human embryonic stem cells with osteogenic potential. *Bone* 2011;**48**:231–41.
134. Jahanbin A, Rashed R, Alamdari DH, et al. Success of maxillary alveolar defect repair in rats using osteoblast-differentiated human deciduous dental pulp stem cells. *J Oral Maxillofac Surg* 2016;**74**:829, e1–9.
135. Rezai-Rad M, Bova JF, Orooji M, et al. Evaluation of bone regeneration potential of dental follicle stem cells for treatment of craniofacial defects. *Cytotherapy* 2015;**17**:1572–81.
136. Fang D, Roskies M, Abdallah MN, et al. Three-dimensional printed scaffolds with multipotent mesenchymal stromal cells for rabbit mandibular reconstruction and engineering. *Methods Mol Biol* 2017;**1553**:273–91.
137. Peltola SM, Melchels FP, Grijpma DW, et al. A review of rapid prototyping techniques for tissue engineering purposes. *Ann Med* 2008;**40**:268–80.
138. Do AV, Khorsand B, Geary SM, et al. 3D printing of scaffolds for tissue regeneration applications. *Adv Healthcare Mater* 2015;**4**:1742–62.
139. Lee CH, Hajibandeh J, Suzuki T, et al. Three-dimensional printed multiphase scaffolds for regeneration of periodontium complex. *Tissue Eng Part A* 2014;**20**:1342–51.
140. Tarafder S, Dernell WS, Bandyopadhyay A, et al. SrO- and MgO-doped microwave sintered 3D printed tricalcium phosphate scaffolds: mechanical properties and in vivo osteogenesis in a rabbit model. *J Biomed Mater Res B Appl Biomater* 2015;**103**:679–90.
141. Riccio M, Maraldi T, Pisciotta A, et al. Fibroin scaffold repairs critical-size bone defects in vivo supported by human amniotic fluid and dental pulp stem cells. *Tissue Eng Part A* 2012;**18**:1006–13.
142. He X, Dziak R, Yuan X, et al. BMP2 genetically engineered MSCs and EPCs promote vascularized bone regeneration in rat critical-sized calvarial bone defects. *PLoS One* 2013;**8**:e60473.
143. Streckbein P, Jackel S, Malik CY, et al. Reconstruction of critical-size mandibular defects in immunoincompetent rats with human adipose-derived stromal cells. *J Craniomaxillofac Surg* 2013;**41**:496–503.
144. Kowalczewski CJ, Tombyln S, Wasnick DC, et al. Reduction of ectopic bone growth in critically-sized rat mandible defects by delivery of rhBMP-2 from kerateine biomaterials. *Biomaterials* 2014;**35**:3220–8.
145. Bolgen N, Korkusuz P, Vargel I, et al. Stem cell suspension injected HEMA-lactate-dextran cryogels for regeneration of critical sized bone defects. *Artif Cells Nanomed Biotechnol* 2014;**42**:70–7.
146. Mishra R, Goel SK, Gupta KC, et al. Biocomposite cryogels as tissue-engineered biomaterials for regeneration of critical-sized cranial bone defects. *Tissue Eng Part A* 2014;**20**:751–62.
147. Saad KA, Abu-Shahba AG, El-Drieny EA, et al. Evaluation of the role of autogenous bone-marrow-derived mesenchymal stem cell transplantation for the repair of mandibular bone defects in rabbits. *J Craniomaxillofac Surg* 2015;**43**:1151–60.
148. Petridis X, Diamanti E, Trigas G, et al. Bone regeneration in critical-size calvarial defects using human dental pulp cells in an extracellular matrix-based scaffold. *J Craniomaxillofac Surg* 2015;**43**:483–90.
149. Chamieh F, Collignon AM, Coyac BR, et al. Accelerated craniofacial bone regeneration through dense collagen gel scaffolds seeded with dental pulp stem cells. *Sci Rep* 2016;**6**:38814.
150. Wang X, Xing H, Zhang G, et al. Restoration of a critical mandibular bone defect using human alveolar bone-derived stem cells and porous nano-HA/collagen/PLA scaffold. *Stem Cells Int* 2016;**2016**:8741641.
151. Bolgen N, Yang Y, Korkusuz P, et al. Three-dimensional ingrowth of bone cells within biodegradable cryogel scaffolds in bioreactors at different regimes. *Tissue Eng Part A* 2008;**14**:1743–50.
152. van Eijk F, Saris DB, Fedorovich NE, et al. In vivo matrix production by bone marrow stromal cells seeded on PLGA scaffolds for ligament tissue engineering. *Tissue Eng Part A* 2009;**15**:3109–17.
153. Tsung LH, Chang K, Chen JP. Osteogenesis of adipose-derived stem cells on three dimensional, macroporous gelatin-hyaluronic acid cryogel. *Biomed Eng Appl Basis Commun* 2011;**23**:127–33.
154. Pallela R, Venkatesan J, Janapala VR, et al. Biophysicochemical evaluation of chitosan-hydroxyapatite-marine sponge collagen composite for bone tissue engineering. *J Biomed Mater Res A* 2012;**100**:486–95.
155. Rodriguez-Lorenzo LM, Saldana L, Benito-Garzon L, et al. Feasibility of ceramic-polymer composite cryogels as scaffolds for bone tissue engineering. *J Tissue Eng Regen Med* 2012;**6**:421–33.
156. Rodrigues SC, Salgado CL, Sahu A, et al. Preparation and characterization of collagen-nanohydroxyapatite biocomposite scaffolds by cryogelation method for bone tissue engineering applications. *J Biomed Mater Res A* 2013;**101**:1080–94.
157. Inci I, Kirsebom H, Galaev IY, et al. Gelatin cryogels crosslinked with oxidized dextran and containing freshly formed hydroxyapatite as potential bone tissue-engineering scaffolds. *J Tissue Eng Regen Med* 2013;**7**:584–8.
158. Kemence N, Bolgen N. Gelatin- and hydroxyapatite-based cryogels for bone tissue engineering: synthesis, characterization, in vitro and in vivo biocompatibility. *J Tissue Eng Regen Med* 2017;**11**:20–33.

159. Ribeiro M, de Moraes MA, Beppu MM, et al. Development of silk fibroin/nanohydroxyapatite composite hydrogels for bone tissue engineering. *Eur Polym J* 2015;**67**:66–77.
160. Ma G, Yang D, Li Q, et al. Injectable hydrogels based on chitosan derivative/polyethylene glycol dimethacrylate/N,N-dimethylacrylamide as bone tissue engineering matrix. *Carbohydr Polym* 2010;**79**:620–7.
161. Mishra R, Kumar A. Effect of plasma polymerization on physicochemical properties of biocomposite cryogels causing a differential behavior of human osteoblasts. *J Colloid Interface Sci* 2014;**431**:139–48.
162. Roether JA, Boccaccini AR, Hench LL, et al. Development and in vitro characterisation of novel bioresorbable and bioactive composite materials based on polylactide foams and Bioglass® for tissue engineering applications. *Biomaterials* 2002;**23**:3871–8.
163. Stipniece L, Narkevica I, Sokolova M, et al. Novel scaffolds based on hydroxyapatite/poly(vinyl alcohol) nanocomposite coated porous TiO2 ceramics for bone tissue engineering. *Ceram Int* 2016;**42**:1530–7.
164. Li W, Nooeaid P, Roether JA, et al. Preparation and characterization of vancomycin releasing PHBV coated 45S5 Bioglass® -based glass–ceramic scaffolds for bone tissue engineering. *J Eur Ceram Soc* 2014;**34**:505–14.
165. Nouri-Felekori M, Sheikh-Mehdi Mesgar A, Mohammadi Z. Development of composite scaffolds in the system of gelatin-calcium phosphate whiskers/fibrous spherulites for bone tissue engineering. *Ceram Int* 2015;**41**:6013–9.