Review Article

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Orthopaedic applications of bone graft & graft substitutes: a review

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Treatment of delayed union, malunion, and nonunion is a challenge to the orthopaedic surgeons in veterinary and human fields. Apart from restoration of alignment and stable fixation, in many cases adjunctive measures such as bone-grafting or use of bone-graft substitutes are of paramount importance. Bone-graft materials usually have one or more components: an osteoconductive matrix, which acts as scaffold to new bone growth; osteoinductive proteins, which support mitogenesis of undifferentiated cells; and osteogenic cells, which are capable of forming bone in the appropriate environment. Autologous bone remains the "gold standard" for stimulating bone repair and regeneration, but its availability may be limited and the procedure to harvest the material is associated with complications. Bone-graft substitutes can either substitute autologous bone graft or expand an existing amount of autologous bone graft. We review the currently available bone graft and graft substitutes for the novel therapeutic approaches in clinical setting of orthopaedic surgery.

Key words Bone graft - orthopaedic surgery - osteoconductive - osteoinductive - osteogenic graft substitute

Several categories of bone graft and graft substitutes exist and encompass a variety of materials, material sources, and origins. The available graft substitutes formed from composites of one or more types of material. These composites are generally built on a base material. Laurencin *et al*[1] classification of grafts and graft substitutes could be modified as follows:

A. ***Harvested bone grafts and graft substitutes*:** bone grafts, endogenous or exogenous, are often essential to provide support, fill voids, and enhance biologic repair of skeletal defects due to traumatic or non-traumatic origin. Limitations of use of endogenous bone substance involve additional surgery; often resulting donor site morbidity and limited availability[2-4] where as allograft have been encountered with risk of disease transmission, immunogenicity[5]. Therefore, there is a growing need for synthesis of allograft bone substitutes used alone or in combination with other materials (*e.g.*, Allogro [AlloSource, Centennial, Colo], Opteform [Exactech, Inc, Gainesville, Fla], Grafton [BioHorizons, Birmingham, Ala], OrthoBlast [IsoTis OrthoBiologics, Irvine, Calif]).

B. ***Growth factor-based bone graft substitutes*:** natural and recombinant growth factors used alone or in combination with other materials such as transforming growth factor-beta (TGF-beta), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and bone morphogenetic protein (BMP).

C. ***Cell-based bone graft substitutes*:** use cells to generate new tissue alone or are seeded onto a support matrix (*e.g.*, mesenchymal stem cells).

D. ***Ceramic-based bone graft substitutes*:** include calcium phosphate, calcium sulfate, and bioglass

used alone or in combination (*e.g.*, OsteoGraf [DENTSPLY Friadent CeraMed, Lakewood, Colo], Norian SRS [Synthes, Inc, West Chester, Pa], ProOsteon [Interpore Cross International, Irvine, Calif], Osteoset [Wright Medical Technology, Inc, Arlington, Tenn]).

E. ***Polymer-based bone graft substitutes*:** degradable and nondegradable polymers, are used alone or in combination with other materials (*e.g.*, Cortoss [Orthovita, Inc, Malvern, Pa], open porosity polylactic acid polymer [OPLA], Immix [Osteobiologics, Inc, San Antonio, Tex]).

F. ***Miscellaneous*:** Various unconventional marine biomaterials are also in use as bone graft substitute which includes coral, chitosan, sponge skeleton *etc*.

Bone grafts and their substitutes can also be divided into osteoinductive agents, osteoconductive agents and osteogenic agents.

- Osteoinductive agents are generally proteins, which induce differentiation of undifferentiated stem cells to osteogenic cells or induce stem cells to proliferate[6].
- Osteoconduction is the process whereby microscopic and macroscopic scaffolding is provided for inward migration of cellular elements involved in bone formation (*e.g.*, mesenchymal cells, osteoblasts, osteoclasts, and vasculature).
- Osteogenesis in a general sense, osteogenesis refers to bone formation with no indication of cellular origin: new bone may originate from live cells in the graft or cells of host origin.

Many other classification systems of graft and graft substitute also exist. However, in this review the modification of Laurencin *et al*[1] is followed. The past and existing bone grafts, graft substitutes and the clinical evidence to support their use in the management of orthopaedic cases are reviewed as also future direction of research (Table).

A. Harvested bone grafts and graft substitutes:

1. Bone grafts:

I.I. Autogenous bone grafts (Bone autografts)

Autogenous bone grafts are considered as the gold standard for bone replacement, mainly because they offer minimum immunological rejection, complete histocompatibility and provide the best osteoconductive, osteogenic and osteoinductive properties[7]. Autografts usually contain viable osteogenic cells, bone matrix proteins and support bone growth[8] which are obtained from vascularized and non vascularized cortical and autologous bone marrow grafts. They offer

Table. Bone graft and graft substitutes

Class	Description	Examples	Properties of action
Autograft based	Used alone		• Osteoconductive • Osteoinductive • Osteogenic
Allograft based	Allograft bone used alone or in combination with other materials	Allegro, Orthoblast, Grafton	• Osteoconductive • Osteoinductive
Factor based	Natural and recombinant growth factors used alone or in combination with other materials	TGF-β, PDGF, FGF, BMP	• Osteoinductive • Both osteoconductive and osteoinductive with carrier materials
Cell based	Cells used to generate new tissue alone or seeded onto a support matrix	Mesenchymal stem cells	• Osteogenic, • Both osteogenic and osteoconductive with carrier materials
Ceramic based	Includes calcium phosphate, calcium sulfate, and bioactive glass used alone or in combination	Osteograf, , Osteoset, NovaBone	• Osteoconductive • Limited osteoinductive when mixed with bone marrow
Polymer based	Includes degradable and nondegradable polymers used alone and in combination with other materials	Cortoss, OPLA, Immix	• Osteoconductive • Bioresorbable in degradable polymer
Miscellaneous	Coral HA granules, blocks and composite	ProOsteon	• Osteoconductive • Bioresorbable

structural support to implanted devices and ultimately become mechanically efficient structures as they are incorporated into surrounding bone through creeping substitution[9]. They also suffer from resorption, limited availability and viability.

Autologous cancellous bone graft has been considered more osteogenic as compared to cortical bone graft because the presence of spaces within their structure, allows the diffusion of nutrients and limited revascularization by microanastomosis of its circulating vessels[10-12]. Cancellous graft is good space filler, but it does not provide substantial structural support. As only the osteoblasts and endosteal cells on the surface of the graft survive the transplant, a cancellous graft acts mainly as an osteoconductive substrate, which effectively supports the ingrowth of new blood vessels and the infiltration of new osteoblasts and osteoblasts precursors[13]. Further, an osteoprogenitor cell, however, is a mesenchymal cell that has acquired the ability to create cells with osteogenic capabilities. Transfer of a single osteoprogenitor cell can, through propagation, produce possibly hundreds of osteoblasts, and thus a considerable amount of bone. The principle advantage of autologous cancellous grafts is the potential to transfer osteoprogenitor cells to the recipient site. Osteoinductive agents, such as bone morphogenic protein (BMP), have varying abilities to induce mesenchymal cells to transform to osteoprogenitor cells and thus produce bone. Cancellous graft does not provide immediate structural support, it integrates quickly and ultimately achieves strength equivalent to cortical graft within six to twelve months[14]. Osteoinductive factors released from the graft during the resorptive process as well as cytokines released during the inflammatory phase may also contribute to the healing of the wound, this is only based on circumstantial evidence; not yet been substantiated by scientific documentation[13,15]. It has been observed that weight bearing capacity of affected limb returned earlier in animals with autograft compared to other types of bone grafts used[16]. In an experimental study dog, it was observed that fresh autogenous bone grafts are incorporated rapidly and possess osteoinductive, osteoconductive and osteogenic properties[17]. Autologous cancellous bone is commonly harvested from iliac crest, sometimes from the distal part of the radius/ tibia. It is widely used for delayed union of long bone fractures and reconstruction of depressed fracture of lateral tibial plateau[18-20].

Autologous cortical grafts have little or no osteoinductive properties and are mostly osteoconductive, but the surviving osteoblasts do provide some osteogenic properties as well[21,22]. Non-vascularized cortical grafts provide immediate structural support; they become weaker than vascularized cortical grafts during the initial six weeks after transplantation as a result of resorption and revascularization[21-23]. Vascularized cortical grafts heal rapidly at the host-graft-interface, and their remodeling is similar to that of normal bone. On the contrary, nonvascularized grafts do not undergo resorption and revascularization and, therefore, they provide superior strength during the first six weeks[21]. Despite their initial strength, cortical graft still must be supported by internal or external fixation to protect them from fracture. Autologous cortical bone grafts are good choices for segmental defects of bone of > 5 to 6 cm, which require immediate structural support. Larger graft requires prolonged time for resorption and fracture of graft may ensue if osteogenesis is not proper. On the other hand, if a bone graft is fragmented into small particles, even cancellous bone is killed and will no longer be osteogenic[24,25]. The main advantages of autologous cancellous graft are their excellent success rate and low risk of disease transmission. However, disadvantages as cited above include potential morbidity at the donor site, availability in limited quantities, and risk of wound infection, increased blood loss and prolonged anaesthetic time[26,27].

Site of grafting is another important factor influencing osteogenesis. Resorption and replacement of a bone graft in a skeletal bed occurs more rapidly at the end of a long bone (cancellous) than at the center of shaft (cortical bone)[24,28]. Accurate contact between a cortical bone graft and its bed is utmost necessary. Bone to bone contact along with low-intensity pulsed ultrasound LIPUS are also necessary in the treatment of delayed union or filling a skeletal defect with percutaneous bone grafts[29,30]. Ultrasound has a significant effect on biological tissues and cells involved in bone healing and in fracture repair and data from the literature support a positive effect on osteogenesis of LIPUS, applied percutaneously, in different experimental and clinical settings. LIPUS significantly stimulates and accelerates fresh fracture healing and is effective in promoting bone healing in aseptic and septic delayed- and nonunions with a healing rate ranging from 70 to 93 per cent in different, nonrandomized, studies. Advantages of the use of this technology is that it may avoid the need for additional complex operations for the treatment of nonunions include efficacy, safety, ease of use and favourable

cost/benefit ratio. Outcome depends on the site of nonunion; time elapsed from trauma, stability at the site of nonunion and host type[28]. Percutaneous bone grafting appeared to be as effective as open techniques, and possessed considerable advantages. It is safe, time saving and economical, it involves minimal trauma at the fracture site and it avoids major donor site problems[29].

Time interval between procurement and transplantation of graft is also an important factor[31]. Autogenous bone grafts retained their viability for two hours when kept in normal saline[32]. Coupland concluded that the graft remained unchanged in shape and act as a passive scaffold for new bone growth to fill the defect even after autoclaving[33]. In another study[34] freeze drying of autogenous bone did not alter the normal repair process associated with fresh autografts.

I.II. Bone marrow

Bone marrow has been used to stimulate bone formation in skeletal defects and nonunion through cytokines and growth factors secreted by the transplanted cells[35]. The main advantage of this technique is that it can be performed percutaneously, without almost any patient morbidity. Centrifugation of aspirated bone marrow at 400 times gravity for ten minutes separates the marrow cells from plasma and preserves the osteogenic potential of the cells, decreasing the volume of material injected[36]. Proliferation and differentiation of stem cells may be increased by adding them into growth factors[37] or by combining them in collagen[38].

The volume of bone marrow to be injected has been more controversial. The larger the volume of aspirate the grater number of alkaline phosphatase- positive colony forming units but they are more diluted[39]. Connolly *et al*[36] have recommended centrifugation of the aspirate to increase the percentage of cells and the efficacy of the aspirate. Curylo *et al*[40] have reported good results as a graft extender (insufficient autograft augmented with bone marrow) in experimental posterolateral spine fusion.

Autologous bone marrow mixed with 10 mg of demineralized bone matrix has been successfully used to fill bone defects[35,41] as demineralized bone matrix is an excellent carrier because of its osteoinductive as well as osteoconductive properties. Injection of autologous bone marrow, with or without a carrier, has been used to treat nonunion and delayed union of several bones. However, it does not promote healing more rapidly or to a greater extent than do traditional bone grafting techniques[42,43].

I.III. Allogenic bone grafts

The limitations associated with the procurement of autograft for bone grafting can be overcome by the use of allografts. Allograft bone is referred to as cadaver, obtained from donor bone and has both osteoinductive (they release bone morphogenic proteins that act on bone cells) and osteoconductive properties, but lack osteogenic properties because of the absence of viable cells[44]. However, harvesting and conservation of allogenic grafts are additional limiting factor[28,45,46]. The major advantage of allograft bone harvested from cadaver sources include its ready availability in various shapes and sizes, avoidance of the need to sacrifice host structures, and no donor site morbidity. Still, there is some controversy regarding association of allograft bone with the transmission of infectious agents, a major concern virtually eliminated through tissue-processing and sterilization.

Allogenic bone is available in many forms: demineralized bone matrix, morselized and cancellous chips, corticocancellous and cortical grafts, and osteochondral and whole-bone segments.

II. Demineralized bone matrix (DBM):

Demineralized bone matrix (DBM) which has been shown to have an osteoconductive and osteoinductive potential[47-51] is an interesting option. DMB provides no structural strength, and its primary use is in a structurally stable environment. DBM also revascularizes quickly and acts as suitable carrier for autologous bone marrow. It does not evoke any appreciable local foreign body immunogenic reaction as antigenic surface structure of bone is destroyed during demineralization[52]. The biologic activity of demineralized bone matrix is presumably attributable to proteins and various growth factors present in the extracellular matrix and made available to the host environment by the demineralization process. The osteoinductive capacity of demineralized bone matrix can be affected by storage, processing, and sterilization methods and can vary from donor to donor.

DMB has been successfully used to induce bone formation in various clinical conditions *viz*., to fill the defects caused by bone cysts and cavities[41,53,54], Cranio-maxillofacial reconstruction[55], bridging of large bone defects and repair of high risk fracture[50] and even very high risk defects[56]. The most successful grafts may

be composites of DMB bone matrix and autologous bone marrow[35,41] in stable fixation cases and human DMB with calcium sulfate ($CaSO_4$) in displaced intra-articular calcaneal fractures[58]. DMB can also augment and expand autologous cancellous bone graft when the supply of autogenous bone is limited or the defect is very large.

DMB has several potential disadvantages. Because it is an allergenic material, there is the potential to transmit human immunodeficiency virus (HIV). Another possible limitation of demineralized bone matrix is that different batches may have different potencies because of the wide variety of donors used to supply the graft.

B. *Growth factor-based bone graft substitutes*

Bone growth factors

The clinical use of growth factors is mainly limited by the problem of delivery[58]. Insulin like growth factor (IGF-1) and TGFβ mostly modulate the synthesis of the cartilage matrix[59,60] while basic fibroblast growth factor (bFGF) has a powerful mitogenic factor which stimulates the differentiation of chondrocytes[61,62]. Platelet-derived growth factor (PDGF) was studied on the bone healing of unilateral tibial osteotomies in rabbits and revealed that it had a stimulatory effect on fracture healing[63]. Basic fibroblast growth factor (bFGF) is produced locally in bone during the initial phase of fracture healing and is known to stimulate cartilage and bone forming cells[64]. Vascular endothelial growth factor, which combined with a coralline scaffold either coated with a control-plasmid DNA (a small cellular inclusion consisting of a ring of DNA that is not in a chromosome but is capable of autonomous replication), VEGF-plasmid DNA, loaded with mesenchymal stem cells (BMSC) transfected with control plasmid or with both stem cells and the VEGF plasmid showed to improve healing in large bone defects, in which bone substitutes will otherwise not be vascularized and replaced by fresh bone[65]. The application of gene transfer, which is a new technology, represents a unique opportunity for the local administration of growth factors[58,66]. Bone morphogenetic proteins (BMPs) are biologically active molecules capable of inducing new bone formation, and show potential for clinical use in bone defect repair. The synthetic biodegradable polymer/ interconnected-porous calcium hydroxyapatite ceramics (IP-CHA) composite is an excellent combination carrier/scaffold delivery system for recombinant bone morphogenetic protein-2 (rhBMP-2), that strongly promotes the clinical effects of rhBMP-2 in bone tissue regeneration[67].

C. *Cell-based bone graft substitutes*:

I. Stem cell

Stem cell research attracts considerable attention because the ethical controversies associated with the destruction of human embryos and the clinical potential of embryonic stem cells in regenerative and reparative therapies. Stem cell is an 'immature' or undifferentiated cell which is capable of producing any identical daughter cells[68,69]. The main sources of stem cells include somatic (Adult) and embryonic stem cells. Somatic stem cells include haematopoietic stem cells, bone marrow stromal (Mesenchymal) stem cells (MSC)[68,70], neural stem cells[71], dermal (Keratinocytes) stem cells[72], stem cells from fetal cord blood[73] and several others. The best options are those derived from the bone marrow which yields two types, the haematopoietic stem cells which gives rise to the entire blood cell lineage and the mesenchymal stem cells from which are derived various connective tissues such as bone and adipose tissues. Mesenchymal stem cells have also been identified and currently being used for the repair and regeneration of bone, cartilage, muscle, tendon and ligament[74]. Embryonic stem cells in mice have been used to generate a range of distinct phenotype including haematopoietic precursor[75], neural cells[76], adipocytes[77], muscle cells[78], myocytes[79], chondrocytes[80], pancreatic islets[81] and osteoblast[82].

The means of delivering factors to stimulate stem cells *in vivo* to initiate a process leading to regeneration has long been sought. Success has been restricted by problems of dosage, lack of full activity of recombinant factors and the inability to sustain the presence of the factor for an appropriate length of time. 'Gene-activated matrices' are being investigated which compromise plasmids coding for factors in a variety of delivery vehicles. Fresh marrow cells or cultured mesenchymal stem cells (MSCs) combined with porous ceramic and implanted into rat[83,84] or canine segmental bone defects[85] have shown osteogenic potential. Repair of cartilage damage or defects is technically challenging because cartilage tissue is relatively thin and avascular. In an attempt to provide regeneration of both cartilage and bone, cultured MSCs were implanted into massive osteochondral defects in the medial condyle of the distal femur of young adult rabbits. The MSCs uniformly formed chondrocytes which served to resurface the condyle[86,87].

Despite the challenges of isolating, expanding and defining stem cell populations, they hold tremendous promise for tissue regeneration at a clinically useful level. There are several examples of the potential use of stem cells in regenerative medicine, but, a thorough research in this area is needed to characterize graft versus host stem cells immune interactions and to identify mechanisms enabling the delivery or homing of the stem cells to the site of interest in clinical context.

II. Collagen

Collagen as an osteoinductive material is due to its osteoconductive property and when it is used in combination with osteoconductive carriers like hydroxyapatite or tricalcium phosphate. These composites are mixed with autologous bone marrow which subsequently provides osteoprogenitor cells and other growth factors. Chapman *et al*[88] conducted a prospective, randomized comparison of autologous iliac crest bone graft and calcium-collagen graft material in the treatment of acute long-bone fractures with both bone-grafting (<30 cm3 volume required) and internal or external fixation. The authors[88] observed no differences between the two groups with regard to the union rate or functional measures, and they concluded that calcium-collagen graft material with autologous bone marrow can be used instead of autologous bone graft for patients who have an acute traumatic defect of a long bone. There is no scientific proof that calcium-collagen graft materials can effectively substitute for autologous bone graft to stimulate healing of nonunion. This material with autologous bone marrow can be used as a replacement for autologous bone graft for acute long-bone fractures with enough comminution or cortical bone loss to require bone-grafting when internal or external fixation is planned[26]. It is not recommended to fill metaphyseal bone defects resulting from articular fractures as it does not offer structural support and also for the treatment of nonunion except in the role of a bone-graft expander when the supply of autologous bone graft is limited[26].

III. Gene therapy

Gene therapy involves the transfer of genetic information to cells. When a gene is transferred to a target cell, the cell synthesizes the protein encoded by the gene. For gene expression, the transferred DNA material must enter the nucleus where it can be transcripted. After transcription, the generated m-RNA is transported outside the nucleus and serves as a matrix for the production of proteins in the ribosome. The gene therapy used for gene induction is short-term and as regional therapy. The gene can be introduced directly to specific anatomic site (*in-vivo* technique) or specific cells can be harvested from the patient, expanded, genetically manipulated n tissue culture and then reimplanted (*ex-vivo* technique). Generally, the direct method is less technically demanding, indirect gene delivery is safer, because, the gene manipulation takes place under controlled conditions outside the organism. Viral and non-viral vectors can be used for the delivery of genetic materials into cells. Non-viral gene transfer systems such as liposomes, naked DNA are usually easier to produce and have a lower toxicity and immunogenicity, but the efficiency of their gene delivery is impeded by a blow rate of infection unless the transduced cells are selected[89,90]. Recently, viral gene vectors, including retrovirus, adenovirus, adeno-associated virus and herpes virus are more efficient method of gene transfer[91].

Tomita *et al*[92] first reported successful delivery of genes into the articular cartilage using a haemagglutinating virus (HVJ; sendai virus) liposome suspension containing the *SV40 large T antigen (SVT)* gene which was injected intra-articularly into the knees of rats. Biological effect of an effective growth factor in cartilage healing was studied using rabbit mesenchymal stem cells transduced with retroviral vectors encoded for the gene of bone morphogenetic protein-7, seeded on polymer scaffold grafts implanted into osteochondral defects in rabbit knees[93].

D. *Ceramic-based bone graft substitutes*

Among different ceramic based graft substitute materials, calcium phosphate based ceramics such as hydroxyapatite (HA), β-tricalcium phosphate (β-TCP) and bioactive glass are used quite substantially for long time. Calcium phosphate ceramics are synthetic scaffolds that have been used in dentistry since the early 1970s and in orthopedics since 1980s[94-97].

I. Calcium hydroxyapatite (HAp)

Hydroxyapatite is a biocompatible ceramic produced through a high-temperature reaction and is highly crystalline form of calcium phosphate. The nominal composition of this mixture is $Ca_{10}(PO_4)_6(OH)_2$ with a calcium-to-phosphate atomic ratio of 1.67. The most unique property of this material is chemical similarity with the mineralized phase of bone; this similarity accounts for their osteoconductive potential and excellent biocompatibility[98-100]. Calcium

hydroxyapatite/tricalcium phosphate (60/40) provide a structure or scaffold which can have a close interface with adjacent bone and have a limited application in the treatment of load-bearing segmental bone defects but did not fail at the early stages of implantation[101]. Hydroxyapatite has been established to be an excellent carrier of osteoinductive growth factors and osteogenic cell populations, which greatly add to their utility as bioactive delivery vehicles in the future[102].

The ideal pore size for a bioceramic should be similar to that of spongious bone. It has been demonstrated that microporosity (pore size <10 μm) allows body fluid circulation whereas macroporosity (pore size >50 μm) provides scaffold (Pore size-100-200 μm and porosity-60-65%) for bone-cell colonization[103,104]. An ideal pore size diameter of 565 μm is reported as the ideal macropore size for bone ingrowth compared to a smaller size (300 μm)[105]. However, in another study by Kuhne *et al*[106] the optimal size of the pores was found to be 500 μm. In an experimental study in goats with porous calcium phosphate ceramics, Toth *et al*[107] found that the ceramic when mixed with autograft in the ratio of 70 (ceramic): 30 (autograft) were effective for anterior cervical interbody fusion, Johnson *et al*[108] found that hydroxyapatite alone gave poor results. The interconnected high porous structure of hydroxyapatite seems to be promising for the environment of posterolateral lumbar intertransverse process spine fusion (PLF) in the point of producing fusion mass with higher cellular viability[109,110].

During recent years, there have been efforts in developing doped bioceramics materials to enhance their mechanical and biological properties as well as cytocompatibility for use in tissue engineering applications[111,112]. Hydroxyapatite (HA) as a synthetic material, usually used as coating for dental and orthopedic implants, are known for its good cytocompatibility properties, but is limited in use due to its moderate to low solubility within the body and mechanical properties that differ from surrounding tissue and bone[111]. Doped HA with manganese and/or zinc as bone substitute have been tried and resulted in faster resorption kinetics[113].

Plasma spray HA coating has been used on metallic femoral stem and cup as a means of fixation in order to avoid complications related to the use of PMMA[114]. Hydroxyapatite-coated pins enhance pin fixation regardless of bone type and loading conditions and reduces the rate of infection and loosening during external fixation[115,116]. Nguyen *et al*[117] studied the effect of sol-gel-formed calcium phosphate coatings on bone ingrowth and osteoconductivity of porous-surfaced Ti alloy implants in rabbit tibia and observed that endosteal bone growth along the porous-surfaced zone was greater with the Ca-P-coated implants compared to the non Ca-P-coated implants and greater bone-to-implant contact within the sinter neck regions of the Ca-P-coated implants.

II. Tri-calcium phosphate (TCP)

Like hydroxyapatite, TCP is bioabsorbable and biocompatible. The chemical composition and crystallinity of the material are similar to those of the mineral phase of bone. The nominal composition of TCP is $Ca_3 (PO_4)_2$. It exists in either α or β-crystalline forms. The rate of biodegradation is higher when compared with HA. Degradability occurs by combined dissolution and osteoclastic resorption[103].

Tricalcium phosphate implants have been used for two decades as synthetic bone void fillers in orthopaedic and dental applications[95,118]. The small particle size and interconnected sponge like microporosity are believed to improve osteoconductive properties and promote timely resorption concomitant with the process of remodeling[100,119-121]. Zhang *et al*[122] reported bone formation with bone marrow stromal cells (BMSCs) and β-tricalcium phosphate (β-TCP) as bone substitute implanted in rat dorsal muscles. Cutright *et al*[123] found 95 per cent absorption of tricalcium phosphate ceramic implants in rat tibias 48 days postoperatively with extensive bone growth and marrow reformation. Cameron *et al*[124] observed both the toxicity and the bone-ingrowth potential of TCP in canine model and reported no untoward tissue or systemic reaction when implanted in cancellous bone; it was rapidly infiltrated with bone and slowly resorbed. Breitbart *et al*[125] conducted experimental trials with TCP ceramic and osteogenin, an osteoinductive protein as an onlay graft substitute in a rabbit calvarial model. Gao *et al*[126] evaluated the effects of biocoral and TCP cylinders in segmental tibial bone defects (16 mm in length) and observed that biocoral is superior to TCP in repair of segmental defects in weight bearing limbs. Recombinant human bone morphogenetic protein (rhBMP)-2 with beta-TCP is a promising composite having osteogenicity and efficient enough for repairing large bone defects[127].

III. Bioactive glass

Bio-active glass ceramics (Bioglass) were first developed by Hench *et al*[128]. This glass is

biocompatible, osteoconductive and bonds to bone without an intervening fibrous connective tissue interface[129,130]. This material has been widely used for filling bone defects[100,131,132] alone and in combination with autogenous and allogenic cancellous bone graft[133]. Bioglass is composed mainly of silica, sodium oxide, calcium oxide and phosphates.

The bone-bonding reaction results from a sequence of reactions in the glass and its surface[134]. After long-term implantation, this biological apatite layer is partially replaced by bone[135]. The behaviour of bioactive glasses is dependent on the composition of the glass[136,137], the surrounding *p*H, the temperature, and the surface layers on the glass[138,139]. The porosity provides a scaffold on which newly-formed bone can be deposited after vascular in growth and osteoblast differentiation. The porosity of bioglass is also beneficial for resorption and bioactivity[140].

In experimental cancellous bone defects in rat models, bioglass was found biocompatible, and the filler effect was greater with bioactive glass than with autogenous bone[141]. Bioglass was found to trigger new bone formation by allogenic demineralized bone matrix, and the biocompatibility of the glass was verified by the absence of adverse cellular reactions[142,143]. Bone-bonding response significantly enhanced with the microroughening of the bioactive glass surface, but the glass composition affected the intensity of the response[144]. In another study, the microroughening of the bioglass surface accelerated temporal changes in the expression of specific genes involved in the bone healing process[145]. Bioactive glasses have shown no or only mild inflammatory responses in the surrounding tissue in histological *in vivo* studies and in 6 months, the glass fiber scaffolds are completely resorbed[146].

Bioactive glasses have been clinically used for tympanoplastic reconstruction[147], as filling material in benign tumour surgery[148], for reconstruction of defects in facial bones[149,150], for treatment of periodontal bone defects[151,152], in obliteration of frontal sinuses[153-155], in repairing orbital floor fractures[156,157], in lumbar fusion[158], and for reconstruction of the iliac crest defect after bone graft harvesting[159].

IV. Calcium phosphate cement

Calcium phosphate ceramics introduced more than three decades ago are considered as bioactive bone substitutes. The paste or injectable calcium phosphates cement offers the advantage of being freely mouldable and adaptable to bone defects. Brown and Chow[160,161] first reported the formation of apatitic cement consisting of a mixture of tetracalcium phosphate (TetCP) and dicalcium phosphate anhydrite (DCPA). Grüninger *et al*[162] introduced the term "calcium phosphate cements (CPC)" and described as: 'a powder or as a mixture of powders which, upon mixing with water or an aqueous solution to a paste, reacts around room or body temperature by the formation of a precipitate containing crystals of one or more calcium phosphates and sets by the entanglement of the crystals of that precipitate'[163]. After implantation, this composition form HAp *in situ* in contact with the physiological fluid. Since its inception CPCs have attracted much attention and different formulations have been put forward[164-168].

The drawback in using these materials was that close proximity to the host bone was necessary to achieve osteoconduction. Even, when this is achieved, new bone growth is often strictly limited because these materials are not osteoinductive in nature. To overcome this limitation, a number of different bone derived-growth factors have been demonstrated to stimulate bone growth, collagen synthesis and fracture repair both *in vitro* and *in vivo*.

The combination of high biocompatibility, easy-to-shape characteristic, and the capacity to self-setting under ambient conditions makes it an asset in the repair of hard tissue defects[169-172] and research and development on CPC have attracted much attention in recent years[172-175]. Based on its flow behavior before setting of slurry, CPC has been used as a root sealer-filler[176] and as an injectable biomaterial[177-181] for bone replacement, especially in percutaneous vertebroplasty[182-184] and kyphoplasty[185-187]. Šiniković *et al*[188] investigated the potential of CPC in the treatment of orbital wall defect fractures in an adult sheep model and compared the same with autologous calvarias split-bone grafts. However, CPC also suffers from its inherent lack of microporosity for tissue invasion[189] and poor injectibility[190]. Pore size and inherent strength play a major role in the ultimate usefulness of calcium phosphate cement. The pore size of Bone Source, a prototype CPC, has been reported to be as small as 2-5 nm[191] to as large as 8-12 μm[189], unsuitable for this particular application. Earlier it was generally believed that calcium phosphate cements are reabsorbed with bone formed via osteoconduction, but, recent studies suggested that calcium phosphate cements directly initiate osteogenesis[189]. Although the mechanism of osteoinduction remains unclear, the ionic exchanges

properties of the calcium phosphate cement with the surrounding milieu have been pointed out as a relevant parameter, among others. High microporosity in CPC is directly correlated with the exposed surface, and therefore an elevated dissolution in the pores where the level of stable critical level of free calcium ions and possibly free orthophosphate ions might trigger cell differentiation into osteogenic lineage. In addition, through a dissolution–precipitation process, the development of a bone-like mineral layer might initiate bone formation either by mimicry with the bone mineral structure or by the presence of osteogenic compounds (for example bone morphogenetic proteins) contained naturally in body fluids that might have concentrated at the newly formed mineral layer.

V. Calcium Sulfate:

Calcium sulfate graft material with a patented crystalline structure described as an alphahemihydrate acts primarily as osteoconductive bone-void filler that completely resorbs as newly formed bone remodels and restores anatomic features and structural properties. Potential application of calcium sulfate graft material includes the filling of cysts, bone cavities[192], benign bone lesions[193] and segmental bone defects; expansion of grafts used for spinal fusion; and filling of bone-graft harvest sites[26]. It is biocompatible, bioactive and resorbable after 12 wk[194]. Significant loss of its mechanical properties occurs upon its degradation; therefore, it is a questionable choice for load-bearing applications.

E. *Polymer-based bone graft substitutes*

Polymers present some options that the other groups do not. Like many polymers are potential candidates for bone graft substitutes represent different physical, mechanical, and chemical properties. The polymers used today can be loosely divided into natural polymers and synthetic polymers. These, in turn, can be divided further into degradable and nondegradable types.

Polymer-based bone graft substitutes include the following:

Healos (DePuy Orthopaedics, Inc, Warsaw, Ind) is a natural polymer-based product, a polymer-ceramic composite consisting of collagen fibers coated with hydroxyapatite and indicated for spinal fusions[195]. Cortoss is an injectable resin-based product with applications for load-bearing sites[196]. Rhakoss (Orthovita, Inc) is a resin composite available as a solid product in various forms for spinal applications[196]. Degradable synthetic polymers, like natural polymers, are resorbed by the body. The benefit of having the implant resorbed by the body is that the body is able to completely heal itself without remaining foreign bodies. To this end, companies have used degradable polymers such as polylactic acid and poly (lactic-co-glycolic acid) as stand-alone devices and grafted with grafted hyaluronic acid for periodontal barrier applications[197]. BoneTec, Inc (Toronto, Canada) has developed a porous poly (lactic-co-glycolic acid) foam matrix by using a particulate leaching process to induce porosity. Immix Extenders (Osteobiologics, Inc), a particulate poly (lactic-co-glycolic acid) product, is used as a graft extender.

F. *Miscellaneous*

I. Coral

Chiroff *et al*[198] first observed that corals from marine invertebrates have skeletons with a structure similar to both cortical and cancellous bone, with interconnecting porosity. Coralline hydroxyapatite is processed by a hydrothermal exchange method that converts the coral calcium phosphate to crystalline hydroxyapatite with pore diameters between 200 and 500 μm and in a structure very similar to that of human trabecular bone. Bucholz *et al*[199] reported that the clinical performance of autologous cancellous bone graft and coralline hydroxyapatite are similar during filling of bone voids resulting from articular surface depression in tibial plateau fractures. More recently, coralline hydroxyapatite has been used as a carrier for some bone derived growth factors. It has been used as a carrier for BMP with success in rabbit model and as a carrier for transforming growth factors and fibroblast growth factors in a rabbit model[200]. To avoid donor site morbidity, coralline hydroxyapatite granules or blocks of various size, depending on the size of the defect can be used to fill metaphyseal defects after reduction of depressed articular segments[26]. Coralline hydroxyapatite bone graft substitute appears to be a clinically effective material for use in foot procedures although the slow resorption is a concerning characteristic of the graft material without any adverse effect[201]. Another contraindication to the use of this material is a joint surface defect that would allow the grafting material to migrate into the joint.

II. Chitosan and Sponge skeleton:

Over the past three decades, an enormous array of biomaterials proposed as ideal scaffolds for cell

growth have emerged, yet few have demonstrated clinical efficacy. Natural marine sponge skeletons[202] and chitosan[203] have proved as effective biomaterials for modern tissue engineering. The abundance and structural diversity of natural marine sponge skeletons and their potential as multifunctional, cell conductive and inductive frameworks along with collagenous composition of the fiber indicate a promising new source of scaffold for tissue regeneration[204]. Chitosan, a natural product derived from the polysaccharide chitin (Aminopolysaccharide; combination of sugar and protein), an abundantly available natural biopolymer found in the exo-skeletons of crustacean like shrimp, crabs, lobster and other shellfish would be an effective material to repair bone defects due to its biocompatibility[203]. In an experimental study, natural hydroxyapatite/chitosan composite were evaluated in reconstruction of bone defects and observed that this composite has good biocompatibility and osteoconduction. It is a potential repairing material for clinical application[205]. The drawback in using these materials was that close proximity to the host bone was necessary to achieve osteoconduction. Advances in tissue engineering and the integration of the biological, physical, and engineering sciences, will create new carrier constructs that regenerate and restore functional state. These constructs are likely to encompass additional families of growth factors, evolving biological scaffolds, and incorporation of mesenchymal stem cells. Ultimately, the development of *ex vivo* bioreactors capable of bone manufacture with the appropriate biomechanical cues will provide tissue-engineered constructs for direct use in the skeletal system.

Conclusions

Future biosynthetic bone implants may obviate the need for autologous bone grafts. There is increasing interest in combining an osteoconductive protein in an osteoconductive carrier medium to facilitate timed-release delivery and/or to provide a material scaffold for bone formation. Further, advances in tissue engineering, “the integration of the biological, physical and engineering sciences” will generate new carrier constructs that repair, regenerate and restore tissue to its functional state. These constructs are likely to encompass additional families of growth factors, evolving biological scaffolds and incorporation of mesenchymal stem cells. Ultimately, the development of *ex vivo* bioreactors capable of bone manufacture with the appropriate biomechanical cues will provide tissue-engineered constructs for direct use in the skeletal system. Finally, as researchers continue to find new materials and biologic approaches to bone repair, the future of bone graft substitutes continues to be an expanding topic of interest.

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