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Clinical applications of bone graft substitutes in spine surgery: consideration of mineralized and demineralized preparations and growth factor supplementation

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Abstract Bone graft substitutes may be broadly classified as mineralized and demineralized preparations. This article reviews the basic science and biology underlying each preparation. A review of the clinical and experimental applications of each preparation follows. The text concludes with a review of growth factors as biological supplements.

Keywords Allograft · Demineralized bone matrix · Ceramics · Growth factors · Spinal fusion

Introduction and background

Tissue and mineral grafts in the spine perform mechanical and biologic functions. Their capacity for each function is dependent upon the structural, cellular, and biochemical properties of the particular graft chosen. Autogenous bone offers an optimal balance of osteogenic, osteoinductive, and osteoconductive capacities, structural stability, and biocompatibility. However, the availability of autogenous bone graft is clearly limited, and the complications of autogenous harvest are well known [6, 16, 17, 19, 23, 29, 30, 36, 46, 78, 95]. In choosing bone graft substitutes for clinical application, the spine surgeon's decision generally involves a compromise of mechanical and biological functional considerations. This article will address the clinical applications of mineralized and demineralized bone graft preparations in spinal surgery, reviewing the basic science and clinical experience supporting the use of these substitutes.

A bone graft material is any implanted material that alone or in combination with other materials promotes a bone healing response by osteogenic, osteoconductive, or osteoinductive activity at a local site [56]. Graft material that is osteogenic contains viable cells at some stage of osteoblastic differentiation and is capable of forming new bone directly. Osteoinductive graft materials contain cytokines capable of inducing differentiation of host cells into bone forming cells. Osteoconductive graft materials provide a biocompatible matrix that supports new bone formation. Substitutes for autogenous bone graft may have variable capacities for each of these functions. In spinal surgery, the ideal bone graft substitute should be osteogenic, biocompatible, bioabsorbable, easy to use, and cost effective and should provide structural support. However, the success of a particular material in achieving these goals depends upon the material and biologic properties of the grafting material as well as the particular host environment in which it is placed.

Mineralized bone matrices

Bone is a composite tissue consisting of mineral and organic phases. While the mineral phase is primarily structural in function, the regulation of bone metabolism and turnover involves a complex interaction between these two phases. The inorganic component of bone is principally composed of a calcium phosphate mineral analogous to crystalline calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$]. Calcium hydroxyapatite exists as plate-like crystals approximately 20–80 nm long and 5 nm thick. The physical properties of apatite, including solubility and stiffness, are determined by its crystalline structure. Significant differences in the crystalline structure of mature bone, immature bone, and synthetic bone substitutes have been demonstrated by P^{32} MRI [94]. These differences, in turn, determine the biologic behavior of the material in the spinal fusion bed.

Mineralized bone matrices provide an osteoconductive matrix for new bone formation. In isolation, mineralized bone matrix has little osteoinductive or osteogenic potential. In situ, its osteoinductivity is dependent upon the biologic capacity of the host bed or the local environment [76]. The potential for osteogenesis relies on cellular and chemical contributions from the host bed. The principle determinants of host-graft union include stability of the construct, contact between host bone and graft, and vascularity of the graft bed. Factors that inhibit bone graft incorporation include histocompatibility mismatch and reduction of the biologic activity of the graft by processing.

The process of graft incorporation involves hemorrhage, inflammation, tissue revascularization, and substitution and remodeling of the graft with locally derived tissue. New bone formation occurs by a process of creeping substitution in which calcium phosphate cements are replaced by bone with normal organic and mineral phases. Resorption of the mineral component of the matrix may be variable. The resorption of implanted hydroxyapatite is dependent upon pore size, crystalline structure, and local host reaction [61]. Overall, mineralized bone matrices have the advantage of offering immediate structural support and, in unstable osseous defects, stabilization may contribute importantly to the creation of a microenvironment that promotes osteoneogenesis [3,86]. However, the mechanical strength and fatigue properties of the mineralized bone substitutes are generally inferior to those of cortical bone. Mineralized bone matrices used in spine surgery include allograft, corraline hydroxyapatite, and synthetic hydroxyapatite. The clinical use of each in spinal surgery will be reviewed below.

Allograft

Allograft is the most commonly used nonautogenous grafting material in spinal surgery, and 35% of all bone transplantations involve the use of human allograft tissues. Mineralized allograft is primarily osteoconductive, with weak osteoinductive capacity and no osteogenic potential because graft cells do not survive processing and transplantation. Allograft used for orthopedic applications is fresh-frozen, freeze-dried, or demineralized. The method of preparation has significant effects on graft strength, immunogenicity, capacity for incorporation, and potential for disease transmission. Fresh-frozen allografts retain much of their original mechanical strength, while freeze-drying can reduce graft strength up to 50% [37,66]. The freezing process also reduces immunogenicity of allografts [39,76].

The effect of immunogenicity in compromising graft incorporation may be significant [73, 72, 75,77]. Transmission of disease from donor to recipient is a problem with human allografts. The principle pathogens involved are human immunodeficiency virus (HIV) and hepatitis viruses B and C. The risk of disease transmission is determined by the rigor of screening procedures for donors and tissue, and the only cases of disease transmission in musculoskeletal allografts from the method of graft preparation to date have involved frozen, unprocessed grafts [82]. Tissue processing techniques include high pressure lavage to clear out marrow elements and donor cells and chemical treatments to eliminate viruses and reduce immunogenicity of the graft. The combination of donor screening, tissue testing, and tissue processing reduces the risk of viral transmission to less than one event per million grafts [5].

Allograft is available in many preparations. However, the majority are composed of primarily cancellous or cortical bone. Cortical allografts provide significant mechanical stability and structural support, while cancellous bone lends little mechanical stabilization on implantation but has a faster rate of incorporation. Cancellous allograft and particulate allograft preparations (cancellous or cortical) incorporate with new bone forming on the surfaces of trabeculae, with a large surface area available for new bone formation [7,74]. In contrast, cortical incorporation occurs slowly via a process of periosteal new bone formation around the allograft as an external callus derived from the host bone [28].

Particulate and structural grafts demonstrate significant differences in the histology of incorporation. Particulate grafts demonstrate more rapid and complete revascularization than structural grafts. Particulate bone remodels completely with time, while cortical bone remains a mixture of necrotic and viable bone. The process of creeping substitution is also differs significantly between these forms of allograft, with new bone formation occurring appositionally followed by resorption in cancellous bone, which process is reversed in cortical allografts [11]. These differences in biologic capacity between graft types lead to significant differences in optimal clinical applications.

The use of bone allografts in the spine has been reviewed previously by the senior author [12]. Structural cortical allografts are most useful in interbody arthrodesis of the lumbar and cervical spine, with low rates of graft

subsidence or resorption. In revision lumbar surgery, tricortical allograft may be as effective as iliac crest in promoting anterior arthrodesis of the spine [13]. Crushed cortical or cancellous allograft may be useful as an autograft extender in posterior spinal fusion. In thoracolumbar deformity, cancellous allograft with instrumentation may give satisfactory results in the pediatric population but yields inferior results in adults. The conclusion from the senior author's experience is that successful use of allograft bone in the spine is dependent on the type of allograft bone used, the anatomic site of fusion, and patient age.

A review of other clinical applications of allograft compared with autogenous bone in spinal surgery is useful. In cervical spine, the use of allograft vs autograft has been debated since the first anterior discectomies and interbody fusions. Smith and Robinson used autogenous iliac crest graft and reported radiographic union in 18/21 patients [71]. Concurrently, Cloward reported resorption of only 3/46 grafts using his dowel technique with fresh-frozen allograft [18]. More recent reviews demonstrated similar fusion rates using autogenous and allogenous grafts in single level cervical surgery but significant differences in multilevel cervical fusions [38, 96,98].

In posterolateral arthrodesis of the lumbar spine, differences in function between allograft and autograft are more significant. In a prospective comparison of autograft and allograft preparations in posterolateral arthrodesis of the spine, differences in fusion mass were radiographically clearly apparent, reliable arthrodesis being achieved with autograft, followed by mixed autograft and allograft, and frozen allograft, and the least reliable graft material was freeze-dried allograft [1]. Similarly, a prospective evaluation of mineralized and demineralized allograft mixed with autogenous bone radiographically demonstrated fusion inferior to that from posterolateral arthrodesis with iliac autograft [44].

In summary, cortical allografts provide a useful structural matrix that is osteoconductive and has limited osteoinductivity. Cortical allografts are less likely than cancellous allograft to incorporate with adjacent host bone, but outcome and fusion rates in anterior lumbar and single level cervical applications are comparable to those with autogenous graft. Cancellous bone is useful as a graft extender in posterolateral arthrodesis of the spine but is an unreliable substitute in instrumented thoracolumbar deformity, especially in adults.

Ceramic matrices

Ceramic matrices include inorganic, ionically bonded preparations that mimic the mineral phase of bone [80]. Ceramic preparations that have been used in spinal reconstructive surgery include hydroxyapatite, tricalcium phosphate (TCP), and combinations of the two. Early formulations of hydroxyapatite used as bone void fillers were sintered by heating the precipitate at temperatures reaching or exceeding 1100°C. Products such as Pro-Osteon and Interpore are hydroxyapatite lattices created from a coralline scaffold. They are created by heating the calcium carbonate skeleton of coral in the presence of an aqueous phosphate solution that drives the exchange of the calcium carbonate of the coral with a calcium phosphate replica. The coral species *Porites astreoides* was chosen because of its theoretically ideal pore size for the ingrowth of bone. On electron microscopic examination, the average pore size of *Porites astreoides* was found to be 153.95±25.36 μm [61,62].

Ceramic matrices provide a biocompatible osteoconductive surface for bone regeneration and may contribute limited structural support [14,43]. Advantages of ceramic matrices include low immunogenicity and toxicity, stability at physiologic pH levels, and the ability to withstand sterilization procedures without losing structural integrity. While the organic phase of bone confers bone stiffness and compressive strength, ceramics are inherently brittle and susceptible to fracture, with elasticity moduli significantly higher than those of cortical and cancellous bone, and with low tensile strength. Bone mineral is distinct from the synthetic or geologic mineral hydroxyapatite. Although the overall crystal structure and composition of bone apatite is similar to that of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], the former displays highly significant functional differences concerning crystal size, short-range order, and the presence of carbonate (CO_3^{-2}) and acid phosphate (HPO_4^{-2}) [93]. This may explain differences observed in remodeling and resorption of synthetic apatite matrices used in spinal arthrodesis. Overall, more crystallization and higher mineral density yield greater mechanical strength and lasting stability. In contrast, an amorphous, simple preparation of calcium phosphate and calcium sulfate may also provide an osteoconductive matrix useful as a bone graft extender in spinal fusion while retaining a rate of resorption that equals the rate of formation. Optimal remodeling of the spinal fusion mass is dependent upon biodegradability of the ceramic, which, depending on the crystalline structure and composition, may take from several months to several years.

There are several currently used preparations of ceramic matrices that differ in biological and mechanical characteristics. Calcium sulfate and calcium phosphate are purely osteoconductive, with resorption profiles closely matching the rate at which new bone is deposited. These materials are replaced by host bone through a process of creeping substitution. These materials exist in many different preparations including powders, pellets, putty, and injectable cements. Tricalcium phosphate [$Ca_3(PO_4)_2$] (TCP) has been used as a bone void filler. It has advantages similar to those of hydroxyapatite since it is biocompatible and bioabsorbable [25,31]. However, it is brittle, with very low impact resistance. Porous TCP contains micropores 3–5 nm in diameter and has compressive and tensile

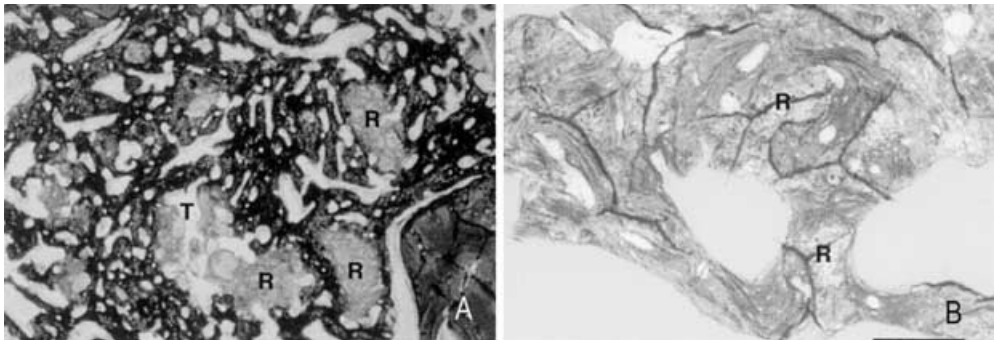

Fig. 1A, B Low (**A**) and high magnification (**B**) histologic sections of femoral slot defects filled with alpha-BSM (courtesy of ETEX)

strengths similar to but lower than those of cancellous bone [43]. It also dissolves more rapidly than hydroxyapatite, especially in an acidic milieu. Collagraft (Zimmer, Warsaw, Ind., USA) is a combination of TCP with hydroxyapatite and type 1 collagen.

Calcium phosphate cements that set in situ are now commercially available or in the final stages of U.S. Federal Drug Administration approval. These include SRS skeletal repair system (Norian, Cupertino, Calif., USA) and alpha-BSM (ETEX, Cambridge, Mass., USA). These calcium phosphate preparations have the advantage of excellent biocompatibility as well as in situ setting without shrinkage or generation of heat. The endothermic setting of these cements makes them ideal carriers for growth factors and cytokines.

The Norian SRS is a biocompatible and resorbable calcium phosphate cement combined from monocalcium phosphate, TCP, calcium carbonate, and a sodium phosphate solution mixed into an injectable paste. Under physiologic conditions, the material hardens within minutes into a dahllite (carbonated hydroxyapatite) in a nonexothermic reaction. It reaches 85–95% of completion within 12 h and has a final compressive strength of 55 MPa [42]. This material appears to offer significant mechanical integrity for the augmentation of fixation devices such as pedicle screws.

Alpha-BSM provides poorly crystalline calcium phosphate apatite with favorable absorption characteristics and easy intraoperative handling characteristics. It is composed of a calcium phosphate material that can be hydrated with saline to form a workable paste. This paste remains formable for hours at room temperature but hardens within 20 min at physiologic body temperature (37°C) and can be prepared to harden to a variety of compressive strengths (5–40 MPa). However, it is still significantly weaker than cortical bone. The poorly crystalline nature of the cement also closely mimics the mineral phase of bone, thus providing an excellent osteoconductive scaffold for cell-mediated absorption and remodeling into natural host bone (Fig. 1).

Clinical applications of ceramics in spinal surgery include animal and human studies. The application of ceramic matrices in animal models of spine fusion has demonstrated mixed results. In lumbar fusion in sheep, a ceramic composite containing 65% hydroxyapatite, 35% tricalcium phosphate, and type 1 bovine dermal collagen (Collagraft, Zimmer) demonstrated radiographic and histologic evidence of better fusion as a bone graft substitute than autogenous bone [88]. However, in a study on dogs, a similar preparation demonstrated union inferior to that of an equal volume of autogenous cancellous bone. In addition, the combination of collagen ceramic composite with autogenous cancellous bone graft significantly reduced the effectiveness of the autogenous bone graft [57]. Studies using arthrodesis in dogs demonstrate the importance of the local environment in promoting incorporation of ceramic matrices in spinal fusion. In one study with a biphasic material composed of 60% hydroxyapatite and 40% TCP, the biomechanical properties of the posterolateral fusion were equal to those with autograft and ceramic grafts, and the amount of new bone formation was related to contact area with bone and decortication of the host graft site [27]. In a study of anterior interbody arthrodesis in the canine thoracic spine, calcium carbonate graft led to arthrodesis that was significantly weaker biomechanically and histologically than with autograft. Internal fixation of the fused segment did, however, significantly improve revascularization and remodeling in both autograft and ceramic graft specimens [34].

In rabbits, the effectiveness of ceramic matrix grafts in promoting spinal arthrodesis was limited. A comparison of natural coral with autogenous bone in rabbit anterior interbody fusion demonstrated no signs of integration of the natural coral, with no evidence of fusion at 3 months. In contrast, autogenous graft promoted reliable arthrodesis [81]. In posterolateral arthrodesis in rabbits, coralline hydroxyapatite as an isolated graft material led to unreliable arthrodesis compared with autograft (Fig. 2). However, supplementation of the ceramic matrix with osteoinductive bone protein extract or with direct current electrical stimulation led to reliable spinal arthrodesis [8, 10, 79].

Although a recent study reported the utility of ceramics in spinal arthrodesis in adolescent idiopathic scoliosis, its efficacy in this situation is still a subject of controversy. In a prospective trial comparing ceramic matrix (porous biphasic calcium phosphate ceramic blocks comprised of

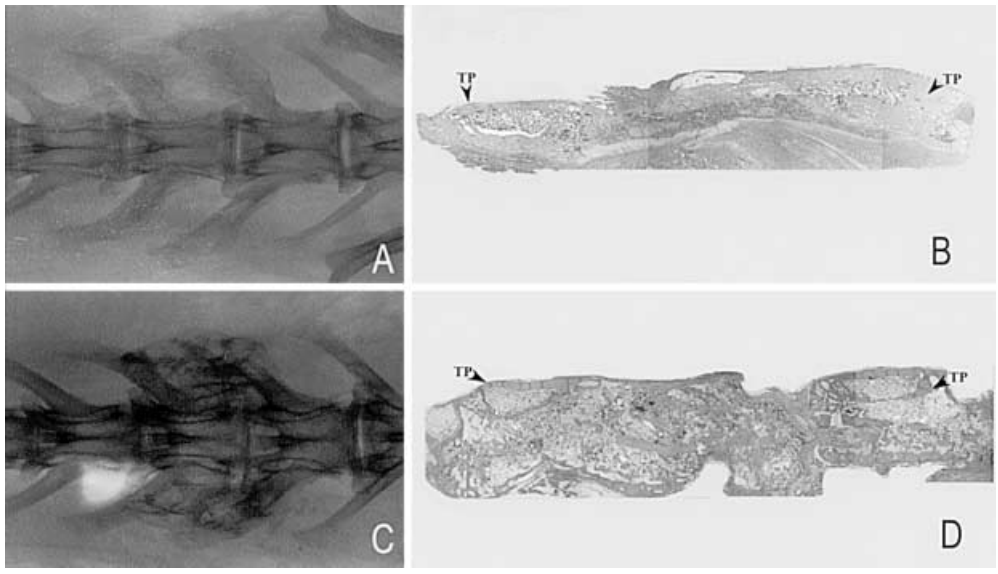

Fig. 2A–D The use of hydroxyapatite matrices in rabbit spinal fusion. **A** Commercially available collagen hydroxyapatite matrix placed in the intertransverse area of a rabbit spine showing no bony fusion at 8 weeks. **B** Sagittal histological cross-section with hematoxylin-eosin staining showing fibrous tissue interposition between the decorticated transverse processes and no evidence of fusion. **C** Autogenous iliac crest graft placed in the intertransverse area showing solid fusion at 8 weeks. **D** Sagittal histological cross-section of the fusion area with hematoxylin-eosin showing a mature cortical rim

hydroxyapatite and TCP) with autograft alone, fusion rates were similar at 1 year, and donor site morbidity and blood loss were lower in the ceramic group [27]. However, these results must be interpreted with caution. In the cases of adolescent scoliosis treated with spinal fusion, the ceramic was used as a bone graft extender to supplement the local bone used for graft materials. In addition, the patient population studied in this trial has a high propensity to healing, even without the addition of bone grafts. As thoracoplasty is used more routinely for correcting the rib hump deformity in children with scoliosis, sufficient bone can be obtained from the resected ribs to eliminate the need for iliac crest bone. This virtually eliminates the morbidity associated with graft harvest from that site. Thus, the utility of ceramics as bone graft extenders in the surgical treatment of children with adolescent idiopathic scoliosis is questionable.

The variability of clinical results for arthrodesis may be related to variance in local environment (segmental stability, exposed bone) and to the significant variation in biological properties (pore size, osteophilicity, resorbability) of the different ceramic preparations. The tissue surrounding the ceramic matrix is the most important determinant of the kinetics of graft incorporation, as this material has weak osteophilic properties and lacks the capacity for osteoinduction [26]. Enhancement of the local environment for osteoneogenesis, including segmental stabilization, optimizing the supply of osteogenic cells, providing osteoinductive material, and possibly adding electrical stimulation, enhances the effectiveness of ceramic matrices in promoting spinal arthrodesis. It is clear that both host and donor cells contribute to the formation of the final fusion mass [35]. Despite the presence of an adequate osteoinductive and osteoconductive environment, the lack of sufficient numbers of osteoprogenitor cells will adversely affect formation of the fusion mass. Further work is needed to determine the optimal biologic characteristics of ceramic matrices with regard to mechanical stability and resorbability [69]. Ceramic composites consisting of an osteoconductive ceramic matrix and an osteoinductive substance (autograft, DBM, growth factors, electrical current) may offer the most effective graft material for promoting arthrodesis [9].

Demineralized bone matrices

The osteoinductive factors of bone are contained within the organic phase. While mineralized matrices have minimal osteoinductive activity, demineralized preparations have demonstrated a potent effect on the differentiation of osteoprogenitor cells into osteoblasts. Marshall Urist first identified a bone morphogenetic substance in his pioneering work using soluble extracts from demineralized bone [84]. The demonstration of osteoneogenesis in response to ectopic submuscular implants of demineralized bone was pivotal in further identification and cloning of the identified bone morphogenetic proteins [93]. De novo bone formation in ectopic, submuscular sites has become the standard means of assessing molecules with bone morphogenetic activity. The capacity of demineralized bone matrix (DBM) to induce new bone formation is well established [83,85]. The primary osteoinductive component of DBM consists of small amounts of glycoproteins in the organic phase of bone, the most important of which are the bone morphogenetic proteins. The major pathway of osteoneogenesis induced by DBM is endochondral in subcutaneous and submuscular implants and by direct induction of resident mesenchymal stem cells to osteoblasts and direct formation of bone without a cartilaginous intermedi-

ate in calvarial defects [90, 91,92]. This difference indicates the importance of the host environment in the process of osteogenesis induced by DBM.

Despite animal data suggesting a positive effect of DBM on spinal fusions, the clinical utilization of DBM in spinal fusion has not demonstrated similar efficacy. In a multicenter prospective comparison of graft incorporation in the cervical spine, allograft with DBM was compared with autograft. Rates of graft collapse and pseudoarthrosis were higher in the allograft with DBM group, suggesting that current demineralized preparations do not offer sufficient osteoinductive capacity to facilitate reliable arthrodesis [2]. The utility of demineralized matrix gel in posterolateral lumbar spinal fusion was demonstrated in rabbits. The DBM appeared more effective than frozen allograft alone in promoting arthrodesis [58]. However, DBM did not increase the frequency of successful arthrodesis when added to the standard amount of autograft. Demineralized bone matrix preparations may be effective as graft extenders in the setting of limited autograft [53] and as graft enhancers when comparing fusion quality to that with autograft alone [50]. A role for DBM as a graft extender was further supported by results of a dog study [33]. In other studies on dogs, DBM has not been shown useful in promoting spinal arthrodesis. Cook et al. demonstrated that DBM alone or in combination with allograft did not produce reliable spinal arthrodesis [22] and may have an inhibitory effect on arthrodesis of the spine compared with autogenous bone alone and with recombinant osteogenic protein (rhOP-1) [20,40]. The differing efficacy of DBM in spinal fusion demonstrated in these studies is likely a result of the different DBM preparations used. It is important to note that the osteogenic activity of a DBM preparation is highly dependent upon the type and specific preparation of bone used. In addition, the carrier with which the DBM is mixed may affect its osteoinductivity. Current preparations of DBM which are mixed with a glycerol carrier (Grafton, Osteotech, Eatontown, N.J., USA) are very acidic. The low pH may have detrimental effects on host cells if it is used in large quantities. Other preparations containing hyaluronic acid (DBX, Synthes) have a more neutral pH and thus may be less harmful to host tissues. In hosts with compromised osteogenic capacity, such as smokers, DBM may be useful as a supplement to autogenous bone graft [70].

In summary, despite good evidence for osteogenic activity in DBM, there is little evidence suggesting its effectiveness as a substitute for autogenous bone graft. There is tremendous variability in the osteoinductive capacity of different commercial demineralized bone graft preparations as assessed by submuscular assay [68], and this may contribute to variability in clinical experience. Demineralized bone matrix offers no structural or mechanical stability independently of its carrier and does not appear to be a reliable substitute for autogenous bone graft. The material may have a role as a graft extender or as a supplement in hosts with compromised bone forming capacity.

Growth factors and composite grafts

Recent advances in cellular and molecular biology led to the identification of specific cytokines that are active in mediating cellular activities including mitogenesis, anabolic activity, and differentiation. The ability to control cellular activity is a potentially powerful tool in the management of orthopedic disorders and surgical reconstructions. Many growth factors and other cytokines have been shown to be osteoinductive in animal models. The growth factors that may enhance bone formation in vitro include insulin-like growth factor (IGF-1), fibroblast growth factor (aFGF, bFGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGF-ß) [4, 15, 45, 48, 51, 54,55]. Bone morphogenetic proteins are a subset of the TGF-ß superfamily and are the only cytokines that demonstrated a capacity to induce new bone formation in vivo [87]. Bone morphogenetic proteins are important mediators of new bone formation and repair in all stages of life from embryonic development to adulthood [41,63]. Recent developments in recombinant techniques permit isolation of BMP in pharmacologic quantities, in contrast to isolation through demineralization, in which less than 20 μg of osteoinductive material could be extracted from 10 kg of bovine cortical bone [89]. Clinical experience with bone morphogenetic proteins in spinal fusion studies suggest a valuable role as bone graft supplements or substitutes [9, 21, 24, 32, 49, 59, 64, 65, 67,97].

Composite grafts permit combination of the osteoinductive and osteogenic capacities of growth factors or autogenous bone with the structural capacity of mineralized matrices [9]. The optimal carrier for bone growth morphogenetic proteins has not been determined but would have a reversible affinity to the glycoprotein, structural characteristics possibly including malleability or mechanical rigidity, limited immunogenicity and toxicity, and resorbability to permit complete replacement by bone. Inorganic carriers of BMP that have demonstrated efficacy in promoting spinal arthrodesis include true bone ceramic (TBC) derived from sintered bovine bone, and hydroxyapatite-TCP [52]. Organic carriers include polylactic acid polymers (PLA), collagen and noncollagenous protein carriers, mineralized or demineralized bone matrix, and autograft [47,60]. Advantages of organic carriers include the capacity for chemical bonding to growth factors and the provision of a biodegradable environment for new bone formation and graft incorporation. However, many organic carriers are weakly immunogenic and lack the osteoconductive function of inorganic bone cements. The structural capacity of inorganic cements is a further advantage of this carrier. Composite grafts offer potential for the design of bone graft substitutes that are specific for the structural and biologic demands of the host, and it is likely that very different composites will be used for anterior interbody arthrodesis than for long-instrumented posterior fusion.

Conclusions

Effective clinical application of mineralized and demineralized bone matrix substitutes requires an understanding of their biologic and structural characteristics. Existing preparations have clear limitations in clinical efficacy. Experience to date suggests that grafting materials and composites will continue to evolve for specific applications, and the choice of bone graft materials will be determined by the properties of the local host environment, including anticipated loading and vascular and soft tissue envelope. The development of growth factors and other cytokines that function as potent induction agents for osteoneogenesis offers tremendous potential for the design of composite materials providing osteoinductive, osteoconductive, and structural functions. New techniques in tissue engineering and gene therapy will add a further osteogenic capacity to future bone graft substitutes.

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