Bone graft substitutes for the promotion of spinal arthrodesis

GREGORY A. HELM, M.D., PH.D., HAYAN DAYOUB, M.D., AND JOHN A. JANE, JR., M.D.

Departments of Neurosurgery and Biomedical Engineering, University of Virginia, Charlottesville, Virginia

In the prototypical method for inducing spinal fusion, autologous bone graft is harvested from the iliac crest or local bone removed during the spinal decompression. Although autologous bone remains the “gold standard” for stimulating bone repair and regeneration, modern molecular biology and bioengineering techniques have produced unique materials that have potent osteogenic activities. Recombinant human osteogenic growth factors, such as bone morphogenetic proteins, transforming growth factor–β, and platelet-derived growth factor are now produced in highly concentrated and pure forms and have been shown to be extremely potent bone-inducing agents when delivered in vivo in rats, dogs, primates, and humans. The delivery of pluripotent mesenchymal stem cells (MSCs) to regions requiring bone formation is also compelling, and it has been shown to be successful in inducing osteogenesis in numerous preclinical studies in rats and dogs. Finally, the identification of biological and nonbiological scaffolding materials is a crucial component of future bone graft substitutes, not only as a delivery vehicle for bone growth factors and MSCs but also as an osteoconductive matrix to stimulate bone deposition directly. In this paper, the currently available bone graft substitutes will be reviewed and the authors will discuss the novel therapeutic approaches that are currently being developed for use in the clinical setting.

**Key Words** • stem cell • spinal fusion • graft

A variety of internal fixation techniques can be used in most patients to achieve immediate spinal stability; however, long-term stability typically requires bone fusion of the involved region. A solid bone fusion is usually achieved after placement of autologous or allogenic bone grafts, each of which has specific advantages and disadvantages. Although autologous bone is the “gold standard” graft material, donor-site morbidity (pain, infection), limited supply, and inconsistent osteogenic activity continue to be associated problems. Allogenic bone grafts are significantly less osteogenic than autologous bone, mainly because allogenic bone typically acts only as a passive scaffold for vascular ingrowth and bone deposition. Because of these limitations, basic science and clinical researchers are aggressively developing biosynthetic bone grafts as an alternative to autologous and allogenic bone grafts. Research has also focused on defining the physiological mechanisms involved in bone repair and regeneration. These mechanisms have only been partially elucidated but appear to involve complex interactions between a variety of different angiogenic and osteogenic growth factors, ECM proteins, and pluripotent MSCs.

Typical biosynthetic bone grafts rely on one, or a combination, of these components of the osteogenic cascade for their activity.

The three processes by which bone can be repaired or regenerated are osteoinduction, osteoconduction, and osteogenesis. Osteoinduction is defined as the ability to stimulate the proliferation and differentiation of pluripotent MSCs. In endochondral bone formation, stem cells differentiate into chondroblasts and chondrocytes, laying down a cartilaginous ECM, which subsequently calcifies and is remodeled into lamellar bone. In intramembranous bone formation, the stem cells differentiate directly into osteoblasts, which form bone through direct mechanisms. Osteoinduction can be stimulated by osteogenic growth factors, although some ECM proteins can also drive progenitor cells toward the osteogenic phenotype. Osteoconduction is defined as the ability to stimulate the attachment, migration, and distribution of vascular and osteogenic cells within the graft material. The physical characteristics that affect the graft’s osteoconductive activity include porosity, pore size, and three-dimensional architecture. In addition, direct biochemical interactions between matrix proteins and cell surface receptors play a major role in the host’s response to the graft material. The ability of a graft material to produce bone independently is termed its direct osteogenic potential. To have direct osteogenic activity, the graft must contain cellular components that directly induce bone formation. For example, a
collagen matrix seeded with activated MSCs would have the potential to induce bone formation directly, without recruitment and activation of host MSC populations. Because many osteoconductive scaffolds also have the ability to bind and deliver bioactive molecules, their osteoinductive potential will be greatly enhanced.

**BONE GRAFT SUBSTITUTES**

**Biodegradable Scaffolds**

Biocompatible matrices are currently being developed to stimulate osteogenesis via osteoconduction and to promote osteoinduction by using osteogenic growth factors, mesenchymal cell implants, and genetically engineered cells.9,15 Many of the scaffolds currently being designed have outstanding osteoconductive properties, rapidly stimulating the migration of fibrovascular and osteoprogenitor cells into a porous structure, which is subsequently incorporated into and replaced by bone tissues. The specific properties of the scaffold, which will optimize bone growth, are dependent on the site requiring bone repair, although the most effective scaffolds have specific characteristics. The material should: 1) readily incorporate and retain mesenchymal cells in tissue culture; 2) rapidly induce fibrovascular invasion from the surrounding tissues; 3) have significant osteoconductive properties to improve incorporation with the host bone; 4) not induce significant acute immune responses or a chronic foreign body response; 5) have biomechanical properties similar to those of normal bone, such as a compressive modulus of 50 MPa and a compressive strength of 5 MPa for human trabecular bone, which will limit stress shielding resulting in bone loss adjacent to the implant; 6) be biodegradable, with a controllable absorption rate that parallels the rate of new bone deposition; 7) have biodegradation products that are nontoxic and easily secreted by normal physiological pathways; and 8) contain sites that can noncovalently bind osteogenic biomolecules to enhance osteoinduction. Numerous materials have been evaluated for their osteoconductive potential, but to date, the optimal scaffold has yet to be developed. The advantages and disadvantages of some of the most interesting osteoconductive substrates will be briefly reviewed.

**Polymers.** Numerous polymeric systems have been studied, including poly-α-hydroxy esters, polydioxanone, propylene fumarate, poly-ethylene glycol, poly-ethers, polyanhydrides and polyurethanes, poly-L-lactic acid, poly-glycolic acid, and poly-lactic-co-glycolic acid, polyanhydrides and polyurethanes, poly-L-lactic acid, propylene fumarate, poly-ethylene glycol, poly-ethers, strates will be briefly reviewed.

Ceramics. Hydroxyapatite and β-TCP have been the two most intensely studied ceramics for bone repair and regeneration.12 Their most unique property is chemical similarity to the mineralization phase of bone; this similarity accounts for their osteoconductive potential and excellent biocompatibility.23 An important difference between these compounds is their resorption rates. In long bone models at 3 months, 85% of β-TCP is resorbed compared with only 5.4% of HA. Since implanted HA requires many years for complete resorption, it may actually prevent newly formed bone within the porous HA from experiencing the mechanical stresses required for bone remodeling. Unlike β-TCP, however, HA possesses a structure that is very similar to natural bone and, therefore, has improved osteoconductive properties. Both HA and β-TCP have been shown to be excellent carriers of osteoinduction growth factors and osteogenic cell populations, which will greatly add to their utility as bioactive delivery vehicles in the future.18 Interestingly, the authors of recent studies have demonstrated that HA can directly induce osteogenic differentiation of MSCs in tissue culture.

**Extracellular Matrix Scaffolds.** Various ECM proteins, including collagen, laminin, fibronectin, and glycosaminoglycans (that is, hyaluronic acid, heparin sulfate, chondroitin sulfate A, and dermatan sulfate), may be fabricated into excellent biological scaffolds.3 These proteins have the advantages of supporting the migration and differentiation of osteoblastic progenitor cells, facilitate the binding of growth factors responsible for osteogenesis, and resorbing within a reasonably short period of time via nontoxic mechanisms. In addition, the matrices can be made porous and biomechanically robust, leading to a variety of clinical applications. In the majority of previous clinical studies concerning biological scaffolds the authors have focused on collagen matrices. Although more than 15 different kinds of collagen have been identified, Type I collagen is the most ubiquitous and has been the most heavily studied as a possible osteogenic scaffold.

At the cellular level, ECM molecules exhibit a variety of activities, including acting as a substrate for cell migration; an adhesive for cell anchorage; a ligand for ions, growth factors, and other bioactive agents; and a signal for contacting cells. Matrix components can be reassembled into a theoretically limitless number of combinations, with varying sizes and shapes, making them ideal to form implantable devices and substrates for cell delivery. The high number of polar and nonpolar residues in collagen matrices provides excellent regions for noncovalent interactions with osteogenic growth factors and can be designed to optimize the amount of “free” and “bound” protein, to control the rate of protein release, and to provide high local concentrations of osteoinductive biomolecules. The engineering of osteoconductive delivery vehicles will be an important component of any successful bone graft substitute.

**Demineralized Bone Matrix**

Prior to the identification and cloning of the human bone growth factor genes, BMPs were isolated from demineralized bone extracts. The DBM production process is fairly straightforward, involving the initial pulverizing of bone specimens into particles 70 to 450 μm in diameter,
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followed by their demineralization with 0.5 N hydrochloric acid and subsequent rinsing with sterile water. The application of DBM to promote spinal arthrodesis has been studied in detail in both preclinical and clinical trials. Oikarinen, et al., initially demonstrated that DBM could be successfully used to fuse the rabbit spine, a finding that was later supported by studies by Ragni and Lindholm. Despite initial enthusiasm for this approach, clinical studies in which the investigators evaluated the use of DBM for inducing spinal fusion have yielded disappointing results. For example, An, et al., performed a prospective, nonrandomized study comparing autologous bone alone with DBM and freeze-dried allogenic bone in anterior cervical fusions. The authors demonstrated that the pseudarthrosis rate was significantly lower in the autograft group (26%) compared with the DBM group (46%). In addition, Jorgenson, et al., demonstrated that when DBM was compared with autologous bone for posterolateral lumbar fusions, the DBM graft was inferior to the autologous bone, as reflected by lower solid fusion rates and radiographically demonstrated bone density. On the other hand another research group demonstrated radiographically in a retrospective study that posterolateral lumbar fusion sites treated with autologous bone alone (54 cases) or with a composite of DBM and autologous bone (36 cases) had similar characteristics at 12 months, although the fusion rates were not determined. The principal limitations of DBM preparations include batch-to-batch variability, suboptimal processing methods, and potential infectious agents contaminating the material. It is probable that delivery of highly concentrated, rhBMPs (alone or in combination) will replace DBM formulations in the near future.

**Bone Growth Factors**

In the last 10 years, remarkable advances have been made in the field of osteoinductive growth factors. The results of numerous studies indicate that bone regeneration involves the complex interaction of a variety of different regulatory factors. The TGFβ superfamily contains some of the most important growth factors involved in bone healing, including TGFβ1 through β5, BMPs, and growth and differentiation factors. In addition, other growth factors, including fibroblast growth factors (acidic and basic), platelet-derived growth factor and insulin-like growth factor, are clearly present at sites undergoing bone healing. Using current molecular biology techniques, recombinant human bone growth factors can now be produced with purity and in high concentrations, and their availability has led to a large number of preclinical and clinical studies to determine the efficacy of each protein.

Bone growth factors are typically delivered on an osteoconductive scaffold, such as Type I collagen, HA, or biodegradable polymers. The peptides that have shown the greatest osteogenic activity to date include two members of the BMP family, BMP-2 and BMP-7, which stimulate specific serine/threonine transmembrane receptors on MSCs and osteoblasts. Activation of the receptors leads to the transphosphorylation of second messengers within the cytosol, which subsequently translocate to the nucleus where they induce the transcription of a variety of genes involved in cellular proliferation and differentiation. Interestingly, these growth factors can initiate the complex cascade of endochondral bone formation. The BMPs initially recruit MSCs to the region via chemotaxis and stimulate their rapid proliferation and differentiation into chondroblasts and chondrocytes. These cells subsequently lay down a cartilaginous matrix, and this calcifies into woven bone. This tissue subsequently remodels, forming mature lamellar bone, which contains active bone marrow elements. In a variety of femoral defect models in rats, rabbits, dogs, sheep, and primates, BMP-2 and BMP-7 delivered on an osteoconductive carrier have been shown to accelerate bone healing significantly. In other studies, numerous groups have also demonstrated the utility of rhBMPs for inducing spinal arthrodesis in a variety of different spine models in rats, rabbits, dogs, and primates. In several studies, the BMPs have been shown to be more effective at fusing the spine—in terms of fusion rate and biomechanical strength—than autologous bone grafts.

Recombinant human bone growth factors have recently been used in clinical trials and have been shown to have osteogenic potential in several locations; however, there do appear to be significant differences in the physiological activity of the various BMPs depending on the clinical situation and the particular patient. Boyne et al., demonstrated that rhBMP-2 delivered on collagen sheets was capable of increasing the height of atrophied maxillary bones, although the grafts were less effective at treating alveolar ridge resorption. Geesink, et al., subsequently demonstrated significant osteogenic activity of BMP-7 delivered on a collagen carrier in five of six patients in whom fibular osteotomies were performed. In the spinal region, Boden, et al., also showed that rhBMP-2 delivered on a collagen sponge placed inside an interbody fusion cage can induce spinal arthrodesis at a rate higher than cages filled with autologous bone. In patients with thoracolumbar burst fractures, Laursen, et al., implanted BMP-7 on a collagen carrier into the lesions and demonstrated that the BMP actually increased early bone resorption at the treatment site, most likely resulting in decreased biomechanical strength at the treatment site. The results of these initial studies clearly indicate that additional work needs to be performed to determine which specific BMPs have the most potent osteoinductive activity and whether combinations of different growth factors will also improve the rate and quality of bone deposition. In addition, determination of the ideal BMP dose, the most effective BMP carrier, and the optimum rate of release of the BMP from the carrier are clearly required before consistent clinical results can be achieved.

**Cell-Based Approaches for Bone Formation**

The osteogenic potential of osteoconductive scaffolds can be increased by seeding the material with cells that have osteoblastic potential. Various cellular approaches have been attempted, including the use of: 1) unfractinated fresh bone marrow; 2) marrow derived–culture expanded MSCs; and 3) MSCs predifferentiated into osteoblasts. As with any tissue-engineering approach, each of these cell-based therapies has its own advantages and disadvantages.

**Fresh Bone Marrow.** The authors of several preclinical
and clinical studies have demonstrated that fresh autologous bone marrow has the potential to induce osteogenesis in a variety of locations, including the spine. The principal advantages of using this approach are that the harvesting/implantation of autologous bone marrow cells from the iliac crest is relatively inexpensive, simple, and not subject to regulation by the Food and Drug Administration. The mechanism by which fresh bone marrow induces bone formation is the proliferation and differentiation of osteoprogenitor cells contained in the marrow cavities. Because only 0.001% of the nucleated cells in normal bone marrow are MSCs, it may be difficult to implant a large enough number of these cells to achieve satisfactory clinical results. In addition, in the debilitated or aged patient, because the number of MSCs in the bone marrow significantly decreases, the use of this approach may be ineffective in this population. In appropriate patients and in selected clinical situations, however, the use of bone marrow aspirates to augment osteoconductive scaffolds or in combination with osteogenic growth factors may be an attractive treatment option.

**Mesenchymal Stem Cells.** Numerous research groups are currently focusing their efforts on the purification and expansion of pluripotent stem cells for the treatment of a variety of disorders, including Parkinson disease, stroke, cardiomyopathy, hepatic failure, and renal disease. Mesenchymal stem cells have also been identified and are currently being developed for the repair and regeneration bone, cartilage, muscle, tendon, and ligament. Mesenchymal stem cells are typically harvested from bone marrow and have been isolated from rodents, canines, and humans. Interestingly, these cells can undergo extensive subcultivation in vitro without differentiation, magnifying their potential clinical utility. Human MSCs can be directed toward osteoblastic differentiation by adding dexamethasone, ascorbic acid, and β-glycerophosphate to the tissue culture media. This osteoblastic commitment and differentiation can be documented by analyzing alkaline phosphatase activity, the expression of bone matrix proteins, and by the mineralization of the ECM.

Human MSCs express a variety of different cell surface proteins, including numerous integrins (α1, α2, α3, α5, α6, αV, β1, β3, and β4), growth factor receptors (basic fibroblast growth factor, platelet-derived growth factor, interleukin-1, TGF-β1 receptors, and TGF-βII receptors) and cell adhesion molecules (intracellular adhesion molecule-1, vascular cell adhesion molecule, activated leukocyte cell adhesion molecule, and L-selection). They should therefore be highly responsive to osteogenic growth factors, as well as to osteoconductive matrices used as cellular delivery vehicles. Although human MSC therapies are theoretically attractive, the development of allogeneic grafts has several potential problems: graft rejection, transmittable diseases, and bacterial and fungal contamination. If these issues are overcome, human MSCs may be ideal for ex vivo BMP gene therapy, because the expressed proteins will not only stimulate the osteogenic cascade by the grafted stem cells but also local host progenitor cells.

Kadiyala, et al., recently demonstrated that MSCs can regenerate bone in a rat femoral defect model. These investigators seeded HA/β-TCP scaffolds with syngeneic marrow-derived MSCs at a concentration of $7.5 \times 10^6$ cells/ml. The MSC-loaded carriers demonstrated significantly improved bone formation compared with the cell-free control implants. In a similar study in canines, Bruder and Fox demonstrated that HA/β-TCP implants seeded with culture-expanded autologous MSCs also induced significantly greater bone formation within the ceramic carrier and appeared to induce a more rapid union with the surrounding host bone compared with control grafts. Human MSCs loaded onto a ceramic carrier in a similar fashion also promoted bone formation in a femoral defect model in athymic nude rodents. Immunocompromised animals were used to attenuate the host immune response against the human xenograft. It remains unclear whether human-derived allogeneic MSCs will also induce significant immune responses when they are applied in clinical trials.

There has been only one reported clinical trial of MSCs for the treatment of human disease. Horwitz, et al., demonstrated that allogeneic bone marrow–derived MSCs transplanted into three children with osteogenesis imperfecta significantly improved bone deposition in trabecular bone. In addition, a mean increase of 28 g in bone mineral content was observed in these patients, compared with predicted values of 0 to 4 g. The results of this study underscore the feasibility of using culture-expanded MSCs for the induction of osteogenesis for chronic bone disease; however, these techniques need further evaluation and refinement prior to widespread clinical application.

**Mesenchymal Stem Cells Predifferentiated Into Osteoblasts.** Several research groups have also studied the ability of cultured marrow MSCs, which had been predifferentiated into osteoblasts, to induce bone regeneration. When undifferentiated MSCs are used to treat bone defects, these cells must first differentiate into their osteoblastic phenotype for bone deposition to occur. If the MSCs are differentiated into osteoblasts with dexamethasone, ascorbic acid, and β-glycerophosphate prior to implantation, the healing process should, at least theoretically, be accelerated. This method has been investigated in both rodents and rabbits, demonstrating improved bone healing with osteoblast-loaded scaffolds compared with cell-free control matrices. The obvious disadvantages to this method are similar to those for allogeneic MSCs—namely problems with cellular rejection, graft contamination, and batch-to-batch variability. These important approaches require additional investigation to determine whether they can become clinically effective in the future.

**CONCLUSIONS**

Future biosynthetic bone implants may obviate the need for autologous bone grafts. Armed with the knowledge of the basic mechanisms involved in bone repair and regeneration, researchers can now maximize the osteoinductive, osteoconductive, and osteogenic properties of the graft material. The optimal graft will most likely be composed of osteoprogenitor cells and osteoinductive growth factors that are delivered on an osteoconductive, resorbable scaffold. The major issues that need to be addressed include limiting the host immune responses directed against allogeneic mesenchymal cell implants, the optimum dose and
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rate of protein release for each osteoinductive growth factor, and the development of appropriate delivery vehicles for each clinical situation. It is anticipated that construction of a material with these three key components should produce a biosynthetic bone graft that possesses performance characteristics superior to autologous bone.

References


Address reprint requests to: Gregory A. Helm, M.D., Ph.D., Department of Neurosurgery, Box 800212, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908. email: gas9r@virginia.edu.