Chapter 31
Graft Substitutes for Use in Spinal Fusions

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Spinal fusion procedures remain a common therapeutic option for various pathologic conditions of the human spine. The most frequent method for inducing spinal fusion uses autologous bone grafts harvested from the iliac crest or from 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 osteoconductive or osteoinductive activities. Recombinant human osteogenic growth factors, such as bone morphogenetic proteins (BMPs), transforming growth factor -ƒÒ (TGF-ƒÒ), and platelet-derived growth factor (PDGF), can now be produced in highly concentrated and pure forms. They have now been shown to be extremely potent when delivered in vivo in rats, dogs, primates, and humans. The delivery of pluripotent genetically modified mesenchymal stem cells (MSCs) to regions requiring bone formation is also compelling and has successfully induced osteogenesis in numerous preclinical studies in rats and dogs. Finally, the identification of biologic and nonbiologic scaffolding materials is an extremely important 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 directly stimulate bone deposition. In this paper, we will review the currently available bone graft substitutes, in addition to novel molecular approaches, that are currently being developed for use in the clinical setting.

Modern internal fixation techniques routinely lead to immediate spinal stability in most patients. However, long-term stability typically requires bony fusion of the involved region. A solid bony fusion is usually achieved with autologous or allogenic bone grafts, each of which have specific advantages and disadvantages. Autologous bone is the gold standard graft material in clinical practice, although donor site morbidity (pain and infection), limited supply, and inconsistent osteogenic activity continue to be problematic (37). Allogenic bone grafts are significantly less osteogenic than autologous bone, mainly because allogenic bone does not contain osteogenic growth factors and typically acts only as a passive scaffold for vascular in-growth and bone deposition. Basic science and clinical researchers are rapidly developing biosynthetic bone grafts as an alternative to autogenous and allogenic bone grafting. Research has also focused on defining the cellular and molecular mechanisms involved in bone repair and regeneration. Many mechanisms have only been elucidated and seem to involve complex interactions between a variety of different mesenchymal, angiogenic, and osteogenic growth factors, extracellular matrix 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 mechanisms 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 intramembranous bone formation, the stem cells differentiate directly into osteoblasts, which form bone through direct mechanisms. In endochondral bone formation, stem cells differentiate into chondroblasts and chondrocytes, subsequently lay down a cartilaginous extracellular matrix, which then calcifies and remodels into lamellar bone. Osteoinduction is routinely stimulated by osteogenic growth factors. In addition, some extracellular matrix 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. Several physical characteristics can affect the graft osteoconductivity, including porosity, pore size, and

three-dimensional architecture. In addition, direct interactions between matrix proteins and their appropriate cell surface receptors play a major role in the host response to the graft material. The ability of a graft material to independently produce bone 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 genetically modified MSCs that secrete osteogenic growth factors would have the potential to directly induce bone formation, without recruitment and activation of host MSCs populations. Many osteoconductive matrices also have the ability to bind and deliver osteogenic molecules, which will greatly enhance their osteoinductive potential.

BIODEGRADABLE SCAFFOLDS

Biocompatible matrices are currently being developed to promote osteogenesis via osteoconduction and to stimulate osteoinduction using osteogenic growth factors and MSC implants (6, 10, 17). Many of the scaffolds that are currently being designed have outstanding osteoconductive properties, rapidly stimulating the migration of osteoprogenitor and fibrovascular cells into a porous structure, which is subsequently incorporated into and replaced by bony tissues. The specific properties of the scaffold that will optimize bone growth is dependent on the site requiring bony repair, although the most effective scaffolds have specific characteristics. The material should readily incorporate and retain MSCs in tissue culture and rapidly induce fibrovascular invasion from the surrounding tissues. The material should also have significant osteoconductive properties to improve incorporation with the host bone and should not induce significant acute immune responses or a chronic foreign body response. The scaffold should have biomechanical properties similar to that of the 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 and be biodegradable, with a controllable absorption rate that parallels the rate of new bone deposition. Finally, the material should have biodegradation products that are nontoxic and easily secreted by normal physiologic pathways and should contain sites that can noncovalently bind osteogenetic biomolecules to enhance osteoinduction. Numerous biologic and synthetic materials have been evaluated for their osteoconductive potential, although 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 materials have been studied, including poly-ƒÑ hydroxy esters, propylene fumarate, polydioxanone, polyethylene glycol, polyanhydrides, polyerthoesters, polyurethanes, poly-L-lactic acid (PLLA), polyglycolic acid (PGA), and poly-lactic-co-glycolic acid (PLGA) (2, 15). PGA, PLGA, and PLLA have the advantages of being already approved for human use and can be constructed with varying porosities and in any three dimensional shape. They have been shown to be an excellent substrate for cellular or bioactive molecule delivery. Perhaps the major drawbacks to these unique polymers is that they do not contain bioactive ligands and may induce mild toxicity from lactic acid release during degradation. In addition, some of the materials may induce a significant foreign body inflammatory response, which may have an inhibitory affect on bone deposition. However, with further preclinical and clinical development, these materials may prove to be outstanding osteoconductive scaffolds and delivery vehicles (22).

Ceramics

ƒÒ-tricalcium phosphate (ƒÒ-TCP) and hydroxyapatite (HA) have been the two most intensely studied ceramics for use in bone repair (14). Their most important property is their chemical similarity to the mineralization phase of normal bone, which account for their osteoconductive potential and excellent biocompatibility (26). The most important difference between these compounds is their resorption rates. In long bone models, 85% of ƒÒ-TCP is resorbed compared with only 5.4% of HA at 3 months. Because 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. However, unlike ƒÒ-TCP, HA has a structure that is very similar to natural bone and, therefore, has improved osteoconductive properties. Both HA and ƒÒ-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 (20). Interestingly, recent studies have demonstrated that HA can directly induce osteogenic differentiation of mesenchymal stem cells in tissue culture, suggesting that they could have a similar response in vivo.

Extracellular Matrix Scaffolds

Various extracellular matrix proteins, including collagen, fibronectin, laminin, and glycosaminoglycans (i.e., hyaluronic acid, heparin sulfate, chondroitin sulfate A, and dermatan sulfate) may be purified and fabricated into excellent biologic 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. Most previous clinical studies concerning biologic scaffolds 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, extracellular matrix molecules have a variety of activities, including acting as a substrate for cell migration, an adhesive for cell anchorage, a ligand for growth factors, and a signal for contacting cells. Matrix components can be combined in a theoretically limitless number of ways, with varying sizes and shapes, which make them ideal to form implantable devices and substrates for cell delivery. The high number of polar and nonpolar residues in collagen matrices provide excellent regions for noncovalent interactions with angiogenic and 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 large local concentrations of growth factors. The engineering of osteoconductive matrices will be an important component of any successful bone graft substitute.

DEMINERALIZED BONE MATRIX

BMPs were initially isolated from extracts from demineralized bone. The demineralized bone matrix (DBM) production process has been fairly well established, involving the initial pulverizing of bone specimens into particles 70¡V450 ƒÝm in diameter, followed by their demineralization with hydrochloric acid and subsequent rinsing with sterile water (35). The application of DBM to promote spinal fusion has been studied in detail in both preclinical and clinical trials (27). Oikarinen (29) initially showed that successful spinal fusions could be achieved in the rabbit using DBM, which was later supported by studies by Ragni and Lindholm (32, 33). However, the initial enthusiasms for using DBM for inducing spinal fusion in the clinical setting have been tempered. An et al. (1) performed a prospective, nonrandomized study comparing autologous bone alone with DBM and freeze-dried allogenic bone in anterior cervical fusions. The study demonstrated that the pseudoarthrosis rate was significantly less frequent in the autograft group (26%) compared with the DBM group (46%). In addition, Jorgenson et al. (23) demonstrated that when DBM was compared to autologous bone for posterolateral lumbar fusions, the DBM was inferior to the autologous bone as assessed by solid fusion rates and radiographic bone density. However, another research group demonstrated in a

retrospective study that posterolateral lumbar fusion sites treated with autologous bone alone (n = 54) or with a composite of DBM and autologous bone (n = 36) had similar radiographic characteristics at 12 months, although the fusion rates were not determined (35). The main limitations of the DBM approach include batch-to-batch variability, substandard processing methods, and potential infectious agents contaminating the material. It is probable that delivery of highly concentrated, recombinant human BMPs (alone or in combination) will ultimately be superior to DBM formulations in the long-term.

BONE GROWTH FACTORS

During the last 10 years, remarkable advances have been made in the field of osteoinductive growth factors (18, 19, 36). Numerous studies indicate that bone regeneration involves the complex interaction of a variety of different regulatory factors. The TGF-ƒÒ superfamily contains some of the most important growth factors involved in bone healing, including TGF-ƒÒ1 through TGF-ƒÒ5, BMPs, and growth and differentiation factors (GDFs). In addition, other growth factors, including fibroblast growth factors (aFGF and bFGF), PDGF, and insulin-like growth factor (IGF), are clearly present at sites undergoing bony healing. Using current molecular biology techniques, recombinant human bone growth factors can now be produced with purity and in large concentrations. Their availability has led to a large number of preclinical and clinical studies to determine each proteins efficacy.

BMPs are typically delivered on osteoconductive scaffolds, such as type I collagen, biodegradable polymers, or hydroxyapatite. The BMPs that have shown the greatest osteogenic activity to date include BMP-2 and BMP-7. BMPs stimulate specific serine/threonine transmembrane receptors on MSCs and osteoblasts (28). 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 (28). BMPs alone 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 (34). These cells subsequently secrete a cartilaginous matrix, which subsequently calcifies into woven bone. This tissue subsequently remodels, forming mature lamellar bone, which contains active bone marrow elements. In larger concentrations, BMPs can also stimulate MSCs to differentiation directly into osteoblasts, which directly induce osteogenesis. 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 improve bone regeneration. Numerous groups have subsequently demonstrated the ability of recombinant human BMPs (rhBMPs) to induce spinal fusions in a variety of different spine models in rats, rabbits, dogs, and primates. In terms of fusion rate and biomechanical strength, several studies have shown that BMPs have been shown to be more effective at fusing the spine than autologous bone grafts.

Recombinant human osteogenic growth factors have recently been advanced to human clinical use and have shown striking bioactivity in several locations. However, there do seem to be significant differences in the physiologic activity of the various BMPs depending on the clinical situation and the particular patient. Boyne et al. (7) 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. (16) subsequently demonstrated significant osteogenic activity of BMP-7 delivered on a collagen carrier in five of six patients undergoing fibular osteotomies. In the spinal region, Boden et al. (5) also showed that rhBMP-2 delivered on a collagen sponge placed inside a interbody fusion cage can induce spinal arthrodesis at a rate faster than cages filled with autologous bone. However, Laursen et al. (25) implanted BMP-7 on a collagen carrier into thoracolumbar burst fractures 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. Poynton and Lane (31) report that both BMP-2 and BMP-7 seem to be safe to use in the spinal regions, although accurate placement is required to prevent central or lateral stenosis. The growth factors also need to be used with caution after durotomy. Burkus et al. (12) demonstrated that rhBMP-2 applied on a collagen sponge in a tapered lumbar interbody cage leads to similar fusion rates compared with autograft while avoiding the 5.9% iliac crest harvest site complication rate. Boden et al. (4) demonstrated that rhBMP-2 delivered on 60% HA and 40% TCP led to a 100% fusion rate, with or without internal fixation, after posterior lateral arthrodesis procedures. Vaccaro et al. (38) reported the results of autograft versus rhBMP-7 putty for posteriolateral lumbar fusions. Clinical success as determined by the Oswestry scale was attained in 73% of the autograft patients and 86% of the BMP-7 putty patients. A solid fusion was achieved in 74% of the BMP-7 putty

patients and 60% in the autograft-treated patients. These initial studies clearly demonstrate that additional preclinical and clinical research 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 formation. In addition, determination of the ideal BMP dose, the most effective BMP carrier, and the optimal rate of release of the BMP from the carrier are clearly required to optimize clinical results.

CELL-BASED APPROACHES FOR BONE FORMATION

The osteogenic activity of osteoconductive scaffolds can be increased by seeding the material with cells with osteoblastic potential. Various approaches are currently being evaluated, including the use of unfractionated fresh bone marrow, marrow derived, culture expanded MSCs, and MSCs predifferentiated into osteoblasts (8). Each of these various cell-based therapies has its own advantages and disadvantages.

Fresh Bone Marrow

A variety of excellent studies have demonstrated that fresh autologous bone marrow has the potential to induce osteogenesis in a variety of locations, including the spine (8, 11, 13). The principle advantages for using this approach are that the harvesting and implantation of autologous bone marrow cells from the iliac crest is relatively inexpensive, straightforward, and not subject to regulation by the Food and Drug Administration. The mechanism by which fresh bone marrow induces osteogenesis is by the proliferation and differentiation of marrow-derived osteoprogenitor cells into bone. Because only a small percentage of the nucleated cells in normal bone marrow are MSCs, it may be difficult to implant enough cells to achieve satisfactory clinical results. In addition, in the debilitated or aged patient, the number of MSCs in the bone marrow significantly decreases, which may make the use of this approach ineffective in this patient population. However, in appropriate patients and in selected clinical situations, the use of bone marrow aspirates to augment osteoconductive scaffolds or in combination with osteogenic growth factors may be an attractive treatment option.

MSCs

Numerous research groups are currently focusing their efforts on developing methodologies for using pluripotent stem cells for the treatment of a variety of disorders, including Parkinson¡¦s disease, stroke, cardiomyopathy, hepatic failure, and renal disease. More specifically, MSCs have also been isolated and are currently being developed for the repair and regeneration of musculoskeletal tissues, including bone, cartilage, muscle, tendon, and ligament (9). MSCs are typically harvested from bone marrow and more recently adipose tissue. They have been isolated from rodents, canines, and humans. Interestingly, these cells can undergo extensive subcultivation in vitro without differentiation, which could significantly increase their potential clinical utility. Human MSCs can be directed toward osteoblastic differentiation by treating the cells with dexamethasone, ascorbic acid, and ƒÒ-glycerophosphate. The cells¡¦ commitment and differentiation into osteoblasts can be documented by analyzing alkaline phosphatase activity, the expression of bone matrix proteins, and by the mineralization of the extracellular matrix.

MSCs typically express a variety of different cell surface proteins, including numerous integrins (ƒÑ1, ƒÑ2, ƒÑ3, ƒÑ5, ƒÑ6, ƒÑV, ƒÒ1, ƒÒ3, and ƒÒ4), growth factor receptors (bFGFR, PDGFR, interleukin-1R, TGF-ƒÒIR, and TGF-ƒÒIIR) and cell adhesion molecules (ICAM-1, VCAM, ALCAM, and L-selection) (8). This explains their high responsiveness to osteogenic growth factors, in addition to osteoconductive matrices used as cellular delivery vehicles. Although human MSC therapies are theoretically attractive, the development of allogenic cellular implants have several potential problems, including graft rejection, transmittable diseases, and microbial contamination. If these issues can be addressed, human MSCs may be ideal for ex vivo BMP gene therapy because the secreted osteogenic proteins will not only stimulate the differentiation of the grafted stem cells, but also local host progenitor cells.

Kadiyala et al. (24) showed that MSCs can regenerate bone in rats in a femoral defect model. In this study, HA/ƒÒ-TCP scaffolds were seeded with syngeneic, marrow derived MSCs at a concentration of 7.5 x 106 cells/mL. The MSC-loaded scaffolds showed significantly improved bone formation compared with the control implants. In a similar study, Bruder and Fox (8) demonstrated that HA/ƒÒ-TCP implants seeded with autologous MSCs also induced

significantly more bone formation within the ceramic carrier and seemed to induce a more rapid union with the surrounding host bone compared with control grafts in a canine model. Human MSCs loaded onto a ceramic carrier were also capable of promoting 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 human clinical trials, although several studies have shown that MSCs secrete immunomodulatory cytokines, which may limit the immune response.

Horwitz et al. (21) demonstrated that allogeneic bone marrow-derived MSCs transplanted into three pediatric patients with osteogenesis imperfecta significantly improved bone deposition in trabecular bone. In addition, the patients had a mean increase of 28 g in their bone mineral content, compared with predicted values of 0 to 4 g. This study demonstrates the possibility of using culture expanded MSCs to induce systemic, and possibly local, bone formation. However, these techniques clearly need further development before they are ready for widespread clinical use.

MSCs Predifferentiated into Osteoblasts

Research groups have also demonstrated the ability of cultured marrow MSCs, which had been predifferentiated into osteoblasts, to induce bone regeneration (30, 39). When undifferentiated MSCs are used to treat bony defects, these cells must first differentiate into their osteoblastic phenotype for bone deposition to occur. If the MSCs are treated with dexamethasone, ascorbic acid, and *f*Ò-glycerophosphate before implantation, the healing process should be accelerated. This approach has been investigated in both rodents and rabbits, demonstrating improved bony healing with osteoblast-loaded scaffolds compared with cell-free control matrices. The obvious disadvantages to this approach are similar to those for allogeneic MSCs, namely, problems with cellular rejection, bacterial and fungal contamination, and batch-to-batch variability. These important approaches require more evaluation to determine whether they can become consistently effective in the clinical setting.

CONCLUSION

Future tissue-engineered implants may obviate the need for autologous bone grafts. Using the known basic mechanisms involved in bone repair and regeneration, researchers can now maximize the osteoinductive, osteoconductive, and osteogenic properties of the graft material. The most effective graft will most likely be composed of osteogenic cells and osteoinductive growth factors, delivered on an osteoconductive, resorbable scaffold. The major issues that need to be addressed include limiting host immune responses directed against allogeneic cellular implants, the optimal dose and rate of protein release for each osteoinductive growth factor, and the development of appropriate scaffold material for each clinical situation. It is anticipated that fabrication of an implant with these three key components should produce a biosynthetic bone graft with performance characteristics equal to and possibly superior to autologous bone.

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