Chapter 205

Bone Grafts, Substitutes and Growth Factors in Spine Surgery

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INTRODUCTION

Bone grafts have been utilized to treat musculoskeletal and dental injuries for centuries. Ancient Romans utilized gold for dental implants, while Mayans used gold, jadeite, and turquoise for jeweled inlays into teeth.[1-3] In the 1600s, a Dutch surgeon named Job-Van Meekeren attempted to use a piece of dog skull for a soldier with a cranial defect.[4,5] The first documented autologous bone graft was used in Germany in 1821 to fill a large bony defect of a humerus, while the first known use of allograft for a tibia was done by Sir William MacEwen in 1881.[6-8]

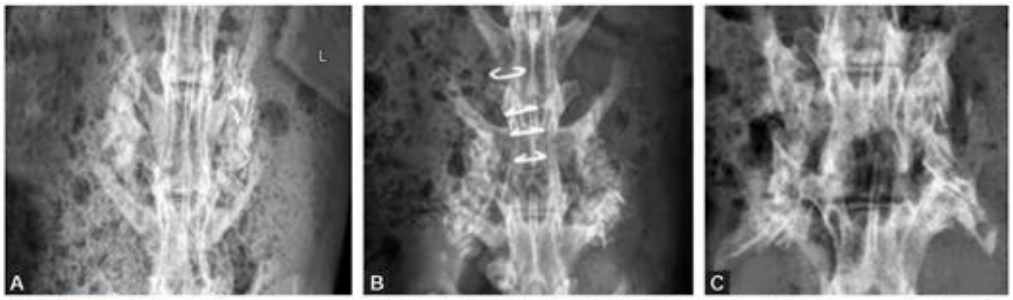
Autografts and allografts are used frequently in operating rooms around the world. Orthopedic procedures (2.2 million) utilize autografts and allografts per year around the world.[9] Approximately, 50% of these procedures are used for spinal arthrodesis.[10]

The use of bone grafts can be separated into three categories: osteoconductive, osteoinductive, and osteogenic.[11] Osteoconductive material, such as fresh allograft is akin to a matrix that allows attachment of bone-forming cells, which will eventually grow into mature skeletal units. These materials work as structural support for eventual bone growth. Osteoinductive materials, such as certain forms of bone morphogenetic protein (BMP) provide factors that induce progenitor cells toward becoming bone-forming cells. Osteogenic grafts like autografts directly provide cells that produce bones.

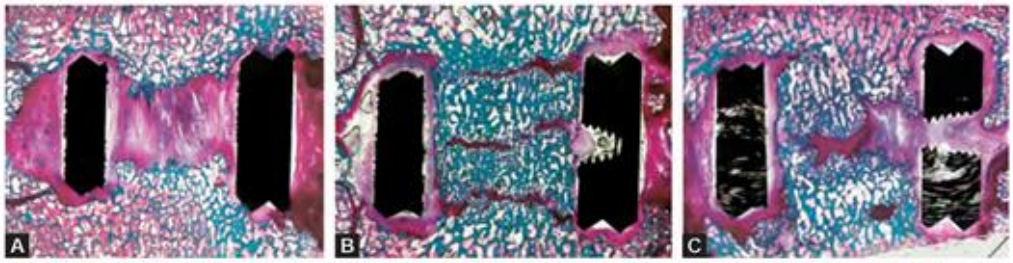
Graft types are categorized based on how they are made and how they are used. Autografts refer to material provided from one anatomic site to support bone healing in another area of the body. Bone from cadaver can be used as an allograft to help in filling bony defects as a largely osteoconductive material with some osteoinductive properties. Synthetic material like ceramic bone grafts are solely osteoconductive and allow for bony growth. Bone growth factors, such as BMP induce the body's multipotent stem cells to branch and become bone forming cells. Finally, there has been significant research into the use of stem cells directly as bone graft material to promote bone healing by directly supplying progenitor cells.

HEALING OF GRAFT

The process of bone healing that occurs with a graft in lumbar fusion surgery was described by Boden et al. with the use of a rabbit model.[12] The first stage is induced by inflammation from decorticating bone in the lumbar spine. This inflammatory phase consists of an initial hematoma formation. This fusion milieu is predominantly made up of macrophages, lymphocytes, and polymorphonucleocytes (PMNs). Eventually this hematoma mass becomes a fibrovascular stroma. The second phase of bone healing consists of resorption of the cortical portion of an iliac crest autograft within the rabbit model. This reparative phase continues up until 6 weeks after the initial rabbit surgery. After 6 weeks, a remodeling phase begins. There is new immature woven bone, which begins forming a peripheral cortical rim around the fusion site. The overall bone



Figs. 205-1A to C: Images of rabbit lumbar spine at various time points after the initial fusion operation. The images show the transitions from a hematoma during the inflammatory phase within the first several weeks to a well-formed bony mass after weeks of remodeling. (A) No significant bony incorporation. (B) Radiograph of a different rabbit 4 weeks after the index procedure shows evidence of new cortical bone, especially along the rabbit transverse processes. (C) 10 weeks after the original operation there is near complete consolidation of the fusion mass between the transverse processes.



Figs. 205-2A to C: Images of pig spines after bony fusion with cages with (A) no bone graft material, (B) autograft, or (C) allograft. There is no bony bridging trabeculae seen in A as there has been no fusion. Both B and C have bridging trabeculae seen via the light green stain. *Source*: Xue Q, Li H, Zou X, et al. Healing properties of allograft from alendronate-treated animal in lumbar spine interbody cage fusion. Eur Spine J. 2005;14(3):222-6.

volume increases during the remodeling phase. The changes seen on radiograph within the rabbit spine model are shown in **(Figs. 205-1A to C)**. Human studies indicate that the process of bony remodeling and fusion does take much longer than 6 weeks. Estimates range from 6 to 18 months.[13] Bony remodeling can be seen on histologic analysis as bridging trabeculae. A section from pig spine shows bridging trabeculae in spinal cages from the cranial and caudal adjacent vertebrae in **(Figs. 205-2A to C)**.[14]

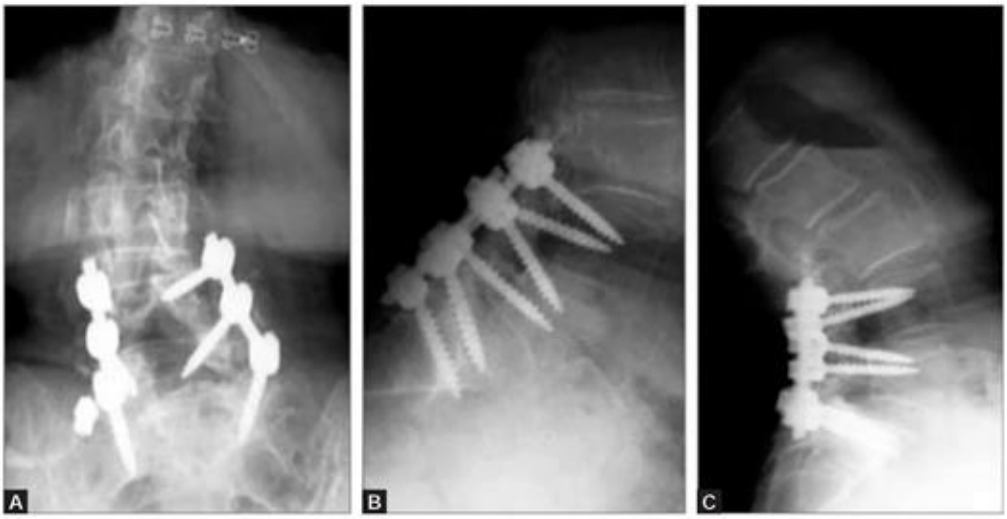
AUTOLOGOUS BONE GRAFTS

The gold standard for bone graft is cancellous autograft for a variety of reasons. The use of autograft for spinal procedures dates back to 1911 with Hibbs using local bone graft for treatment of Pott's disease.[13,15] Whether from the iliac crest, tibia, fibula or ribs, this graft material has a unique complement of live cells, osteogenic, osteoinductive, and osteoconductive properties. This allows it to support bone regeneration while also bringing stem cells that may differentiate into osteoblasts. Hydroxyapatite (HA) and collagen both provide scaffold for eventual bone growth, providing osteoconductive properties. Within cancellous bone there is also a host of native growth factors including BMPs and transforming growth factor-β (TGF-β) that work as osteoinductive agents recruiting progenitor cells to differentiate into bone forming cells. Cancellous autograft provides osteogenic properties early in the healing phase as mesenchymal stem cells from cancellous autograft begin to differentiate into osteogenic cells.

Cortical bone provides another source of autograft material to be used in spine surgery. Cortical bone provides

Figs. 205-3A to J: Ten cage variants. (A) BAK cage (Zimmer Spine). (B) Ray Threaded Fusion Cage (Stryker Spine). (C) LT-CAGE. (D) INTER FIX device (panels C and D; Medtronic Sofamor Danek). (E) Harms cage (DePuy Spine). (F) PEEK cage (Medtronic Sofamor Danek). (G) JAGUAR I/F CAGE (Brantigan Device; DePuy Spine). (H) BOOMERANG. (I) Bone Dowel. (J) Femoral Ring. *Source*: (H to J courtesy of Medtronic Sofamor Danek): Williams AL, Gornet MF, Burkus JK. CT evaluation of lumbar interbody fusion: current concepts. Am J Neuroradiol. 2005;26(8):2057-66.

more structural support as compared to cancellous bone. There are, however, fewer stromal cells and fewer growth factors. Due to its osteoconductive properties, it is able to integrate bone-forming cells at both ends of the graft. Osteoclast tunneling and resorption of the cortical graft simultaneously occurs with bone remodeling around the cortical graft. This "creeping substitution" eventually replaces the cortical graft entirely with viable new bone. Autograft material can be placed in anterior spinal support devices or cages; these devices are shown in **(Figs. 205-3A to J)**. These devices with autograft can further facilitate the bony ingrowth of trabeculae to create a favorable environment for fusion. Initial repair is greatest at the host-graft junction due to this substitution occurring transversely



Figs. 205-4A to C: Solid fusion mass created with the assistance of Healos graft carrier with bone marrow aspirate. (A) A solid fusion mass without any radiolucencies. There are no signs of instability in either the flexion. (B) or extension. (C) X-rays.
Source: Ploumis A, Albert TJ, et al. Healos graft carrier with bone marrow aspirate instead of allograft as adjunct to local autograft for posterolateral fusion in degenerative lumbar scoliosis: A minimum of 2-year follow-up study. J Neurosurg Spine. 2010;13(2):211-5.

and parallel to the long-axis of the graft.[7] Given the process of slow degradation of nonvascularized cortical graft and replacement with new bone, remodeled bone is significantly weaker for months to years.[16,17] Nonviable bone from the original graft will persist well after bony fusion.

Vascularized bone grafts, while more technically challenging, offer several advantages to nonvascularized options in the spine. These grafts have been used in patients with bone loss from osteomyelitis or tumor resection. Placement of bone graft both anterior and posterior in the spine has been utilized.[18] In these clinical situations, the soft tissue bed may be compromised by infection, radiation treatment, and/or wide oncologic resections. The time it would take for the creeping substitution needed for nonvascularized bone graft incorporation in these situations may be too long for adequate stability to be maintained. Vascularized grafts provide faster ingrowth and resistance to higher biomechanical loads within the spine.[19-21] Ackerman inserted vascularized fibular graft in either an anterior or posterior position in seven patients with either chronic infection or after tumor resection and achieved bony union at an average of 3.2 months in all but one.

The most common graft still used for spinal surgery today, and the gold standard, is autologous iliac crest bone graft (ICBG). The techniques for the harvest of these grafts and how to avoid complications are discussed in detail in Chapter 13. For additional information, *see* Refs 24–29.

Bone marrow aspirate contains osteoprogenitor cells and have been used for years in a variety of orthopedic applications, including spine surgery and tibial nonunions.[22] See Chapter 13 for a discussion of bone marrow grafting and the technique of harvest. A solid lumbar fusion X-ray using bone marrow aspirate is shown in **Figures 205-4A to C.**

Autologous platelet concentrate or platelet-rich plasma is a plasma sample that is enriched with particular growth factors. By sequestering and concentrating platelets, compounds, such as TGF-β, vascular endothelial growth factor and other growth factors are collected.[23] These factors have been utilized in a variety of clinical settings, such as lateral epicondylitis and Achilles tendinopathy.[24,25] Platelet-rich plasma has not been found to be beneficial, however, when attempting to stimulate bony growth during revision acetabular procedures.[26] Platelet-rich plasma has failed to show a benefit in enhancing bony integration when used with grafts, graft substitutes, or implants.[27]

Reamer irrigation aspirate is a method to collect high volumes of bone graft and mesenchymal stem cells from the medullary canal of the femur using a reamer-aspirator system.[28-33] This is discussed in detail and the surgical technique is presented in Chapter 13.

ALLOGRAFTS

Allografts refer to bone obtained from a cadaver to be used for replacement and/or augmentation of a patient's native bone healing. Over 35% of all bone transplants utilize human allograft tissue.[34] There are a variety of forms of allograft. Fresh allograft, fresh frozen allograft, and demineralized bone graft have all been described for surgical use. These allografts have both osteoconductive and weak osteoinductive properties.[35] Certain allografts do contain BMPs, GDFs (growth differentiation factors), and possibly TGF-β.[36] Fresh frozen allograft retains much of their original mechanical strength, although these grafts harbor much more of an immune response. The risk of disease transmission is highest for fresh allografts. Freeze drying these specimens, however, does reduce immunogenicity of the graft significantly.

The preparation of allograft bone is vital to decrease the immunogenicity of the graft to help with osseous integration. Frozen grafts are stored in –60°C, and freeze-dried allografts are those in which all the water is removed followed by vacuum packaging. The goal of graft preparation is to retain as much of the osteoinductive and osteoconductive properties as possible while reducing immunogenicity to safe levels. Low-dose irradiation, physical debridement, ultrasound or pulsatile washes, ethanol treatment, and antibiotic soaking all can be used for graft preparation.[7] This sterilization process leaves allograft bone with limited osteoinductive capability.[11] Furthermore, the Food and Drug Administration (FDA) requires further testing after sterilization for HIV-1 (human immunodeficiency-1), HIV-2, and hepatitis C.

A specific set of responses occurs as a result of the body's immune response to allograft implantation. The extent to which an immune response occurs is proportional to the residual proteins that are present on allograft bone.[37] This reaction is largely a cell-mediated response with Major Histocompatibility Complex I and II molecules present on specific cells to activate the immune response. Alloantigens from allograft bone are recognized by both these T cells and macrophages. The resultant cytokine cascade also assists with the process of bony incorporation.[38,39]

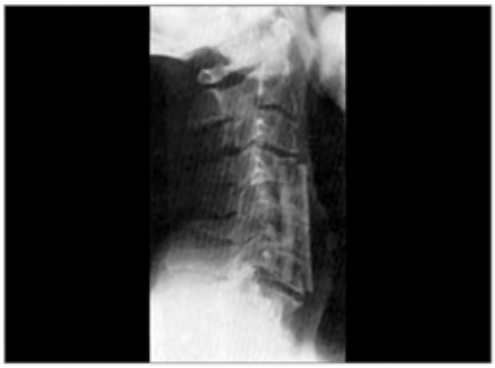


Fig. 205-5: Lateral X-ray 3 months after a multilevel anterior cervical corpectomy without instrumentation. Note the location of the fibular allograft to assist in alignment of the cervical spine.
Source: Muschler G, Lane J. Orthopaedic surgery. In: Habal MB, Reddi AH (Eds). Bone grafts and bone substitutes. Philadelphia, PA: WB Saunders; 1992. pp. 384-95.

Fresh frozen and freeze-dried allograft has been utilized in a variety of clinical settings to augment bony fusion. Within the lumbar spine there have been studies indicating that autograft outperforms allograft alone when used for posterior spinal fusion. Allograft may be more useful in this clinical scenario in the anterior spine.[40] In the cervical spine, structural allograft was associated with a high rate of union when used for occipitocervical fusion when compared to traditional autograft techniques.[41] Long-term follow-up of patients with allograft and plate constructs for an ACDF procedure revealed a satisfactory rate of fusion.[42] Another study looking at freeze-dried fibular allograft also showed a high rate of fusion for one- and two-level surgery.[43] When used for bone loss after cervical corpectomy, allograft fibula did have successful outcomes as well.[44] Figure 205-5 shows an example of an allograft fibula used after a corpectomy. Not all studies indicated complete success with allografts when compared with autograft material. In a study comparing allograft fibula to autograft fibula, it was found that autograft fibula did have a 27% rate of nonunion as compared to 41% in the allograft group.[45]

Demineralized bone matrix (DBM) is a form of allograft bone with osteoconductive properties as well as trace amounts of osteoinductive chemicals. To create DBM, allograft bone is first decalcified with acid. This material can then be crushed into a powder form or mixed with a carrier compound to form DBM. These carriers include hyaluronic acid, glycerol, or gelatin. The processed DBM

Table 205-1	Variable concentration of osteoinductive BMP between lots of demineralized bone matrix (DBM).			
	Lot no. 1 ng/g DBM	*Lot no. 2 ng/g DBM*	*Lot no. 3 ng/g DBM*	*CV*
ELISA analysis of BMP-2 ng/g DBM				
Allomatrix® C bone graft putty[7]	97.5	30.1	28.2	76.01%
DBX® DBM putty[8]	51.4	40.9	36.6	17.72%
DynaGraft® II osteoinductive gel[9]	49.2	38.8	25.4	31.56%
DynaGraft® II osteoinductive putty[11]	39.5	30.8	29.5	16.34%
Grafton® gel[12]	85.6	33.6	20.2	74.35%
Grafton® putty[13]	61.3	51.9	29.0	35.05%
Grafton® crunch (written communication, February 2004)	40.8	30.5	29.0	19.21%
InterGro® DBM putty (written communication, November 2003)[14]	89.7	50.5	33.0	50.29%
Osteofil® allograft paste[15]	120.6	48.4	28.4	73.71%
BMP-2, lots: F = 15.12, P < 0.0002; products: F= 1.29, NS	70.6	39.5	28.8	
ELISA analysis of BMP-7 ng/g DBM				
Allomatrix® C bone graft putty[7]	118.8	67.8	66.3	35.45%
DBX® DBM putty[8]	179.7	94.1	90.9	41.43%
DynaGraft® II osteoinductive gel[9]	188.9	95.6	54.2	61.11%
DynaGraft® II osteoinductive putty[11]	226.8	67.9	55.0	82.08%
Grafton® gel[12]	70.5	69.9	60.3	8.56%
Grafton® putty[13]	84.7	80.0	78.6	3.95%
Grafton® crunch (written communication, February 2004)	73.5	68.1	66.9	5.06%
InterGro® DBM putty (written communication, November 2003)[14]	77.5	72.7	72.7	3.71%
Osteofil® allograft paste[15]	81.6	68.1	66.5	11.51%
BMP-7, lots: F = 6.43, P < 0.01; products: F = 1.19, *NS*	122.4	76.0	67.9	

(NS: Not significant; CV: Coefficient of variation).
Source: Bae HW, Zhao L, Kanim LE, et al. Intervariability and intravariability of bone morphogenetic proteins in commercially available demineralized bone matrix products. Spine. 2006;31(12):1299-306.

is porous and can therefore facilitate ingrowth of vessels, although it provides limited mechanical support. This form of DBM does carry compounds such as BMP, making it osteoinductive. The amount of osteoinductive material, however, can vary greatly between lots of DBM.[46] The variability in DBM composition is shown in **Table 205-1** as well. When DBM is combined with autograft, ceramics and/or bone marrow fusion rates are satisfactory.[47,48] Patients with adolescent idiopathic scoliosis treated with autograft and DBM/autologous bone marrow were also found to have equivalent rates of fusion.[49]

GROWTH FACTORS

Bone morphogenetic protein was originally described by Marshall Urist.[50,51] It belongs to the TGF-β superfamily of protein (except for BMP-1). The BMP signaling is required for endochondral bone formation and also plays a significant role in embryonal development. Within an embryo, BMP helps establish the dorsal ventral axis and differentiation of ectodermal cells.[52,53] Signal transduction involves the intracellular cytoplasmic protein family named SMAD.[54,55] Downstream signaling eventually results in changes in gene expression for cell differentiation. These signaling pathways are critical for heart, cartilage, neural, and bone development. Specific in bony development, the SMAD-BMP combination signals progenitor cells to develop into osteoblasts and dormant osteoblasts to become active.

In order for BMP to be harnessed to induce bone growth in situ, an effective manner of delivery to the host tissue is needed. This requires a carrier for delivery of BMP. Delivery of BMP must occur over a prolonged period of time as well. The carrier substance must not be immunogenic. Absorbable collagen from bovine tendon and collagen-based matrix derived from extracted bone are the most common carriers of BMP.[56-58] Radiographs