Chapter 6

Bone Grafts, Bone Morphogenetic Proteins, and Bone Substitutes

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I. Bone Healing

A. Primary bone healing—Occurs with constructs that provide absolute stability. When open reduction and internal fixation is performed, the initial fracture hematoma is disrupted, possibly slowing repair via removal of the fibrin scaffold and loss of early infiltrating cells and cytokines.

1. Fixation of bony surfaces enables primary healing by creating a low-strain environment.
2. Bone heals directly by cortical remodeling.
3. Areas not in direct apposition may be filled by woven bone that is subsequently remodeled to lamellar bone.

B. Secondary bone healing—Involves responses in the periosteum and surrounding soft tissues. Two types of secondary healing occur: endochondral and intramembranous. Typically, both types occur concurrently at a fracture site.

C. Fracture healing is classically described in three phases.

1. Inflammation (early phase)
   a. Hematoma formation and inflammation occur rapidly in the early phases of bone repair.
   b. Surface osteocytes may survive and are important in synthesizing new bone.
   c. Inflammatory cells migrate to the fracture site, followed by fibroblasts and chondrocytes.
   d. Vasodilation increases local flow and angiogenesis is stimulated, with capillary ingrowth primarily from the periosteum.
   e. Bone morphogenetic proteins (BMPs) are thought to play an important role in inducing host mesenchymal stem cell migration and differentiation at the repair site.
2. Reparative (middle phase) (**Figure 1**)
   a. New bone formation takes the form of immature woven bone (soft callus).
   b. Seams of osteoid surround the core of necrotic

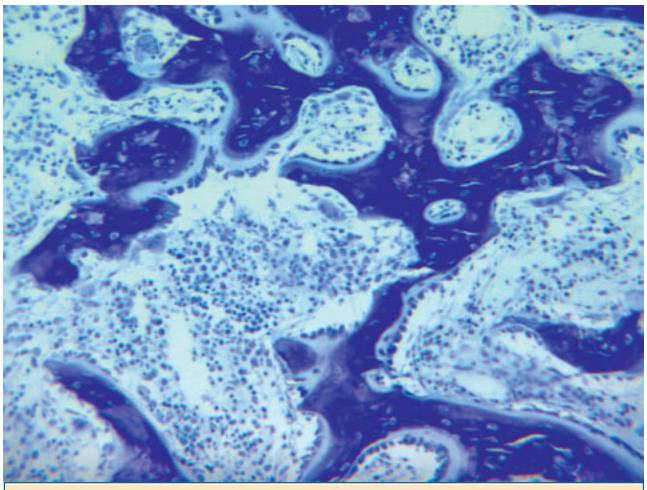

Figure 1 Histology shows reparative bone healing with osteoblasts lining new trabecular bone spicules.

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bone and form viable new bone (hard callus).

c. During endochondral ossification, hyaline cartilage provides a framework bridging fracture fragments.

d. Cartilage is subsequently calcified by osteoblasts.

3. Remodeling (late phase)

a. Coupled resorption and formation occur.

b. Remodeling is influenced by the Wolff law and is usually complete by 1 year.

D. Endochondral versus intramembranous bone healing

1. Healing occurs via endochondral ossification, which directly bridges the fracture gap, and intramembranous bone formation subperiosteally adjacent to the fracture.

2. Factors that impair bone healing

a. Excessive instability at the fracture site or nonapposition of bone

b. Lack of blood supply because of the local vascular anatomy or periosteal stripping from injury/dissection

c. Anti-inflammatory medications (NSAIDs, steroids)

d. Smoking

e. Systemic disease: metabolic bone conditions

II. Role of Bone Grafts

A. Multiple clinical problems may indicate the use of bone graft.

1. Fracture healing, treatment of delayed unions or nonunions

2. Arthrodesis (**Figure 2**)

3. Replacement of osseous defects occurring as a result of trauma, tumor, or wear

B. Bone grafts perform one or more physiologic mechanisms.

1. Osteogenesis

a. Osteogenic graft material directly provides cells that are capable of in vivo bone formation.

b. Osteoprogenitor cells can proliferate and differentiate to osteoblasts and eventually to osteocytes. Mesenchymal stem cells are multipotent and may be induced to differentiate into bone-forming cells by the local environment.

c. Examples: Autologous bone graft, bone marrow aspirate

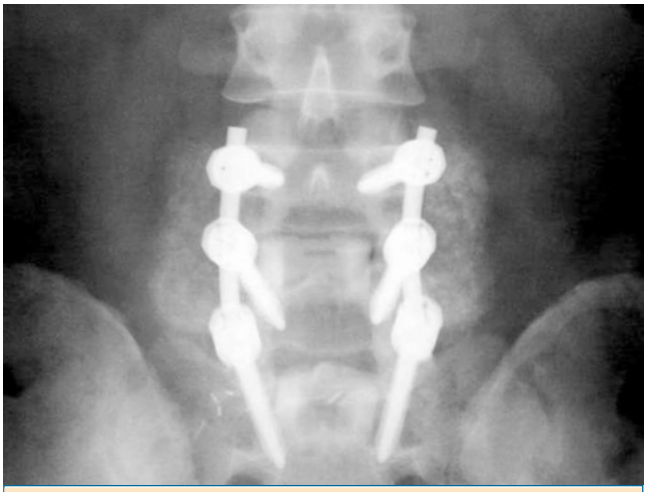

Figure 2 AP radiograph shows L4-S1 instrumented posterior fusion with bone graft placed in the posterolateral gutters.

2. Osteoinduction

a. Osteoinductive graft material has factors that induce progenitor cells down a bone-forming lineage via cytokines acting as chemoattractants and differentiation factors.

b. Example: BMPs

3. Osteoconduction

a. Osteoconductive materials serve as a mechanical scaffold into which new bone can form.

b. The three-dimensional configuration and building-block material dictates osteoconductive properties.

c. Cancellous bone has greater bone-forming potential than cortical bone because of its greater surface area, increased porosity for cellular infiltration, and space for angiogenesis; however, it provides less immediate structural support.

d. Examples: Acellular cancellous chips

III. Bone Graft Materials

A. Bone graft materials come from a variety of sources and exhibit heterogenous properties (**Table 1**). Different materials can be combined into a composite graft.

B. Autograft is tissue transferred from one site to another in the same individual and has classically been the gold standard of bone graft material. It is still the gold standard by which other grafting materials are measured. Often, the supply may be limited, and procurement can result in donor site morbidity.

1. Autograft is osteogenic, osteoinductive, and osteoconductive.
2. Autograft may be cortical, cancellous, or corticocancellous; it also may be nonvascularized or vascularized.
   a. Cortical autograft provides structural support, but its high inorganic density means it provides fewer cells and factors for osteogenesis.
   b. Cancellous autograft provides less structural support but greater osteoconduction and greater osteogenesis and osteoinduction because of its greater cellular and organic density.
3. Iliac crest bone graft (ICBG) is the most frequent autograft.
   a. It has the potential to provide abundant cancellous and/or cortical graft.
   b. Studies have shown complications associated with ICBG, including chronic pain, hematoma formation, injuries to the lateral femoral cutaneous or cluneal nerves, infection, fracture, and scars. The rate of complications is contested in the literature, with studies conducted before 2000 demonstrating major complication rates between 0.76% and 25.0% and minor complication rates, including pain, between 9.4% and 24.0%. More recent studies have shown variable complications rates but seem to suggest lower rates with current techniques (not harvesting the full outer iliac table).
4. Local autograft may be found at the surgical site. In spinal surgery, bone from the lamina is often a source of local autograft after a laminectomy; however, the quantity available may be limited.
5. Other bone graft sources include the ribs, fibula, and tibial metaphysis. The fibula and rib are the most common potentially vascularized options considered.

C. Allograft is tissue harvested from a cadaver, processed, and then implanted into another individual of the same species. Because of its availability, it is the most frequently used bone-graft alternative in the United States.
1. Allograft can be cortical, cancellous, or corticocancellous.
2. Most allograft lacks viable cells and therefore does not provide osteogenic properties. Osteoinductive factors are reduced because of the sterilization needed to reduce the risk of disease transmission and avoid host immune responses.
3. Allograft products containing viable cells or enhanced with viable cells are new to the market and may offer another grafting option. Tissues selected from a highly screened donor pool are cryopreserved in a process that maintains cellular viability. Few independent published clinical studies on efficacy exist. This class of allograft remains primarily osteoconductive but may have osteogenic and osteoinductive properties.
4. Disease transmission is exceedingly rare in bone allografts. Precise incidence data are not available. However, with recommended donor screening the estimated chance of obtaining a graft from an HIV-positive individual is less than 1 in 1.67 million. The last reports of viral transmission are of hepatitis C in 2002, and human T-cell lymphocytic virus in 1991.
5. The extent of osteoconductive properties, as well as mechanical strength, depends in part on the method of graft processing (fresh, frozen, or freeze-dried form) and whether it is cortical or cancellous.
6. Several different types of allograft may be considered.
   a. Fresh allograft
      - Rarely used because of the potential for an immune response and disease transmission
      - Fresh allograft may be processed to remove cells and reduce host immune reaction. This process has been shown to improve incorporation.
   b. Frozen allograft
      - Reduces immunogenicity; frozen allograft requires cold storage logistics and rewarming before implantation to avoid brittleness, which could result in longitudinal fractures.
      - Maintains the osteoconductive properties and potentially some limited osteoinductive capabilities
      - The shelf life of fresh-frozen bone maintained at –20°C is 1 year; 5 years if kept at –70°C.
   c. Freeze-dried allograft has properties similar to frozen allograft, with a few exceptions.
      - Prepared by freeze-drying
      - Stored at room temperature
      - The shelf life of freeze-dried bone is indefinite but the sterilization of the packaging may expire.
      - Biomechanical studies in the 1980s demonstrated a similar compressive and tensile strength as frozen but reduced torsional/bending strength.
   d. Demineralized bone matrix (DBM)

Table 1

Characteristics of Bone Grafts and Grafting Substitutes

Grafting Modality	Substance/ Implant	Osteogenic	Osteoinductive	Osteoconductive
Autografts	Cancellous bone Morcellized iliac crest Metaphyseal long bone	+++	++	+++
	Cortical bone Local bone Iliac crest FIbula	+	+/–	+/–
	Cellular Bone marrow aspirate	++	+/–	–
Allografts	Fresh	–	+/–	++
	Frozen	–	+/–	+
	Freeze-dried Cortical cancellous chips	–	+/–	+
	Demineralized bone matrix Various preparations	–	+/–	+
Growth factors	rhBMP-2 rhBMP-7	–	+++	–
Ceramics	Hydroxyapatite Tricalcium phosphate	–	–	+
Collagen	Absorbable collagen hemostatic sponge	–	–	–

- DBM is allograft processed with a mild acid extraction to remove the mineral content of bone but leave behind the collagenous structure (mostly type I, with some types IV and X) and noncollagenous proteins.
- DBMs are combined with carriers such as collagen, gelatin, hyaluronic acid, and glycerol into DBM-based products.
- The antigenic potential is low because of sterilization and other processing.
- Theoretically, DBMs have osteoinductive activity, but the level of activity depends on the sterilization process, processing methods, particle size/surface area, and geometry. Significant interproduct (between products) and interlot (between lots) variability exists because each production lot is derived from a single patient.
- DBM-based products are osteoconductive and serve as a scaffold for new bone, but they lack structural support.
- The most popular format is a moldable putty containing a DBM base powder mixed with a carrier. The proportion of the DBM base compared with that of the carriers tends to be low in some products.

D. Autologous bone marrow aspirate

1. Bone marrow aspirate is a potential source of mesenchymal stem cells and osteoprogenitors.
2. It may be aspirated percutaneously from the iliac crest, vertebral body, or other sources. Bone marrow aspirate can then be mixed with other bone graft extenders and ceramics to create a composite graft material.
3. The number of cells varies depending on host

Table 1

Characteristics of Bone Grafts and Grafting Substitutes (*continued*)

Donor Site Morbidity	Immunogenicity	Absorption/ Remodeling Rate	Immediate Structure/ Torque Strength	Typical Orthopaedic Applications
++++	–	+++	–	Lumbar spine Cervical spine Long bones
++++	–	++	++	Spine Tibial nonunion
+/–	–	–	–	Augmentation of other grafting materials Spine Long-bone fracture
–	++	+	++	Spine Long-bone fracture
–	+	–	++	Spine Long-bone fracture
–	+/–	–	+	Spine Long-bone fracture
–	+	–	–	Spine Long-bone fracture
–	–	–	–	Spine Long-bone fracture Nonunions
–	–	–	+/–	Spine Coating for fixation/arthroplasty devices
–	+	–	+/–	Functions poorly alone but functions well coupled with BMPs

BMP = bone morphogenetic protein, rh = recombinant human, – = not present, +/– = variable, +, ++, +++ = is present, qualitative importance.

characteristics such as age and sex.

4. It has been suggested that the potency of marrow aspirates could be increased via selective precursor selection, centrifugation, or clonal expansion.

E. Collagen

1. Collagen contributes to mineral deposition, vascular ingrowth, and growth factor binding, providing a favorable environment for bone regeneration. It does not provide structural support but may carry immunogenic potential.

2. Collagen functions poorly alone but is used as a nonstructural carrier for BMPs, DBMs, or other graft materials.

F. Inorganic compounds and synthetic bioceramics

1. Bioceramics are ionically or covalently bonded calcium phosphate compounds composed of metallic and nonmetallic elements. The most commonly used are alumina, zirconia, bioactive glass, hydroxyapatite (HA), and tricalcium phosphate (TCP). Bioceramics are relatively inert. They demonstrate material properties that are strong in compression but weak in tension. Bioceramics easily bond with living tissues. They may provide good scaffolds for the addition of potentially osteogenic cells in skeletal tissue engineering applications.

2. Several classes of ceramic materials are available.

a. Synthetic HA has the chemical composition $Ca_{10}(PO_4)_6(OH)_2$. It typically is used in its crystalline form and is slow to resorb or remodel. For orthopaedic implants, HA usually is coated onto a metal core or incorporated into

polymers as composites. HA provides a substrate for bony apposition and ongrowth.

b. β-TCP has the chemical composition $Ca_3(PO_4)_2$. Because of its porous nature and chemical composition, it is faster. It is typically developed for orthopaedic implants as a porous biphasic calcium phosphate scaffold with 60% HA and 40% β-TCP.

c. Other materials, such as bioactive glass, contain silicone salt combinations. These materials typically interact with the body fluids and, through a salt exchange, form an amorphous layer of HA, which provides the scaffold for bony apposition.

3. Bioceramics alone possess no osteogenic or osteoinductive properties, and they have variable immediate structural support secondary to resorption. They provide an osteoconductive effect or scaffold, which varies based on the pore size of the synthesized material. Larger pore sizes (>50 μm) provide more space for the migration and ingrowth of osteogenic cells and vascular supply. A typical preparation is HA and TCP, sometimes mixed with autograft.

IV. Bone Morphogenetic Proteins

A. This family of proteins (**Table 2**) contains at least 20 unique peptides that have now been identified and include members of the transforming growth factor-β (TGF-β) superfamily. Only certain BMPs are osteoinductive; others are unrelated to bone formation or have alternative functions. BMPs play a key role in normal embryonic development.

B. Recombinant human forms of these two types of BMPs are currently available: recombinant human BMP-2 (rhBMP-2) and rhBMP-7. These rhBMPs are highly water soluble; without a carrier, they will rapidly diffuse from a wound bed and may be washed away by irrigation. rhBMP-2 is indicated for use in spinal fusion and tibial shaft fractures. It is delivered in a purified absorbable collagen sponge. rhBMP-7 is available only under a humanitarian device exemption and may be indicated for use in recalcitrant long-bone nonunions.

C. The current use of BMPs in spinal fusion is controversial. Multiple studies from 2011to 2013 have debated the safety of rhBMPs for spinal fusion; rhBMP-2 is considered equivalent to ICBG in the formation of bone.

D. Because of the controversies surrounding safety and the possible bias in the reporting of adverse advents during the approval of rhBMP-2, two independent studies reviewed the available data on the safety and effectiveness of rhBMP-2.

1. Studies indicated that clinical outcomes or success scores were not clinically different between patients treated with rhBMP-2 and those treated with ICBG. However, one of the studies showed that radiographic fusion was 12% more common in patients treated with rhBMP-2 than in those treated with ICBG.

2. ICBG and rhBMP-2 are associated with similar complication rates when used as a graft material for anterior lumbar interbody fusions, including the rates for retrograde ejaculation.

3. RhBMP-2 is associated with higher complication rates in anterior cervical fusion and higher ectopic bone formation in posterior lumbar interbody fusions.

4. Posterior lateral lumbar fusions using rhBMP-2 are associated with increased transient leg/back pain.

5. A risk of local swelling exists in anterior cervical fusions in which rhBMP-2 is used.

E. Bone graft materials may be used together to combine required properties or because of limited availability of one component.

1. A combination of bone graft materials allows products having different properties to be obtained (for example, structural cortical allografts augmented by DBM and local bone graft).

2. Available grafts such as ICBG or local bone graft that is in limited supply may be augmented by other grafting materials. Example: The supply of autogenous local bone graft or ICBG providing osteogenesis, osteoinduction, and osteoconduction may be limited; therefore, augmentation by osteoconductive DBM may be performed to obtain sufficient volume.

V. Other Modalities to Enhance Bone Healing

A. Electromagnetic stimulation

1. Bone tissue has bioelectric potential.

a. Bioelectric potential is electronegative in areas of growth or healing. The area returns to neutral or electropositive as healing progresses.

b. Bioelectric potential is electronegative in areas of compression and electropositive in areas of tension.

2. Efficacy

a. Trials have shown significant variability in outcomes, with overall efficacy unclear. Benefits have been seen in single studies for some fracture types. Because of the low morbidity of the treatment, the clinical use of electromagnetic

Table 2

A Comparative Overview of Bone Morphogenetic Proteins

BMP	Synonyms	Function	Knockout Phenotype in Mice	Chromosomal Location in Humans	Chromosomal Location in Mice
BMP-1	hTld1	Induction of cartilage, metalloprotease	Reduced ossification	8p21.3	14 32.5 cM
BMP-2	BMP2A	Cartilage and bone formation	Embryonic lethal, heart defect and lack of amnion	20p12	2 76.1 cM
BMP-3	Osteogenin	Negative regulator of bone development	Increased bone mass, bone volume	4q21	5 55.0 cM
BMP-4	BMP2B	Bone and teeth	Embryonic lethal, heart defect, lack of allantois	14q22-q23	14 15.0 cM
BMP-5		Cartilage development	Loss of one pair of ribs in rib cage, short ear	6p12.1	9 42.0 cM
BMP-6	Vgr-1	Liver and joint development	Delayed sternum ossification	6p24-p23	13 20.0 cM
BMP-7	OP-1	Kidney development	Renal defects	20q13	2 102.0 cM
BMP-8	OP-2	Cartilage and bone formation	Spermatogenesis defects	1p35-p32	Not known
BMP-9	Gdf-2	CNS and liver development and angiogenesis	Postnatal retinal vascular remodeling	10q11.22	Chromosome 14
BMP-10		Heart development	Proliferation defects in embryonic cardiomyocytes	2p13.3	6 D2
BMP-11	Gdf-11	CNS development	Skeletal *A–P* axis growth pattern abnormalities	12q13.2	Chromosome 10
BMP-12	Gdf-7, Cdmp3	Tendon and cartilage development	Abnormal skull development	2p24.1	Chromosome 12
BMP-13	Gdf-12, Cdmp2	BMP inhibitor in tendon development	Abnormal skull, bone fusions at wrist and ankle	8q22.	Chromosome 4
BMP-14	GDF-5, CDMP1	Cartilage development	Delay in fracture healing	20q11.2	11 50.5 cM
BMP-15	Gdf-9	Oocyte development	Decreased ovulation and fertilization	Xp11.2	X 0.5 cM

BMP = bone morphogenetic protein, CNS = central nervous system.

Adapted with permission from Bandyopadhyay A, Yadav PS, Prashar, P: BMP signaling in development and diseases: A pharmacological perspective. *Biochemical Pharmacology* 2013;85(7):857-864.

stimulation is common despite its unclear efficacy.

3. Types
   a. Pulsed electromagnetic field—Alternating current is delivered through an external coil used intermittently during the treatment period.
   b. Capacitively coupled electrical stimulation—Current is delivered between two plates that form a magnetic field over the site of healing.
   c. Direct current electrical stimulation— Direct current is delivered through implanted electrodes.

B. Low-intensity ultrasound may affect bone healing, but it is not in widespread clinical use.

Top Testing Facts

1. Bone healing progresses through three stages: early (inflammation), middle (reparative), and late (remodeling).
2. Bone grafts may be osteogenic, osteoinductive, and/or osteoconductive.
3. Autograft is the gold standard of bone graft materials.
4. Disease transmission is exceedingly rare in bone allografts. It is estimated that with donor screening protocols there is less than a 1 in 1.6 million chance of a false-negative result for HIV status.
5. DBM-based products have been shown to have significant interproduct (between products) and interlot (between lots) donor-specific variability. DBMs are predominantly osteoconductive.
6. Bone marrow aspirates provide potential access to osteogenic mesenchymal precursor cells.
7. Bioceramics are inorganic compounds consisting of metallic and nonmetallic elements held together by ionic or covalent bonds.
8. BMPs (BMP-2, -4, -6, and -7) are potent osteoinductive factors of the TGF-β superfamily.
9. Hyaline cartilage serves as the precursor for bone formation via endochondral ossification.

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