Bone Grafting 101

UNDERSTANDING BONE GRAFTS AND BONE GRAFT SUBSTITUTES

Dr. Salvatore J. Monopoli, DABPS, FACFAS
Alumnus, Cambridge Hospital of Harvard Medical School
Parent Orthopedic Dept Mass General, Foot & Ankle sub division
Clinical Training Manager
BioSurgery US Sales Training

Objectives

- Rationale
- Epidemiology
- Terminology
- Existing Materials
- Characteristics and Challenges
The Need for Bone Grafts and Bone Graft Substitutes

Bone grafts are used to:

- **Fuse joints to prevent movement**
  - Spine and extremities
- **Repair injured bone that has not healed**
  - Delayed union or non-union
- **Repair fractures that have bone loss**
  - Accelerate bone healing
- **Repair bone voids**
  - Surgical, traumatic, disease, infection

Bone Graft Need

Percentage of total number of bone grafting procedures performed in the U.S.

- Thoracolumbar fusion: 11%
- Cervical fusion: 3%
- Trauma surgery: 8%
- Large joint revision and reconstruction: 28%
- CMF surgery: 18%
- Extremity surgery: 1%
Mechanisms of Bone Formation

**Osteoconduction:**
Biocompatible material that provides a physical structure into and along which bone may grow.

**Osteoinduction:**
Capable of inducing bone formation in a non-bony site by recruiting and inducing (pluripotent) cells to become osteoblasts.

**Osteogenesis:**
Graft already contains the cells required to produce bone.

Bone Graft Characteristics / Types

**Osteogenic**
- Autograft

**Osteoconductive**
- Allograft (Cortical & Cancellous)
- Calcium Sulfate Ceramics
- Collagen Composites
- Calcium Phosphate (CaP) Ceramics

**Osteoinductive**
- Recombinant Human Bone Morphogenetic Proteins (rhBMP)
- Demineralized Bone Matrix (DBM)
- Allografts
REVIEW OF BONE GRAFTS AND SUBSTITUTES

The following overview of characteristics and considerations is not meant to be a comprehensive list, practitioners should evaluate each bone graft carefully.

**Autografts**

**Characteristics**
- Established historical standard of bone grafting
- Autograft exhibits all three bone formation mechanisms: osteoconduction, osteoinduction, and osteogenesis
- No potential for disease transmission or immunogenic response

**Considerations**
- Time of overall surgical procedure
- Second site surgery to procure autograft or ICBG
- Limited quantity of 50-55 cm³
- Complication rates reported include:
  - Blood loss
  - Hematoma and arterial injury
  - Nerve injury and numbness
  - Hernia formation
  - Infection
  - Fracture and pelvic instability
  - Cosmetic defects
  - Chronic pain at donor site

**Proposed Mechanism of Action:** Presence of osteoblasts provides direct osteogenesis, presence of growth factors permits osteoinduction, bone tissue allows osteoconduction.
Allograft

**Characteristics**
- Available in various geometric and shapeable forms
- Cortical allografts may provide structural support and may be immediately load bearing
- Osteoconductive and weakly osteoinductive if growth factors remain after processing (cancellous)

**Considerations**
- Potential for disease transmission and immune rejection
- Variable clinical results and inconsistent graft incorporation
- Must have reputable tissue bank
- Freeze-dried allograft has low compressive strength
- FDA classification of "reprocessed human tissues" allows clearance of DBM products without proof of efficacy. Some have 510K clearance

**Proposed Mechanism of Action**: Primarily acts through osteoconduction. Cancellous allograft may have some osteoinductive potential but it will vary depending on the source and how it was processed and sterilized

DBM (Allograft Sub-set)

Cortical bone with mineral removed (via acid extraction) leaving collagenous and non-collagenous proteins with a low concentration of growth factors

**Characteristics**
- Contains type I collagen, non-collagenous proteins, and a low concentration of growth factors (BMP) which may impart osteoinductivity
- Osteoinductivity of DBMs have been proven in animal studies
- DBM is the least immunogenic of the allograft types due to the extensive processing

**Considerations**
- Availability of prospective randomized studies to support osteoinductive claims in humans
- Bone producing ability depends on processing methods which vary from product to product
- FDA classification of "reprocessed human tissues" allows clearance of some DBM products without evidence of efficacy. Some have 510K clearance

**Proposed Mechanism of Action**: Primarily acts through osteoconduction, studies report that the presence of growth factors (e.g. BMP) imparts osteoinductivity
Growth Factor Based (BMP, etc.)

Growth factors bind to receptors on cell surfaces stimulating the formation of proteins to be used inside the cell or externally (e.g. formation of extracellular matrices like bone tissue)

For example ...

- Bone Morphogenetic Proteins (BMPs) (aka recombinant human bone morphogenetic protein or rhBMP)
- Insulin-Like Growth Factor 1 & 2 (IGF-1), (IGF 2)
- Transforming Growth Factor Beta (TGF-β)
- Platelet Derived Growth Factor (PDGF)
- Fibroblast Growth Factor (FGF)

How does rhBMP work?

Mesenchymal stem cells (MSCs) have the ability to change or “differentiate” into various types of cells which make different types of tissues.

Bone Morphogenetic Proteins (BMPs) direct MSCs to differentiate into an osteogenic bone line.
Growth Factor Based (BMP)

Characteristics
- Osteoinductive\(^\text{14}\)
- rhBMP-2 and rhBMP-7 have published clinical efficacy studies\(^\text{14}\)
- rhBMP-2 clinical studies have shown that BMP + collagen worked as good, if not better, than autograft\(^\text{14}\)

Considerations
- Optimal BMP dosage and carrier yet unknown\(^\text{14}\)
- Most common complications include osteolysis, swelling/edema, heterotopic bone formation, and antibody reaction\(^\text{16}\)
- Precise rate of complications and strategy to reduce number of complications is still not known\(^\text{16}\)

Proposed Mechanism of Action: Acts via osteoinduction, BMP binds to mesenchymal stem cell receptors resulting in proliferation and differentiation into osteoblasts.\(^\text{14}\)

Cellular Based: Bone Marrow Aspirate

Characteristics
- Autogenic source of osteogenic precursor cells\(^\text{10}\)
- Has been used alone or in conjunction with other bone graft substitutes\(^\text{10,18}\)
- Minimally invasive harvesting technique reduces donor site morbidity\(^\text{10}\)
- Fractures/non-unions can be treated by percutaneous injection of marrow\(^\text{18}\)

Considerations
- Failures can occur due to inconsistent aspiration methodology\(^\text{18}\)
- Osteoprogenitors may only represent ~0.001% of nucleated cells in healthy adult marrow\(^\text{19}\)
- It may be difficult to obtain enough bone marrow with sufficient number of osteoprogenitor cells for bone healing\(^\text{19}\)
- Aging or disease may reduce the number of osteogenic precursor cells in marrow\(^\text{19}\)

Proposed Mechanism of Action: Bone marrow aspirate contains osteoprogenitor cells with the potential to differentiate into osteoblasts able to produce new bone tissue\(^\text{19}\)
Cellular Based: (PRP)

Platelet Rich Plasma (PRP) is obtained by fractioning whole autologous blood by centrifugation. 20

Considerations

- Outcome is donor dependent and varies with preparation technique.
- Proteases present in the platelet fraction may degrade some of the growth factors.
- Addition of PRP to bone graft seems to reduce the spinal fusion rate in animals as well as in humans.
- Ideal factor concentration and delivery system still not known.

Characteristics

- PRP contains PDGF, EGF, and FGF-2 which stimulate proliferation of osteoblast progenitors.
- Also contains TGF-β which increases type I collagen synthesis.
- VEGF and FGF-2 factors in PRP can potentially enhance early angiogenesis and vascularization.

Proposed Mechanism of Action: Platelet gels provide a rich source of growth factors that direct mechanisms involved in bone healing and subsequent osteogenesis. 18

Bone Graft Alternatives

Ceramics
- Calcium Sulfate
- Calcium Phosphate
  - Synthetic Hydroxyapatite
  - Coraline Hydroxyapatite
  - Tricalcium Phosphate (TCP)
- Calcium Phosphate Cement
- Silicon containing BGS

Collagen Based Materials
- Typically composites with ceramic materials

Baxter
Collagen Based

**Characteristics**
- Collagen is the most abundant protein found in bone tissue.
- Osteoconductive matrix with a favorable physical and chemical matrix for bone regeneration.
- Collagen contributes to mineral deposition, vascular ingrowth, and growth factor binding.

**Considerations**
- Animal derived.
- Potential for immunogenicity.
- Weak structural and mechanical properties.
- Collagen is usually coupled with other bone substitutes (HA, β-TCP, bone marrow, etc.).
- Collagen composites have had mixed results.

**Proposed Mechanism of Action:** Primarily acts through osteoconduction and/or via mechanisms from the component that is added to it.

---

Bioactive Materials - attributes

**Bioactive material** — elicits a specific biologic response at the interface of the material, which results in the formation of a bond between the tissue and the material with a strength equal to or greater than bone.

- Interfacial bone bonding occurs because of the biological equivalence of the inorganic portion of bone and the growing HCA (hydroxyl-carbonate apatite) layer on the bioactive implant.

**Osteostimulatory** — Osteostimulation is a property of some bioactive materials to enhance, actively stimulate both the proliferation and differentiation of progenitor cells (e.g., mesenchymal stem cells).

- **Class B Bioactive particulates**: Osteoconductive, i.e., characteristic of bone growth and bonding along a surface.
- **Class A Bioactive particulates**: Osteoconductive and Osteostimulative. Slow resorption of Class A Bioactive particles permits enhanced proliferation and differentiation of osteoprogenitor cells.
CLASS B BIOACTIVE MATERIALS

Ceramic Based: Calcium Sulfate (Plaster of Paris)

Characteristics
• Osteoconductive matrix with long clinical history
• Can be used in the presence of infection
• Resorption profile (5-7 week period) is ideal for antibiotic delivery

Proposed Mechanism of Action: Calcium sulfate serves as an osteoconductive matrix for the ingrowth of osteogenic cells.

Considerations
• Due to dissolution rate, mechanical properties are variable and implantation should be limited to confined defects
• Complications associated with inflammatory reactions have been reported with calcium sulfate
• In-vivo animal studies have shown that quick resorption and inflammatory response may preclude adequate bone formation

CaSO₄
Ceramic Based: β-Tricalcium Phosphate (β-TCP)

**Characteristics**
- Osteoconductive matrix with a long clinical history
- Available as porous or solid, and as granules or blocks
- Undergoes resorption via dissolution and fragmentation over a 6-18 month period

**Considerations**
- Difference in β-TCP resorption rate and new bone formation rate (usually less bone volume produced versus β-TCP volume resorbed)\(^{21}\)
- Brittle and breakable under tension and shear loads\(^{24}\)
- In-vivo animal studies have showed that β-TCP particles formed during dissolution may cause an inflammatory response and bone resorption\(^{26}\)

**Proposed Mechanism of Action:** The resorption of β-TCP stimulates cellular activity\(^{27}\) and bone formation while the β-TCP scaffold provides an osteoconductive matrix.\(^{24}\)

Ceramic Based: Synthetic Hydroxyapatite

**Characteristics**
- Osteoconductive matrix with a long clinical history\(^{24}\)
- Non-sintered HA is readily reabsorbed in-vivo\(^{24}\)
- After bone incorporation and healing, HA implants attain mechanical strength similar to cancellous bone\(^{11}\)

**Considerations**
- HA products have variable bone formation rates depending on crystallinity, density, and stoichiometry\(^{12}\)
- Sintered HA preparations are resistant to in-vivo resorption (1-2% per year)\(^{24}\)
- Due to its brittle nature and slow bone formation, HA is commonly combined with various grafting agents when being used as an osteoconductive bone substitute\(^{12}\)

**Proposed Mechanism of Action:** HA bonds to bone, stimulates cellular activity\(^{27}\), and provides an osteoconductive matrix for subsequent bone formation.\(^{17}\)
Ceramic Based: Coralline Hydroxyapatite

Coral (calcium carbonate) is converted into hydroxyapatite via hydrothermal processing.10

**Characteristics**
- Osteoconductive matrix
- Interconnected porous matrix is structurally similar to cancellous bone
- Pore size encourages bone ingrowth

**Considerations**
- Must be shielded from loading until bone ingrowth occurs.10
- Typically used in non-weight bearing applications such as maxillofacial, periodontal augmentation, and distal radial fractures.24
- Brittle properties make it difficult to shape and handle.24

**Proposed Mechanism of Action:** HA bonds to bone, stimulates cellular activity,27 and the interconnected porous structure provides an osteoconductive matrix for subsequent bone formation.24

Ceramic Based: Calcium Phosphate Cement

**Characteristics**
- Components are mixed with water into a paste, injected into a defect, and then hardens into a porous, hydroxyapatite.24
- Sets into porous HA which is osteoconductive.21
- Conformable paste can custom-fill defects.12
- Sets in the physiological environment without producing heat.21

**Considerations**
- Resorption and remodeling rate occurs over ~2 years.24
- Potential risk of the paste extruding into surrounding soft tissues.12
- Resultant matrix has a small pore size (1 μm) which limits rapid bone ingrowth.21
- To be used in either low or non-load bearing applications or in combination with metal fixation.21

**Proposed Mechanism of Action:** Porous HA that forms bonds to bone, stimulates cellular activity, and provides an osteoconductive matrix for subsequent bone formation.17
CLASS A BIOACTIVE MATERIALS

Ceramic Based: Silicon(Si) containing Bone Graft Substitutes

Bioactive glass \((\text{SiO}_2)_{46.1} (\text{CaO})_{20.3} (\text{Na}_2\text{O})_{24.4} (\text{P}_2\text{O}_5)_{2.6}\)

- There are two types of Bioactive glasses:
  - Sol-gel derived
  - Melt-derived
    - a specific composition of melt-derived bioactive glass, called Bioglass is used as a bone filling material.\(^{23}\)

- Existing products:
  - 45S5 Bioglass\(^ {23}\)
  - 45S5 Bioglass + Bovine Type I Collagen + β-TCP \(^ {28}\)

Silicate Substituted HA (new class): Several methods for the synthesis have been reported including solgel, hydrothermal, solid-state reaction, chemical precipitation, and crystallization. Chemical precipitation and crystallization are the most commonly used methods.

- Existing products:
  - Si-HA \((\text{Ca})_{10}(\text{PO}_4)_{(6-x)}(\text{SiO}_4)_x(\text{OH})_{(2-x)}\) \(^ {9}\)
Ceramic Based: 4S5S Bioglass

**Characteristics**
- In a physiologic environment in-vitro, Bioglass induces formation of an HCA layer\textsuperscript{23}.
- Osteostimulatory - Stimulation of osteoblast proliferation and differentiation during in vitro osteoblast cell culture studies, as evidenced by increased DNA content and elevated osteocalcin and alkaline phosphatase levels.\textsuperscript{30}
- Particles of 100 µm in diameter have been found to either absorb or are phagocytized by macrophages in vivo\textsuperscript{23}.

**Considerations**
- Osteoblast apoptosis has been identified in vitro on the bioactive substrate, possibly due to high Si release.\textsuperscript{30,33}
- Pores have been introduced into melt-derived bioactive glasses but the pores are few in number and interconnectivity is limited\textsuperscript{32}
- Particles of 100 µm in diameter have been found to either absorb or are phagocytized by macrophages in vivo\textsuperscript{23}.

**Proposed Mechanism of Action:** Osteoconductive matrix. In vitro assays have revealed genes associated with osteoblast growth and differentiation, maintenance of extracellular matrix, and promotion of cell-cell and cell-matrix adhesion were up-regulated in vitro by conditioned cell culture media containing the dissolution products of bioactive glass.\textsuperscript{30,34}

\[
\text{SiO}_2 \cdot 46.1 \quad \text{CaO} \cdot 26.9 \quad \text{Na}_2\text{O} \cdot 24.4 \quad \text{P}_2\text{O}_5 \cdot 2.6
\]

### Ceramic Based: 4S5S Bioglass + Bovine Type I Collagen + β-TCP

**Characteristics**
- See 45S5 slide for Bioglass shared characteristics.
- β-TCP and 45S5 have a long clinical history as osteoconductive matrices\textsuperscript{24}.
- (β-TCP) scaffold is engineered to mimic the structure of cancellous bone.\textsuperscript{28}
- Animal testing resulted in bone that was 23% and 30% Stronger at 12 and 24 weeks respectively, compared to just Collagen + β-TCP.\textsuperscript{28}

**Considerations**
- in a rabbit model, healing of defect sites were nearly identical to adjacent cancellous bone after 24 weeks but also between Collagen + β-TCP and Collagen + β-TCP + Bioglass.\textsuperscript{28}
- Tissue analysis showed an increase in new bone formation in the Collagen + β-TCP + Bioglass group vs Collagen + β-TCP at 12 & 24 weeks, however, the results were not statistically significant.\textsuperscript{28}
- Animal studies have showed that β-TCP particles formed during dissolution may cause an inflammatory response and bone resorption\textsuperscript{26}

**Proposed Mechanism of Action:** Osteoconductive matrix. Per in-vitro studies, ionic constituents are released into the local environment\textsuperscript{28} stimulating osteoblasts\textsuperscript{35,36} to the scaffold surface\textsuperscript{35}, increasing osteoblast proliferation\textsuperscript{35} and protein production\textsuperscript{35}, resulting in increasing the rate of bone formation.\textsuperscript{28}
Ceramic Based: Synthetic Si substituted Calcium Phosphate

Characteristics

- Osteostimulatory - cell culture studies demonstrated that the time to new apatite surface layer formation was reduced by 29% when compared to an identical calcium phosphate material that did not contain 0.8wt% silicate.37
- Structure and chemistry permits adsorption of extracellular proteins critical in promoting osteogenic cell attachment6
- In vitro, increased strut microporosity permitted capillary penetration at earlier time points, promoting apposition of greater volumes of dense, new bone.28

Proposed Mechanism of Action: In vitro, the interconnected porous structure and silicate substituted chemistry demonstrates adsorption of key proteins6, osteoblasts7 and mesenchymal stem cells8; as well as differentiation of mesenchymal stem cells to an osteogenic cell line.17 Vascularized bone is formed that can be resorbed via cell-mediated remodeling.9

Considerations

- The bioactive and osteostimulatory nature have not been correlated with human clinical experience.37
- Limited to non-load bearing applications or in combination with metal fixation37
- Si release has not been measured39
- Not indicated to be mixed with autograft bone37

Summary

<table>
<thead>
<tr>
<th>Graft</th>
<th>Overview</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autograft</td>
<td>Osteogenic, Osteoconductive, Osteoinductive4</td>
</tr>
<tr>
<td>Allograft</td>
<td>Cortical - Osteoconductive (moderately)41</td>
</tr>
<tr>
<td></td>
<td>Osteoinductive (weakly)41</td>
</tr>
<tr>
<td></td>
<td>Cancellous - Osteoconductive (weakly)41</td>
</tr>
<tr>
<td>DBM</td>
<td>Osteoconductive, Osteoinductive (variable)18</td>
</tr>
<tr>
<td>BMP</td>
<td>Osteoinductive18</td>
</tr>
<tr>
<td>BMA</td>
<td>Osteogenic, Osteoinductive18</td>
</tr>
<tr>
<td>Collagen</td>
<td>Osteoconductive27</td>
</tr>
<tr>
<td>Class B Bioactive:</td>
<td>Osteoconductive27</td>
</tr>
<tr>
<td>Ceramics</td>
<td></td>
</tr>
<tr>
<td>Class A Bioactive:</td>
<td>Osteoconductive18, Osteostimulatory23</td>
</tr>
<tr>
<td>(Bioglass and Si</td>
<td></td>
</tr>
<tr>
<td>substituted HA) Si</td>
<td></td>
</tr>
<tr>
<td>containing BGS</td>
<td></td>
</tr>
</tbody>
</table>
Choices

So what material do we use where?

• Selection should be based on reasoned burdens of proof. These include examination of the product claims and whether they are supported by preclinical and human studies in site-specific locations where they are to be utilized in surgery. It is imperative to appreciate the level of evidence claimed in the latter studies.

• Different healing environments (e.g. metaphyseal defect, long bone fracture, segmental diaphyseal defect, interbody spine fusion, posterolateral spine fusion) may have differing levels of difficulty in forming new bone.

• Validation of any bone graft substitute in one clinical anatomic site may not be predictive of its performance in another location.

• Currently marketed products are variable in their composition and their claimed mechanisms of action. It is reasonable that not all bone-graft substitute products will perform the same.

References

References

37. Actifuse 510K  K090850
39. Actifuse IFU. Baxter Biosurgery 2009
41. BONE-GRAFT SUBSTITUTES:FACTS, FICTIONS & APPLICATIONS AMERICAN ACADEMY OF ORTHOPAEDIC SURGEONS 75TH ANNUAL MEETING 2008

Baxter is a registered trademark of Baxter International Inc
All other products or trademarks appearing herein are the property of their respective owners