Biology of Bone Repair

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Types of Bone

• Lamellar Bone
  – Collagen fibers arranged in parallel layers
  – Normal adult bone

• Woven Bone (non-lamellar)
  – Randomly oriented collagen fibers
  – In adults, seen at sites of fracture healing, tendon or ligament attachment and in pathological conditions
Lamellar Bone

- Cortical bone
  - Comprised of osteons (Haversian systems)
  - Osteons communicate with medullary cavity by Volkmann’s canals

Picture courtesy Gwen Childs, PhD.
Haversian System

osteocyte

osteon

Haversian canal

Volkmann’s canal

Picture courtesy Gwen Childs, PhD.
Lamellar Bone

- Cancellous bone (trabecular or spongy bone)
  - Bony struts (trabeculae) that are oriented in direction of the greatest stress
Woven Bone

- Coarse with random orientation
- Weaker than lamellar bone
- Normally remodeled to lamellar bone

Figure from Rockwood and Green’s: Fractures in Adults, 4th ed
Bone Composition

- **Cells**
  - Osteocytes
  - Osteoblasts
  - Osteoclasts

- **Extracellular Matrix**
  - Organic (35%)
    - Collagen (type I) 90%
    - Osteocalcin, osteonectin, proteoglycans, glycosaminoglycans, lipids (ground substance)
  - Inorganic (65%)
    - Primarily hydroxyapatite $\text{Ca}_5(\text{PO}_4)_3(\text{OH})_2$
Osteoblasts

- Derived from mesenchymal stem cells
- Line the surface of the bone and produce osteoid
- Immediate precursor is fibroblast-like preosteoblasts

Picture courtesy Gwen Childs, PhD.
Osteocytes

- Osteoblasts surrounded by bone matrix
  - trapped in lacunae
- Function poorly understood
  - regulating bone metabolism in response to stress and strain

Picture courtesy Gwen Childs, PhD.
Osteocyte Network

• Osteocyte lacunae are connected by canaliculi
• Osteocytes are interconnected by long cell processes that project through the canaliculi
• Preosteoblasts also have connections via canaliculi with the osteocytes
• Network probably facilitates response of bone to mechanical and chemical factors
Osteoclasts

- Derived from hematopoietic stem cells (monocyte precursor cells)
- Multinucleated cells whose function is bone resorption
- Reside in bone resorption pits (Howship’s lacunae)
- Parathyroid hormone stimulates receptors on osteoblasts that activate osteoclastic bone resorption

Picture courtesy Gwen Childs, PhD.
Components of Bone Formation

- Cortex
- Periosteum
- Bone marrow
- Soft tissue
Prerequisites for Bone Healing

• Adequate blood supply
• Adequate mechanical stability
Mechanisms of Bone Formation

• Cutting Cones
• Intramembranous Bone Formation
• Endochondral Bone Formation
Cutting Cones

- Primarily a mechanism to remodel bone
- Osteoclasts at the front of the cutting cone remove bone
- Trailing osteoblasts lay down new bone

Courtesy Drs. Charles Schwab and Bruce Martin
Intramembranous (Periosteal) Bone Formation

- Mechanism by which a long bone grows in width
- Osteoblasts differentiate directly from preosteoblasts and lay down seams of osteoid
- Does NOT involve cartilage anlage
Intramembranous Bone Formation

Picture courtesy Gwen Childs, PhD.
Endochondral Bone Formation

- Mechanism by which a long bone grows in length
- Osteoblasts line a cartilage precursor
- The chondrocytes hypertrophy, degenerate and calcify (area of low oxygen tension)
- Vascular invasion of the cartilage occurs followed by ossification (increasing oxygen tension)
Endochondral Bone Formation

Picture courtesy Gwen Childs, PhD.
Blood Supply

- Long bones have three blood supplies
  - Nutrient artery (intramedullary)
  - Periosteal vessels
  - Metaphyseal vessels

Figure adapted from Rockwood and Green, 5th Ed
Nutrient Artery

• Normally the major blood supply for the diaphyseal cortex (80 to 85%)
• Enters the long bone via a nutrient foramen
• Forms medullary arteries up and down the bone
Periosteal Vessels

• Arise from the capillary-rich periosteum
• Supply outer 15 to 20% of cortex normally
• Capable of supplying a much greater proportion of the cortex in the event of injury to the medullary blood supply
Metaphyseal Vessels

- Arise from periarticular vessels
- Penetrate the thin cortex in the metaphyseal region and anastomose with the medullary blood supply
Vascular Response in Fracture Repair

• Fracture stimulates the release of growth factors that promote angiogenesis and vasodilation

• Blood flow is increased substantially to the fracture site
  – Peaks at two weeks after fracture
Mechanical Stability

- Early stability promotes revascularization
- After first month, loading and interfragmentary motion promotes greater callus formation
Mechanical Stability

- Mechanical load and small displacements at the fracture site stimulate healing.
- Inadequate stabilization may result in excessive deformation at the fracture site interrupting tissue differentiation to bone (soft callus).
- Over-stabilization, however, reduces periosteal bone formation (hard callus).
Stages of Fracture Healing

- Inflammation
- Repair
- Remodeling
Inflammation

• Tissue disruption results in hematoma at the fracture site
• Local vessels thrombose causing bony necrosis at the edges of the fracture
• Increased capillary permeability results in a local inflammatory milieu
  – Osteoinductive growth factors stimulate the proliferation and differentiation of mesenchymal stem cells
Repair

• Periosteal callus forms along the periphery of the fracture site
  – Intramembranous ossification initiated by preosteoblasts

• Intramedullary callus forms in the center of the fracture site
  – Endochondral ossification at the site of the fracture hematoma

• Chemical and mechanical factors stimulate callus formation and mineralization
Repair

Figure from Brighton, et al, JBJS-A, 1991.
Remodeling

- Woven bone is gradually converted to lamellar bone
- Medullary cavity is reconstituted
- Bone is restructured in response to stress and strain (Wolff’s Law)
Mechanisms for Bone Healing

• Direct (primary) bone healing
• Indirect (secondary) bone healing
Direct Bone Healing

- Mechanism of bone healing seen when there is no motion at the fracture site (i.e. absolute stability)
- Does not involve formation of fracture callus
- Osteoblasts originate from endothelial and perivascular cells
Direct Bone Healing

- A cutting cone is formed that crosses the fracture site
- Osteoblasts lay down lamellar bone behind the osteoclasts forming a secondary osteon
- Gradually the fracture is healed by the formation of numerous secondary osteons
- A slow process – months to years
Components of Direct Bone Healing

• Contact Healing
  – Direct contact between the fracture ends allows healing to be with lamellar bone immediately

• Gap Healing
  – Gaps less than 200-500 microns are primarily filled with woven bone that is subsequently remodeled into lamellar bone
  – Larger gaps are healed by indirect bone healing (partially filled with fibrous tissue that undergoes secondary ossification)
Direct Bone Healing

Figure from http://www.vetmed.ufl.edu/sacs/notes
Indirect Bone Healing

• Mechanism for healing in fractures that have some motion, but not enough to disrupt the healing process.
• Bridging periosteal (soft) callus and medullary (hard) callus re-establish structural continuity
• Callus subsequently undergoes endochondral ossification
• Process fairly rapid - weeks
Local Regulation of Bone Healing

- Growth factors
- Cytokines
- Prostaglandins/Leukotrienes
- Hormones
- Growth factor antagonists
Growth Factors

- Transforming growth factor
- Bone morphogenetic proteins
- Fibroblast growth factors
- Platelet-derived growth factors
- Insulin-like growth factors
Transforming Growth Factor

- Super-family of growth factors (~34 members)
- Acts on serine/threonine kinase cell wall receptors
- Promotes proliferation and differentiation of mesenchymal precursors for osteoblasts, osteoclasts and chondrocytes
- Stimulates both endochondral and intramembranous bone formation
  - Induces synthesis of cartilage-specific proteoglycans and type II collagen
  - Stimulates collagen synthesis by osteoblasts
Bone Morphogenetic Proteins

• Osteoinductive proteins initially isolated from demineralized bone matrix
  – Proven by bone formation in heterotopic muscle pouch

• Induce cell differentiation
  – BMP-3 (osteogenin) is an extremely potent inducer of mesenchymal tissue differentiation into bone

• Promote endochondral ossification
  – BMP-2 and BMP-7 induce endochondral bone formation in segmental defects

• Regulate extracellular matrix production
  – BMP-1 is an enzyme that cleaves the carboxy termini of procollagens I, II and III
Bone Morphogenetic Proteins

- These are included in the TGF-β family
  - Except BMP-1
- Sixteen different BMP’s have been identified
- BMP2-7,9 are osteoinductive
- BMP2,6, & 9 may be the most potent in osteoblastic differentiation
  - Involved in progenitor cell transformation to pre-osteoblasts
- Work through the intracellular Smad pathway
- Follow a dose/response ratio
### Timing and Function of Growth Factors

Table 2. Temporal and functional characteristics of members of the TGF-β superfamily observed during fracture healing in animal models

<table>
<thead>
<tr>
<th>Member of the TGF-β superfamily</th>
<th>Time of expression</th>
<th>Specific responses in vivo and in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDF-8</td>
<td>Restricted to day 1(^{20})</td>
<td>Potential function as a negative regulator of skeletal muscle growth(^{20})</td>
</tr>
<tr>
<td>BMP-2</td>
<td>Days 1–2(^{10,20}) (the earliest gene to be induced and second elevation during osteogenesis)</td>
<td>Recruitment of mesenchymal cells Chondrogenesis May initiate the fracture healing cascade and regulate the expression of other BMPs BMP-2, -6, -9 may be the most potent to induce osteoblast lineage-specific differentiation of MSCs(^{19})</td>
</tr>
<tr>
<td>BMP-3, -8</td>
<td>Days 14–21(^{10}) (restricted expression during osteogenesis)</td>
<td>Temporal data suggest a role in the regulation of osteogenesis</td>
</tr>
<tr>
<td>BMP-4</td>
<td>Transient increased expression in the surrounding soft tissues 6 h to day 5(^{9}) Days 14–21(^{10}) Through out fracture healing(^{10})</td>
<td>Involvement in the formation of callus at a very early stage in the healing process In vitro: BMP-3 and -4 stimulate the migration of human blood monocytes(^{61})</td>
</tr>
<tr>
<td>BMP-7</td>
<td>Days 14–21(^{10}) From the early stages of fracture healing(^{9})</td>
<td>Regulatory role in both types of ossification In vitro: stimulation of relative mature osteoblasts(^{19})</td>
</tr>
<tr>
<td>GDF-10, BMP-5, -6</td>
<td>Days 3–21(^{20})</td>
<td>Regulatory role in both types of ossification BMP-6 may initiate chondrocyte maturation(^{19})</td>
</tr>
<tr>
<td>GDF-5, 1</td>
<td>Day 7 (maximal) to day 14(^{10}) (restricted expression during chondrogenic phase) GDF-1 at extremely low levels</td>
<td>GDF-5 an exclusive involvement in chondrogenesis is suggested Stimulation of mesenchymal aggregation and induction of angiogenesis through chemotaxis of endothelial cells and degradation of matrix proteins</td>
</tr>
<tr>
<td>GDF-3, GDF-6, 9</td>
<td>No detectable levels within the fracture callus(^{20})</td>
<td>GDF-6 may be expressed only in articular cartilage(^{20}) and with GDF-5, 7 more efficiently induce cartilage and tendon-like structures in vivo(^{19})</td>
</tr>
<tr>
<td>TGF-β1, -β2, -β3</td>
<td>Days 1–21(^{10}) Days 3–14(^{10}) Days 3–21(^{10})</td>
<td>Potent chemotactic for bone forming cells and macrophages Proliferation of undifferentiated mesenchymal and osteoprogenitor cells, osteoblasts, chondrocytes</td>
</tr>
</tbody>
</table>

Table from Dimitriou, et al., Injury, 2005
Clinical Use of BMP’s

- Used at doses between 10x & 1000x native levels
- Negligible risk of excessive bone formation
- rhBMP-2 used in “fresh” open fractures to enhance healing and reduce need for secondary procedures after unreamed IM nailing
  - BESTT study also had a lower infection rate in Type IIIA & B open fractures with application of rhBMP-2
- BMP-7 approved for use in recalcitrant nonunions in patients for whom autografting is not a good option (i.e. medically unstable, previous harvesting of all iliac crest sites, etc.)
BMP Future Directions

• BMP-2
  – Increased fusion rate in spinal fusion
• BMP-7 equally effective as ICBG in nonunions (small series: need larger studies)
• Must be applied locally because of rapid systemic clearance
• ? Effectiveness in acute fractures
• ? Increased wound healing in open injuries
• Protein therapy vs. gene therapy
• Credibility of researchers compromised
BMP Antagonists

• May have important role in bone formation
• Noggin
  – Extra-cellular inhibitor
  – Competes with BMP-2 for receptors
• BMP-13 found to limit differentiation of mesenchymal stromal cells
  – Inhibits osteogenic differentiation
Fibroblast Growth Factors

- Both acidic (FGF-1) and basic (FGF-2) forms
- Increase proliferation of chondrocytes and osteoblasts
- Enhance callus formation
- FGF-2 stimulates angiogenesis
Platelet-Derived Growth Factor

• A dimer of the products of two genes, PDGF-A and PDGF-B
  – PDGF-BB and PDGF-AB are the predominant forms found in the circulation
• Stimulates bone cell growth
• Mitogen for cells of mesenchymal origin
• Increases type I collagen synthesis by increasing the number of osteoblasts
• PDGF-BB stimulates bone resorption by increasing the number of osteoclasts
Insulin-like Growth Factor

- Two types: IGF-I and IGF-II
  - Synthesized by multiple tissues
  - IGF-I production in the liver is stimulated by Growth Hormone
- Stimulates bone collagen and matrix synthesis
- Stimulates replication of osteoblasts
- Inhibits bone collagen degradation
Cytokines

- Interleukin-1, -4, -6, -11, macrophage and granulocyte/macrophage (GM) colony-stimulating factors (CSFs) and Tumor Necrosis Factor
- Stimulate bone resorption
  - IL-1 is the most potent
- IL-1 and IL-6 synthesis is decreased by estrogen
  - May be mechanism for post-menopausal bone resorption
- Peak during 1st 24 hours then again during remodeling
- Regulate endochondral bone formation
# Specific Factor Stimulation of Osteoblasts and Osteoclasts

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Bone Formation</th>
<th>Bone Resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>TNF-α</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>TNF-β</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>TGF-α</td>
<td>--</td>
<td>+++</td>
</tr>
<tr>
<td>TGF-β</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>PDGF</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>IGF-1</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>IGF-2</td>
<td>+++</td>
<td>0</td>
</tr>
<tr>
<td>FGF</td>
<td>+++</td>
<td>0</td>
</tr>
</tbody>
</table>
Prostaglandins / Leukotrienes

- Effect on bone resorption is species dependent and their overall effects in humans unknown
- Prostaglandins of the E series
  - Stimulate osteoblastic bone formation
  - Inhibit activity of isolated osteoclasts
- Leukotrienes
  - Stimulate osteoblastic bone formation
  - Enhance the capacity of isolated osteoclasts to form resorption pits
Hormones

• Estrogen
  – Stimulates fracture healing through receptor mediated mechanism
  – Modulates release of a specific inhibitor of IL-1

• Thyroid hormones
  – Thyroxine and triiodothyronine stimulate osteoclastic bone resorption

• Glucocorticoids
  – Inhibit calcium absorption from the gut causing increased PTH and therefore increased osteoclastic bone resorption
Hormones (cont.)

- **Parathyroid Hormone**
  - Intermittent exposure stimulates
    - Osteoblasts
    - Increased bone formation

- **Growth Hormone**
  - Mediated through IGF-1 (Somatomedin-C)
  - Increases callus formation and fracture strength
Vascular Factors

• Metalloproteininases
  – Degrade cartilage and bones to allow invasion of vessels

• Angiogenic factors
  – Vascular-endothelial growth factors
    • Mediate neo-angiogenesis & endothelial-cell specific mitogens
  – Angiopoietin (1&2)
    • Regulate formation of larger vessels and branches
Local Anatomic Factors That Influence Fracture Healing

- Soft tissue injury
- Interruption of local blood supply
- Interposition of soft tissue at fracture site
- Bone death caused by radiation, thermal or chemical burns or infection
Systemic Factors That Decrease Fracture Healing

• Malnutrition
  – Reduces activity and proliferation of osteochondral cells
  – Decreased callus formation

• Smoking
  – Cigarette smoke inhibits osteoblasts
  – Nicotine causes vasoconstriction diminishing blood flow at fracture site

• Diabetes Mellitus
  – Associated with collagen defects including decreased collagen content, defective cross-linking and alterations in collagen sub-type ratios

• Anti-Inflammatory Medications
  – Cause (at least a temporary) reduction in bone healing
Electromagnetic Field

- Electromagnetic (EM) devices are based on Wolff’s Law that bone responds to mechanical stress: In vitro bone deformation produces piezoelectric currents and streaming potentials.
- Exogenous EM fields may stimulate bone growth and repair by the same mechanism.
- Clinical efficacy very controversial
  - No studies have shown PEMF to be effective in “gap healing” or pseudarthrosis.
Types of EM Devices

- Microamperes
- Direct electrical current
- Capacitively coupled electric fields
- Pulsed electromagnetic fields (PEMF)
PEMF

• Approved by the FDA for the treatment of non-unions
• Efficacy of bone stimulation appears to be frequency dependant
  – Extremely low frequency (ELF) sinusoidal electric fields in the physiologic range are most effective (15 to 30 Hz range)
  – Specifically, PEMF signals in the 20 to 30 Hz range (postural muscle activity) appear more effective than those below 10 Hz (walking)
Ultrasound

• Low-intensity ultrasound is approved by the FDA for stimulating healing of fresh fractures
• Modulates signal transduction, increases gene expression, increases blood flow, enhances bone remodeling and increases callus torsional strength in animal models
Ultrasound

• Human clinical trials show a decreased time of healing in fresh fractures treated nonoperatively
  – Four level 1 studies show a decrease in healing time up to 38%

• Has also been shown to decrease the healing time in smokers potentially reversing the ill effects of smoking
Summary

• Fracture healing is influenced by many variables including mechanical stability, electrical environment, biochemical factors and blood flow

• Our ability to enhance fracture healing will increase as we better understand the interaction between these variables

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