Biology of Bone Repair

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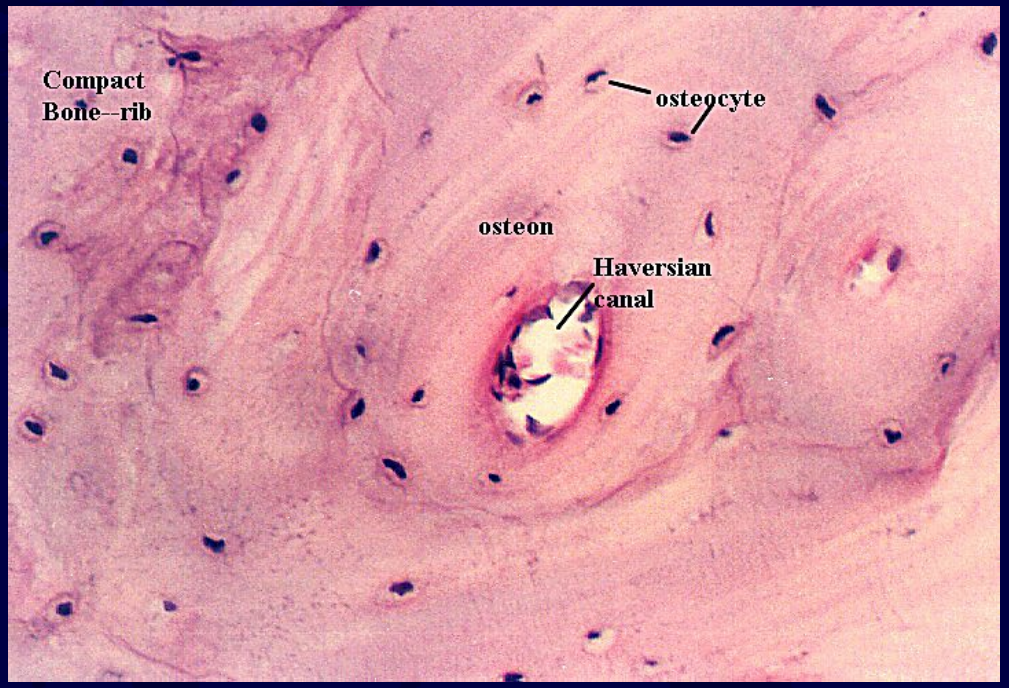
Types of Bone

- 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

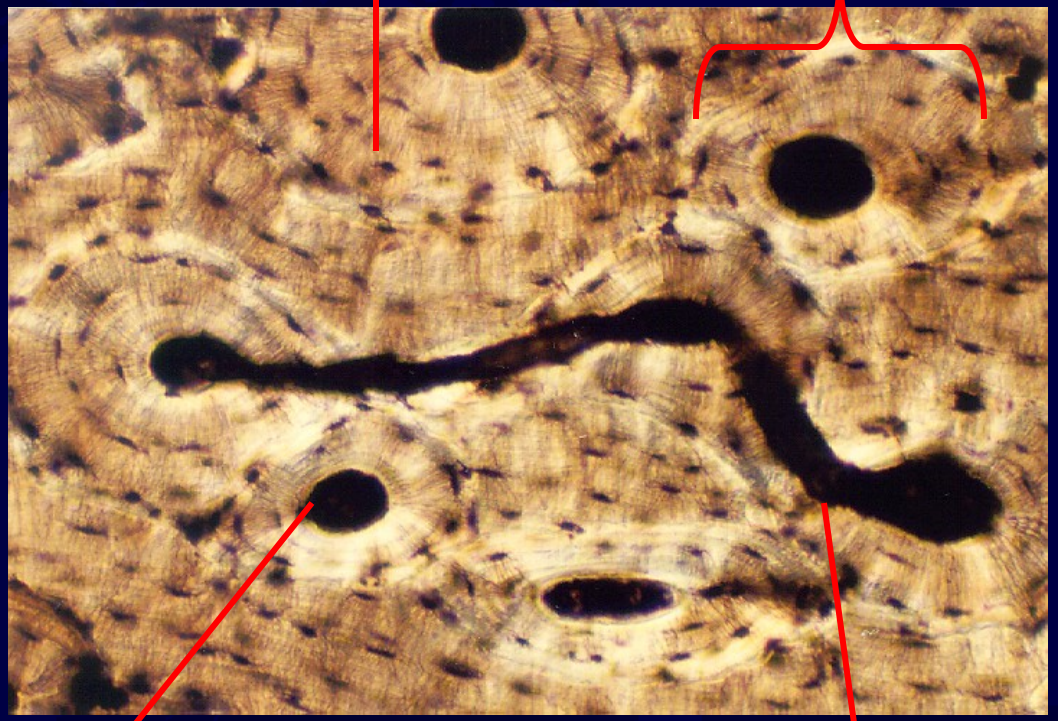
Lamellar Bone

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



Picture courtesy Gwen Childs, PhD.

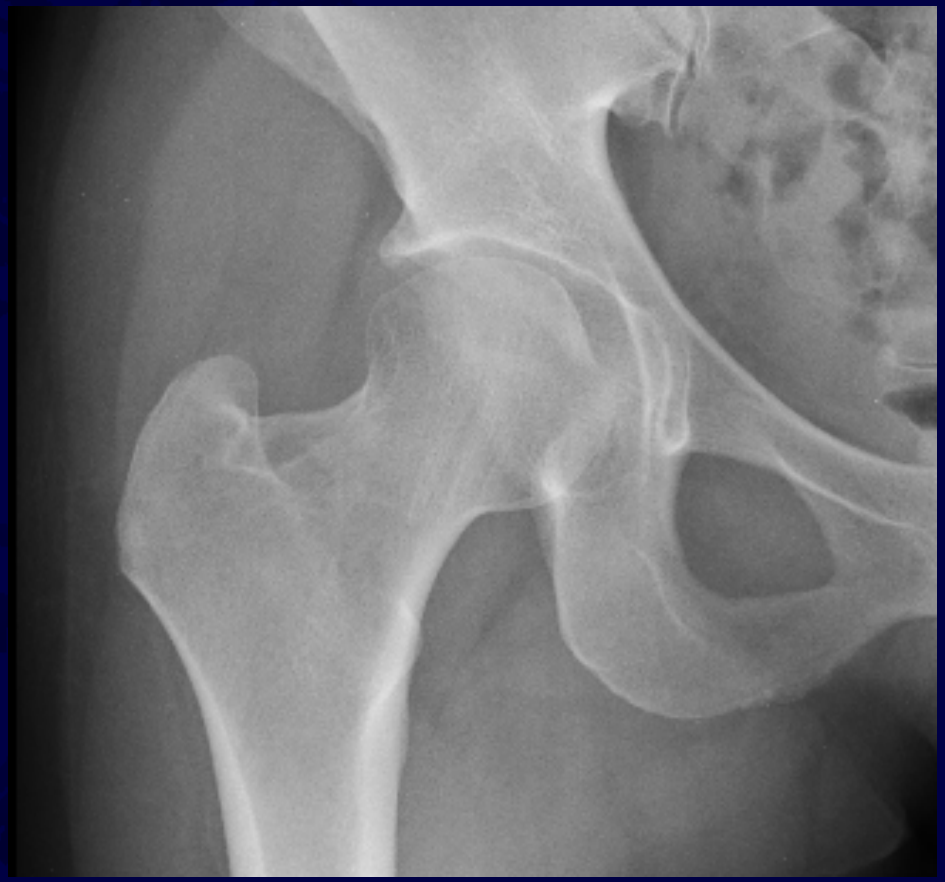
Haversian System



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

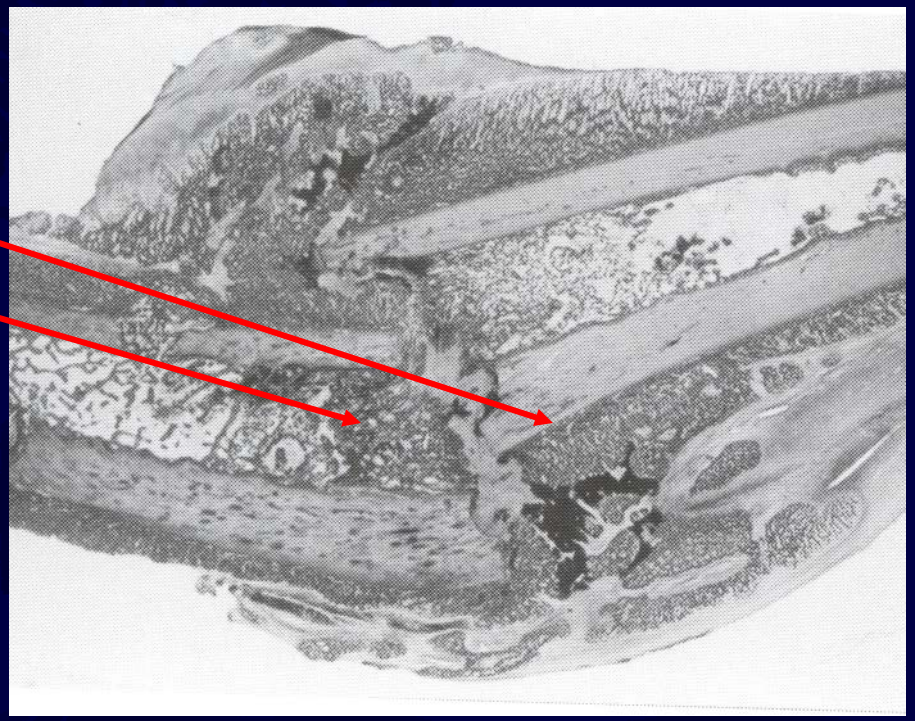


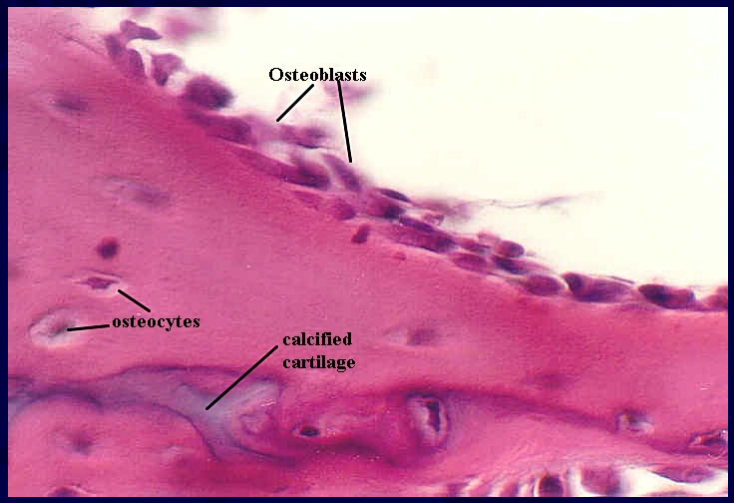
Figure from Rockwood and Green's: Fractures in Adults, 4th ed

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 $Ca_5(PO_4)_3(OH)_2$

Osteoblasts

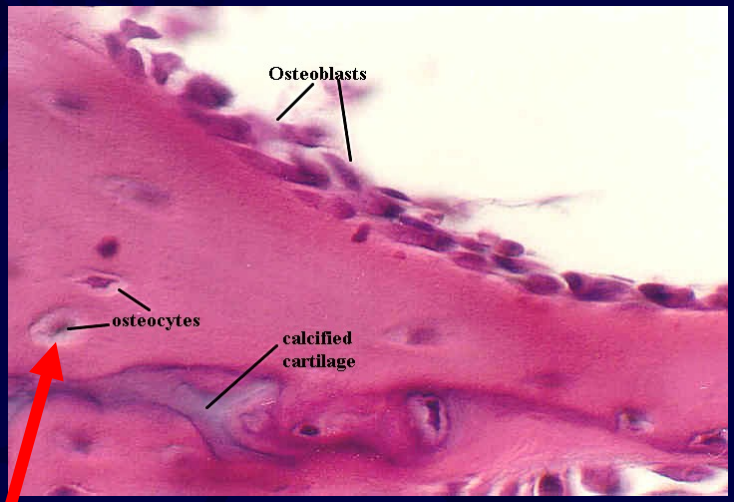
- Derived from mesenchymal stem cells
- Line the surface of the bone and produce osteoid
- Immediate precursor is fibroblast-like preosteoblasts



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



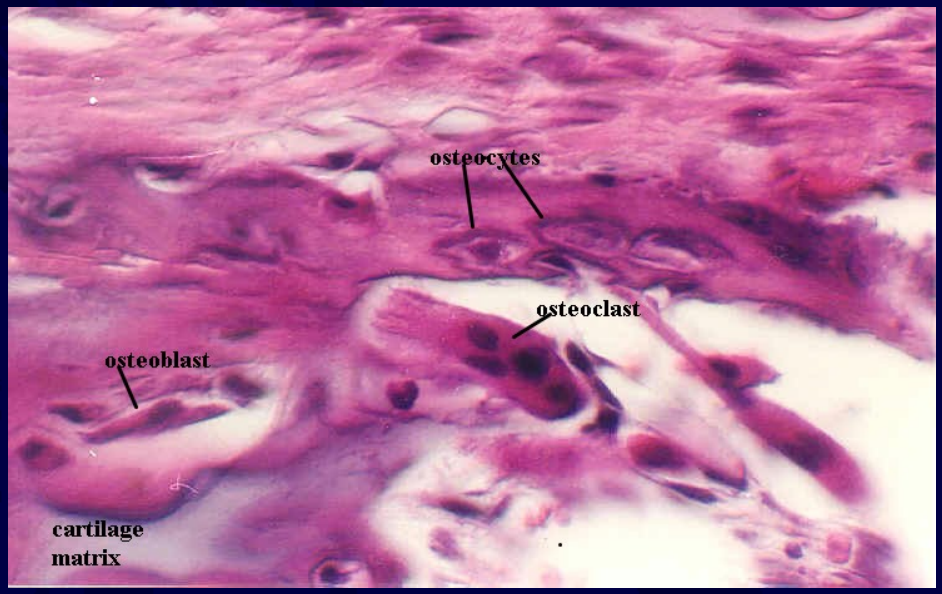
Picture courtesy Gwen Childs, PhD.

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

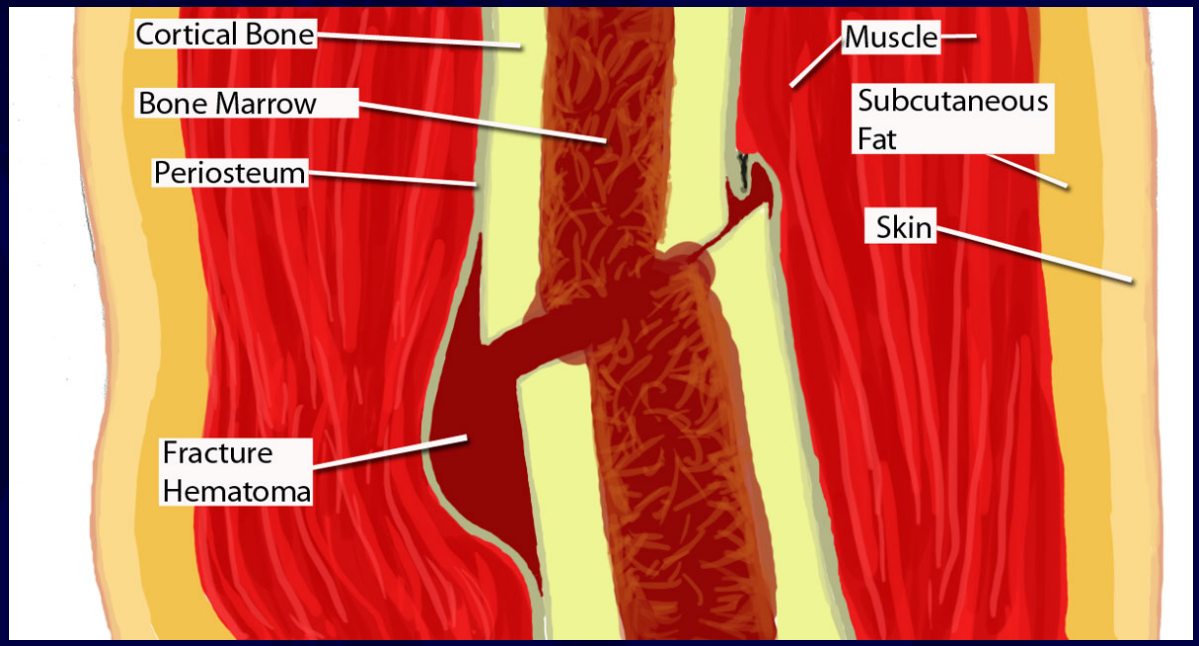
- Derived from hematopoietic stem cells (monocyte precursor cells)
- Multinucleated cells whose function is bone resorption
- Reside in bone resorption pits (Howship's lacunae)
- Parathyroid hormone stimulates receptors on osteoblasts that activate osteoclastic bone resorption



Picture courtesy Gwen Childs, PhD.

Components of Bone Formation

- Cortex
- Periosteum
- Bone marrow
- Soft tissue



Prerequisites for Bone Healing

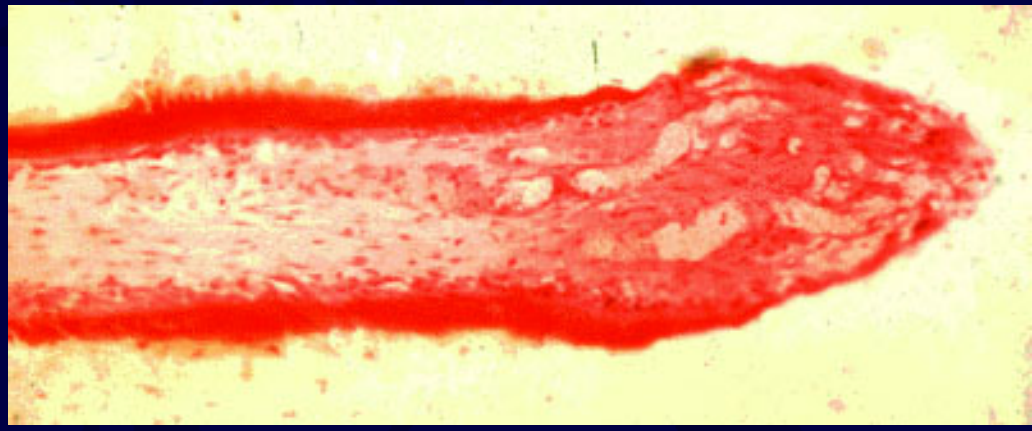
- Adequate blood supply
- Adequate mechanical stability

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

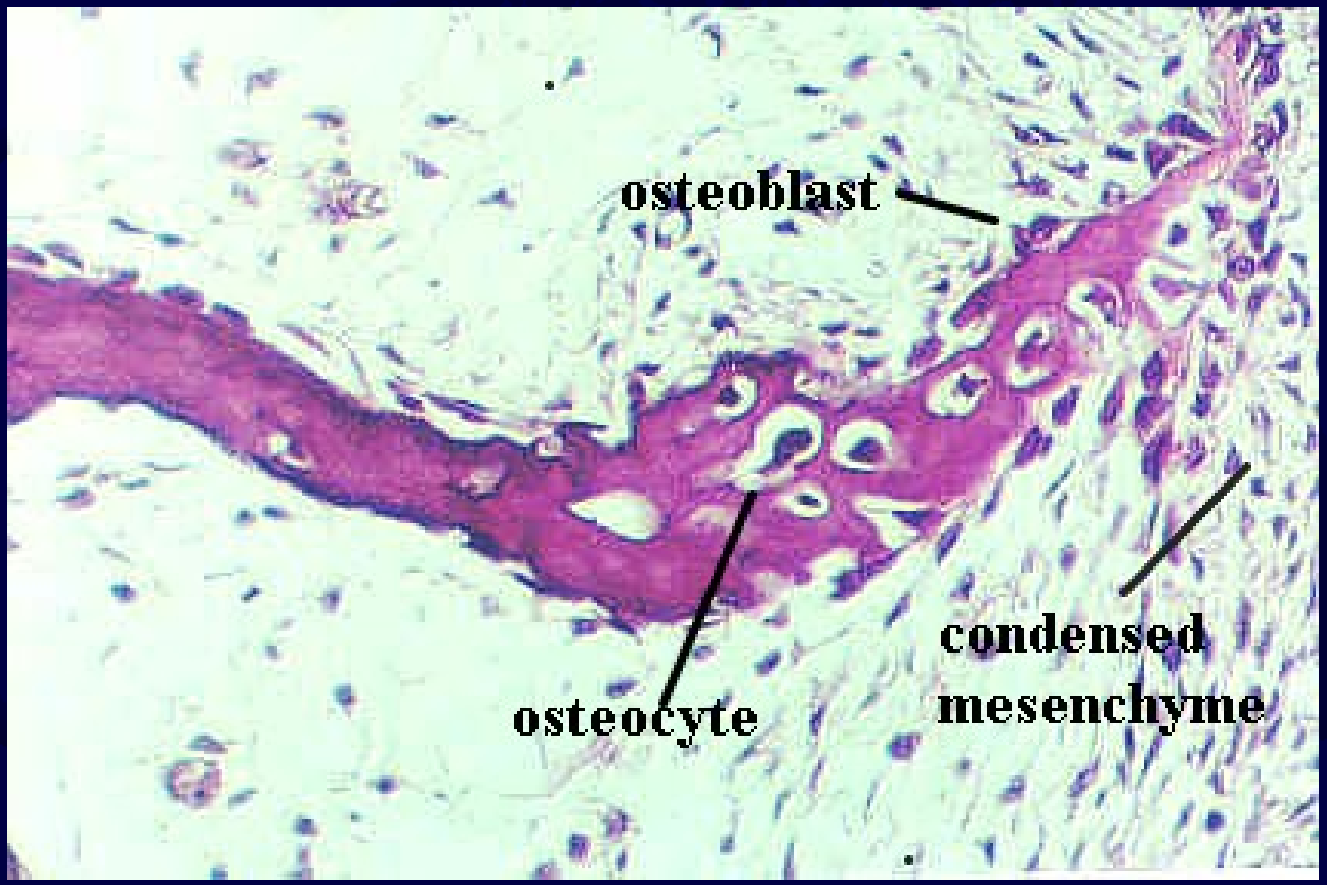


Courtesy Drs. Charles Schwab and Bruce Martin

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

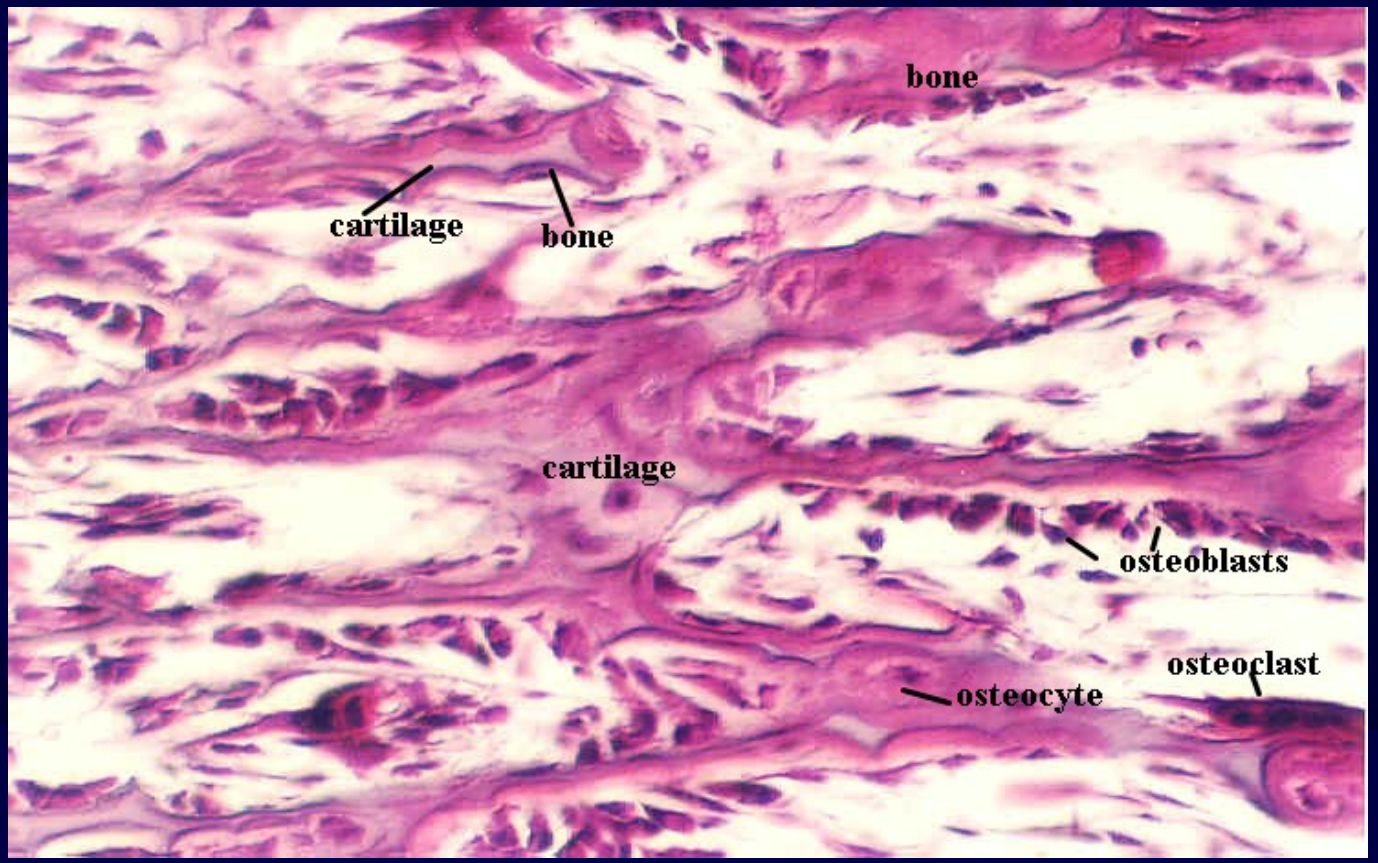


Picture courtesy Gwen Childs, PhD.

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

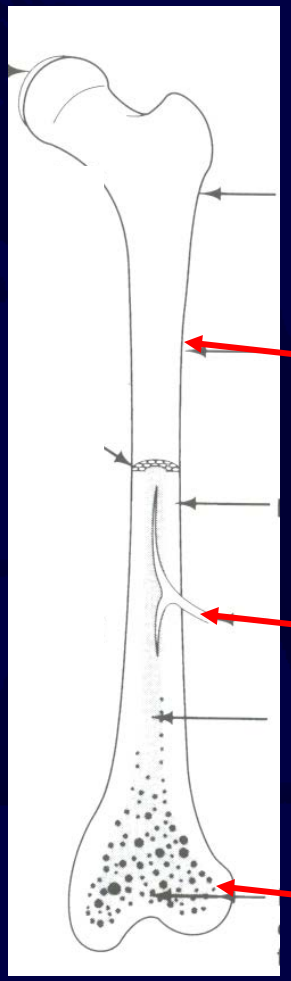


Figure adapted from Rockwood and Green, 5th Ed

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

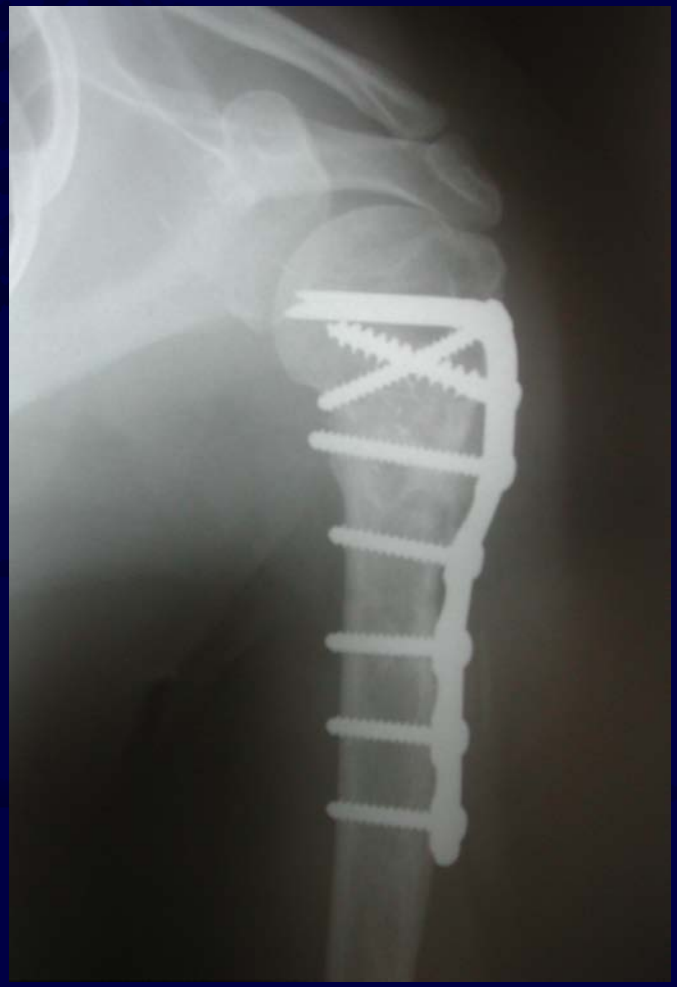
- Arise from periarticular vessels
- Penetrate the thin cortex in the metaphyseal region and anastomose with the medullary blood supply

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

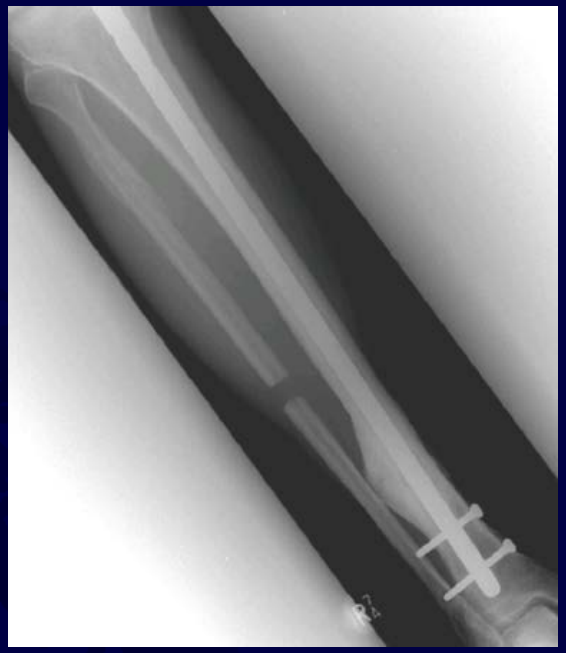
- Inflammation
- Repair
- Remodeling

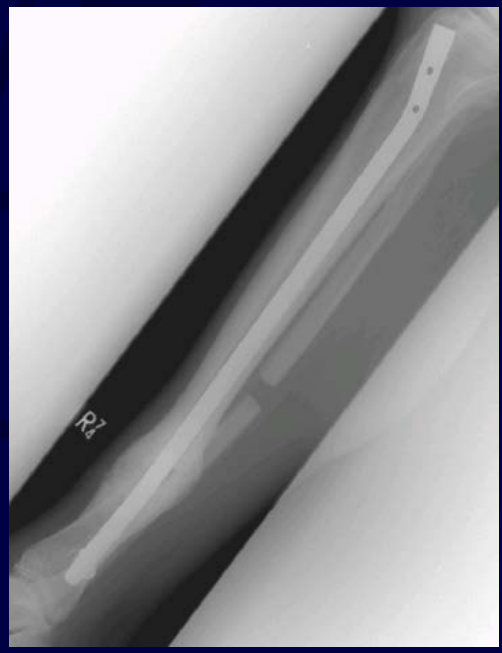
Inflammation

- 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

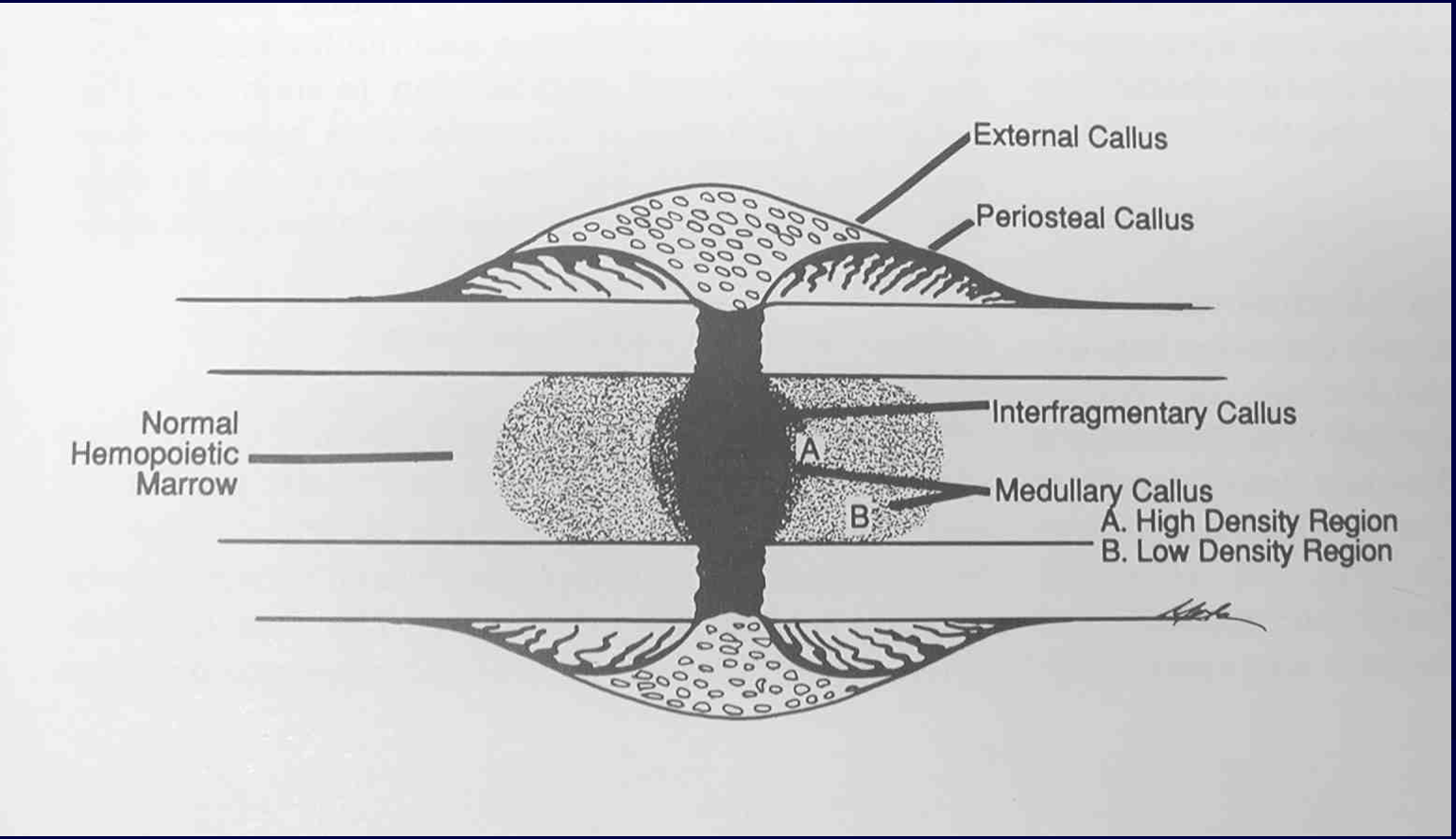
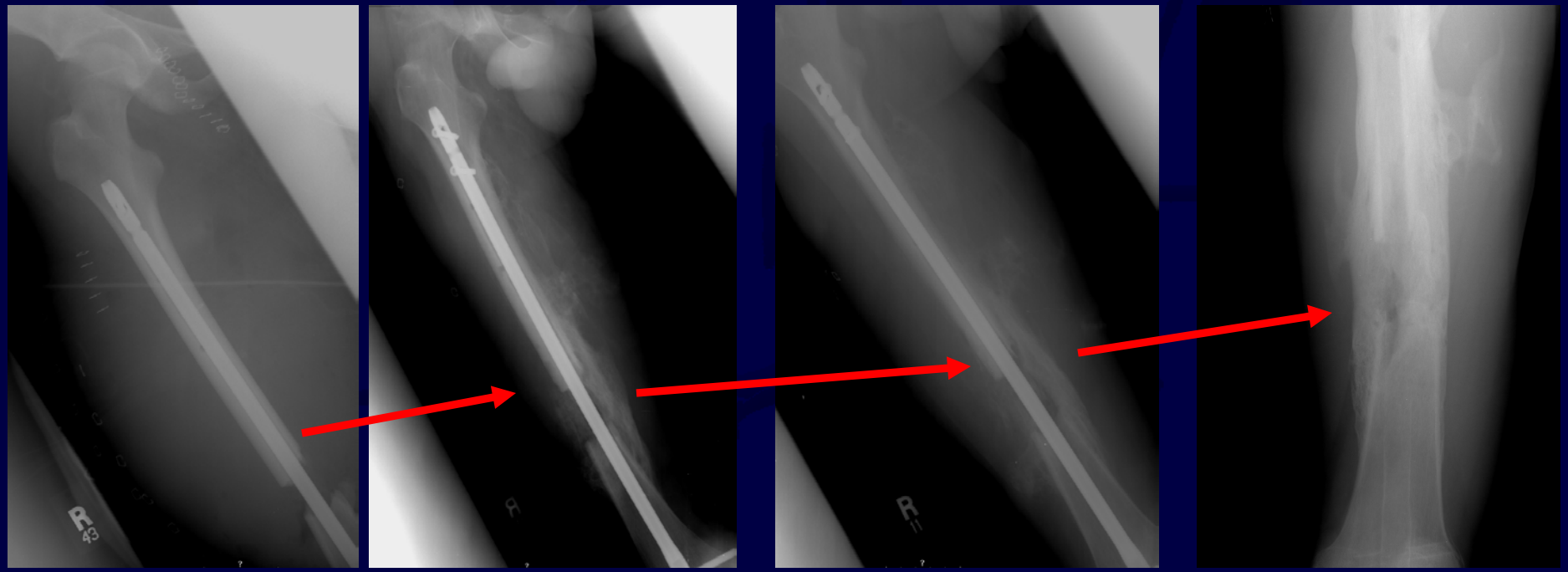


Figure from Brighton, et al, JBJS-A, 1991.

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

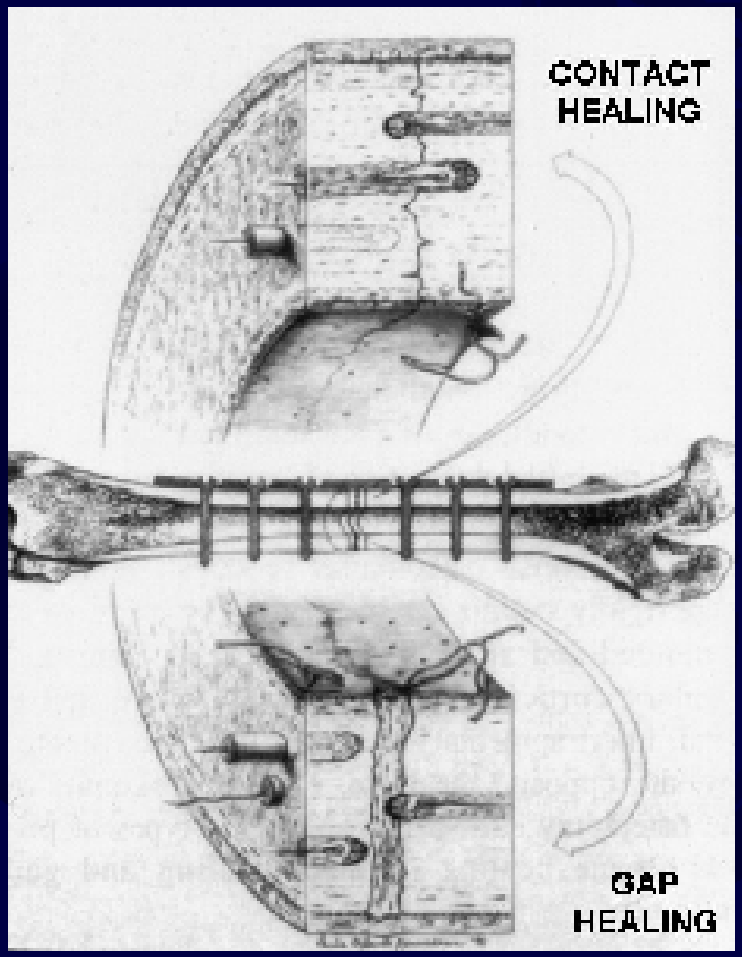
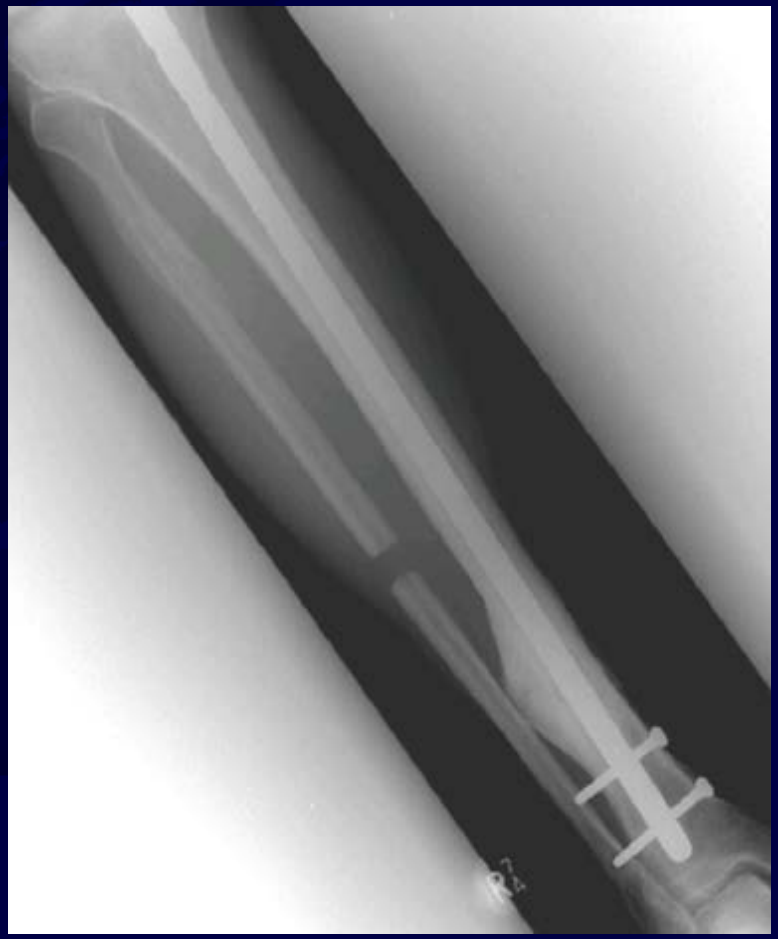


Figure from http://www.vetmed.ufl.edu/sacs/notes

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 ntramembranous 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

Member of the TGF-β superfamily	Time of expression	Specific responses in vivo and in vitro
GDF-8	Restricted to day 1[20]	Potential function as a negative regulator of skeletal muscle growth[20]
BMP-2	Days 1–21[10,20] (the earliest gene to be induced and second elevation during osteogenesis)	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]
BMP-3, -8	Days 14–21[20] (restricted expression during osteogenesis)	Temporal data suggest a role in the regulation of osteogenesis
BMP-4	Transient increased expression in the surrounding soft tissues 6 h to day 5[9] Days 14–21[20] Through out fracture healing[10]	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[63]
BMP-7	Days 14–21[20] From the early stages of fracture healing[9]	Regulatory role in both types of ossification In vitro: stimulation of relative mature osteoblasts[19]
GDF-10, BMP-5, -6	Days 3–21[20]	Regulatory role in both types of ossification BMP-6 may initiate chondrocyte maturation[20]
GDF-5, 1	Day 7 (maximal) to day 14[20] (restricted expression during chondrogenic phase) GDF-1 at extremely low levels	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
GDF-3, GDF-6, 9	No detectable levels within the fracture callus[20]	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[28]
TGF-β1, -β2, -β3	Days 1–21[20] Days 3–14[20] Days 3–21[20]	Potent chemotactic for bone forming cells and macrophages Proliferation of undifferentiated mesenchymal and osteoprogenitor cells, osteoblasts, chondrocytes

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

Cytokine	*Bone Formation*	*Bone Resorption*
IL-1	+	+++
TNF-α	+	+++
TNF-β	+	+++
TGF-α	--	+++
TGF-β	++	++
PDGF	++	++
IGF-1	+++	0
IGF-2	+++	0
FGF	+++	0

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.)

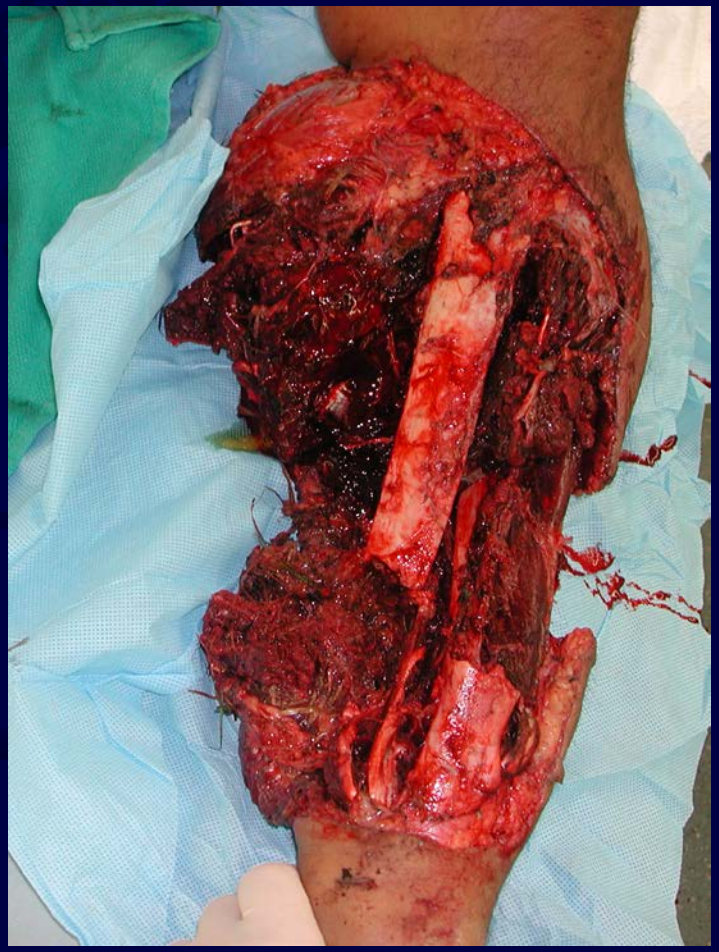
- Parathyroid Hormone
  - Intermittent exposure stimulates
    - Osteoblasts
    - Increased bone formation
- Growth Hormone
  - Mediated through IGF-1 (Somatomedin-C)
  - Increases callus formation and fracture strength

Vascular Factors

- Metalloproteinases
  - 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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