

Biology of Bone Repair

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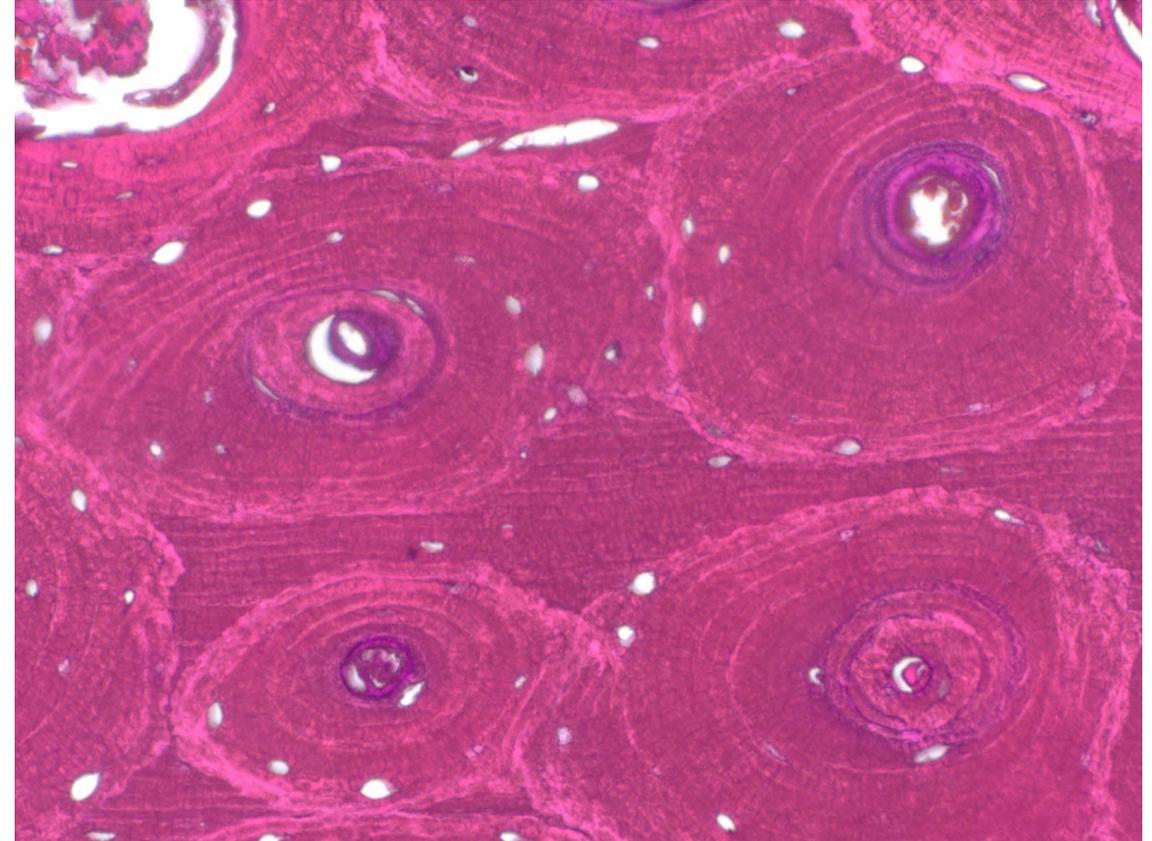


Objectives

- Bone Composition
- Bone Types
- Bone Healing
- Stages of Fracture Healing
- Factors that affect Bone Healing

Bone Composition

- Cells
 - Osteocytes
 - Osteoblasts
 - Osteoclasts
- Extracellular Matrix
 - Organic Portion (35%)
 - Collagen Type 1 90%
 - Osteocalcin, Osteonectin
 - Proteoglycans, glycosaminoglycans
 - Inorganic Portion (65%)
 - Calcium Hydroxyapatite
 - Calcium Phosphate

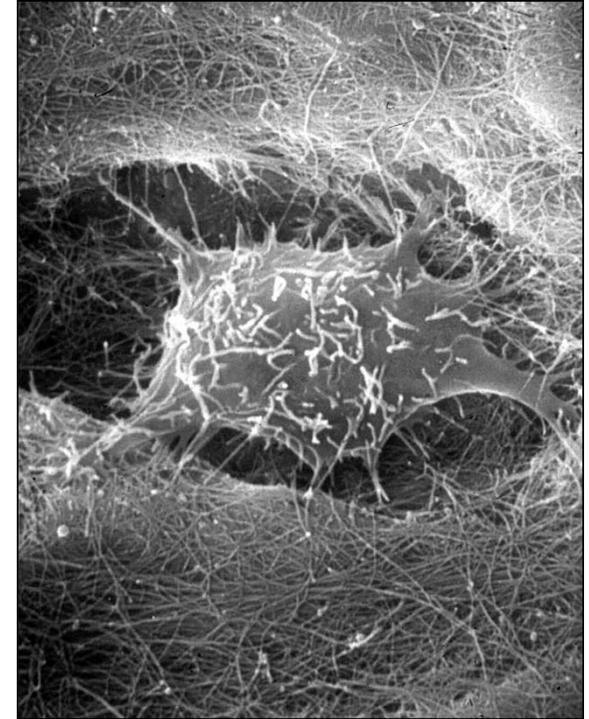


Normal Cortex.

Courtesy of Andrew Rosenberg, MD

Osteocytes

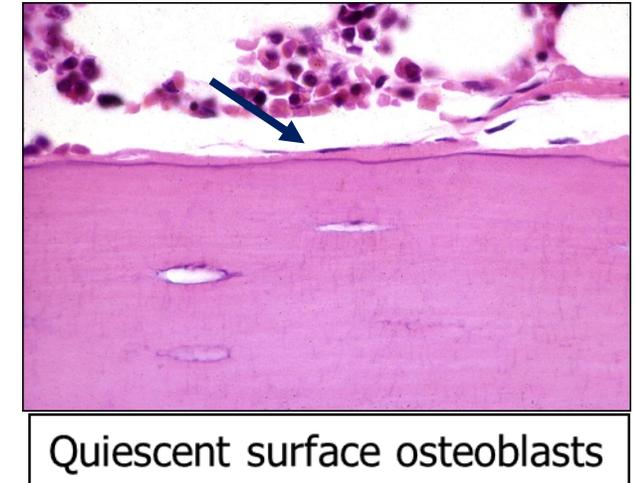
- About 90% of cells in the mature skeleton
- Osteocytes live in lacunae
- Previous osteoblasts that get surrounded by the new formed matrix
- Control extracellular calcium and phosphorus concentration
- Stimulated by calcitonin
- Inhibited by PTH



Electron microscope picture of osteocyte.
Courtesy of Andrew Rosenberg, MD

OsteoBlasts

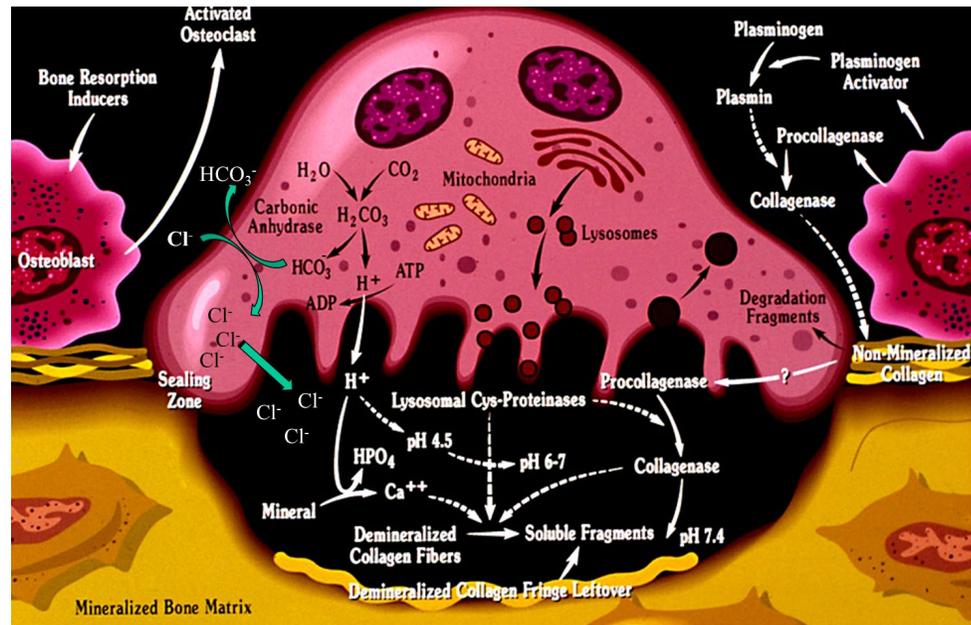
- Derives from undifferentiated **Mesenchymal Stem Cells**
 - RunX2 directs mesenchymal cells to osteoblast lineage
- Line the surface of bone and produce osteoid
- Functions:
 - Form Bone
 - Regulate osteoclastic activity
- Osteoblasts produce Type 1 collagen, RANKL, and Osteoprotegrin
- Osteoblasts are activated by intermittent PTH levels
- Inhibited by tumor necrosis Factor (TNF- α)



Courtesy of Andrew Rosenberg, MD

Osteoclasts

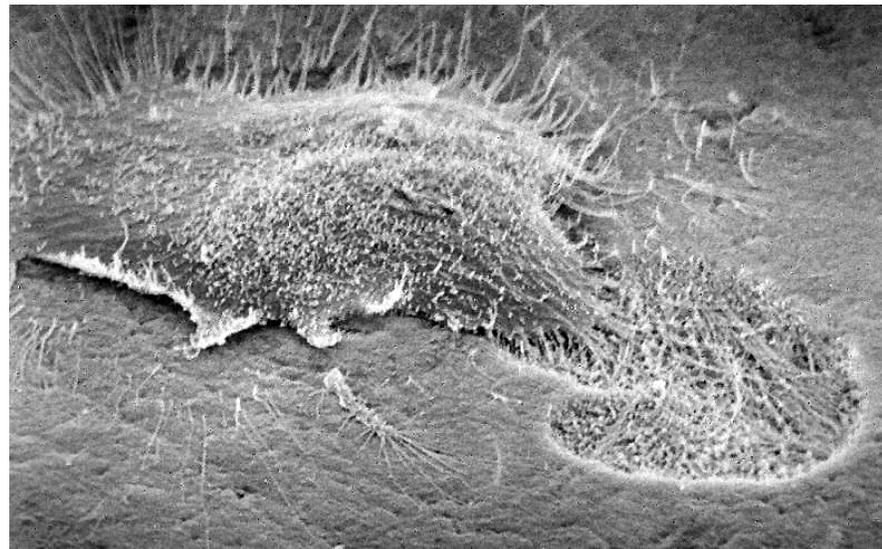
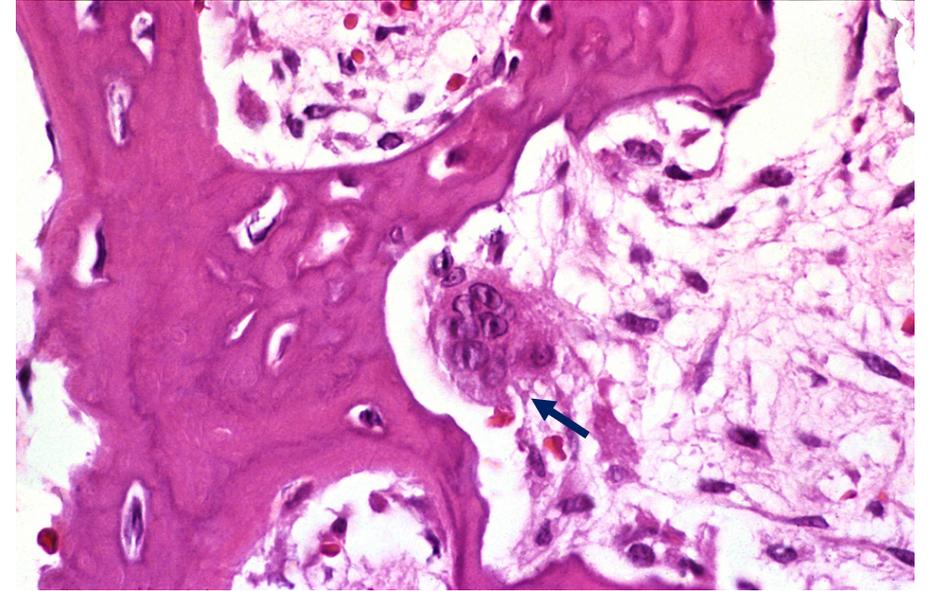
- Derived from **Hematopoietic stem cell** (monocyte precursor cells)
- Multinucleated cells
- Function to resorb bone and release calcium
- Parathyroid Hormone stimulates receptors on osteoblasts that activate osteoclasts



Courtesy of Andrew Rosenberg, MD

Osteoclasts

- Found in bone resorption craters called Howship Lacunae
 - Uses ruffled borders which increases surface area
 - Produces hydrogen ions through carbonic anhydrase
 - The lower pH increases the solubility of hydroxyapatite crystals

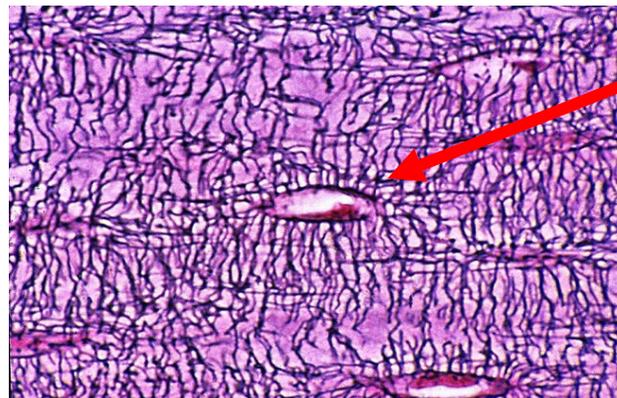


Above- Osteoclast in Howship Lacuna (blue arrow)
Left- Electron Microscope of the same.

Courtesy of Andrew Rosenberg, MD

Osteocyte Network

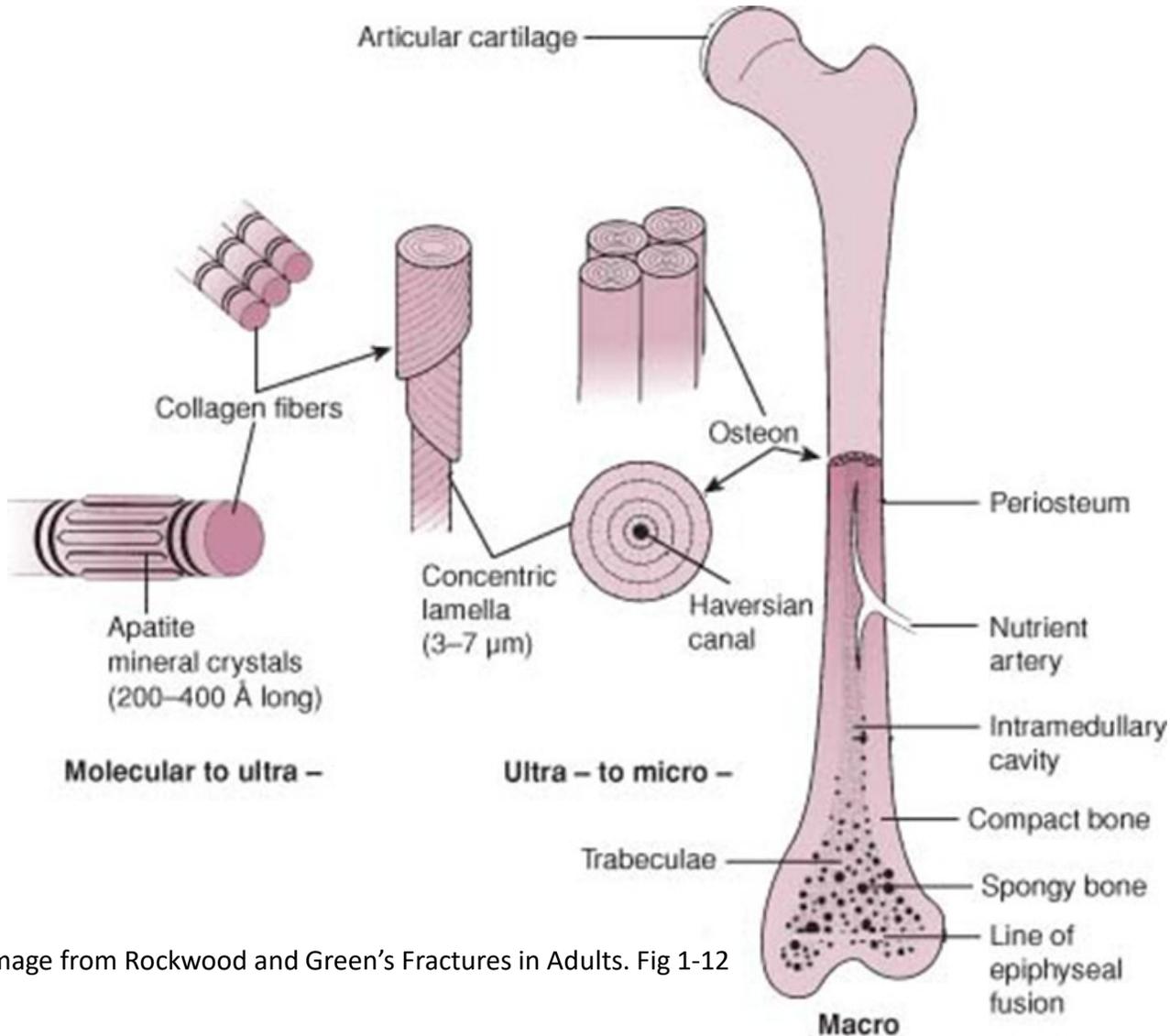
- Osteocyte lacunae are connected by canaliculi
- Osteocytes are interconnected by long cell processes that project through the canaliculi
- Preosteoblasts also have connections via canaliculi with the osteocytes
- Network facilitates response of bone to mechanical and chemical factors



Osteocyte

Courtesy of Andrew Rosenberg, MD

Osteon



- Basic unit of bone
- Consists of
 - Lamella- extracellular matrix made up of collagen fibers. Parallel to each other
 - Osteocytes in their lacunae
 - Vessels in the center in the Haversian Canal

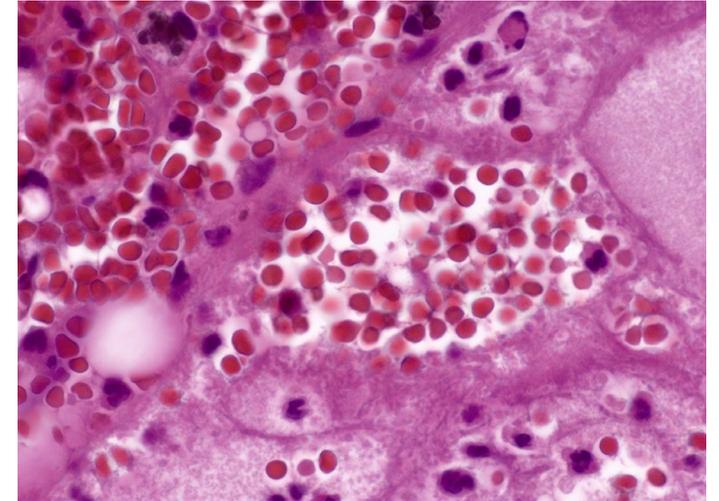
Image from Rockwood and Green's Fractures in Adults. Fig 1-12

Extracellular Matrix

- Organic Components
 - Collagen- mostly Type 1 Collagen which provides tensile strength
 - Proteoglycans
 - Matrix proteins
 - Osteocalcin-most abundant noncollagenous protein
 - Growth Factors
 - Cytokines
- Inorganic Components
 - Calcium hydroxyapatite
 - Calcium Phosphate

Blood Supply

- About 5-10% of a person's cardiac output gets sent to the skeletal system
- Long Bones Receive blood from three sources
 - Nutrient artery system
 - Metaphyseal-epiphyseal system
 - Periosteal system
- Blood flow is one the most important factors in bone healing along with stability
- During fracture healing blood flow peaks at two weeks

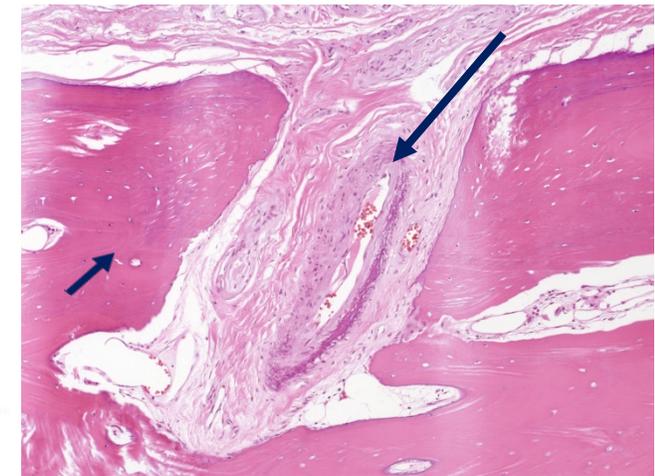


Acute Fracture Callus with Red Blood Cell and Neutrophil infiltration.

Courtesy of Andrew Rosenberg, MD

Nutrient Artery

- Artery enters the nutrient foramen in the diaphysis
- Branches into ascending and descending arteries through medullary canal
- This extends to the endosteum and supplies about 2/3 of the bone



Nutrient artery entering cortex
(Long arrow artery, short arrow cortex)

Metaphyseal Vessels

- Arise from the periarticular vessels (ex. Geniculate arteries)
- Penetrate the metaphyseal region and anastomose with the medullary blood supply

Periosteal Vessels

- Capillaries that supply the outer portion of the bone
- Arise from the periosteum which surrounds the cortex
- Supplies outer 1/3 of bone
- Can supply greater amount if endosteal supply is damaged.

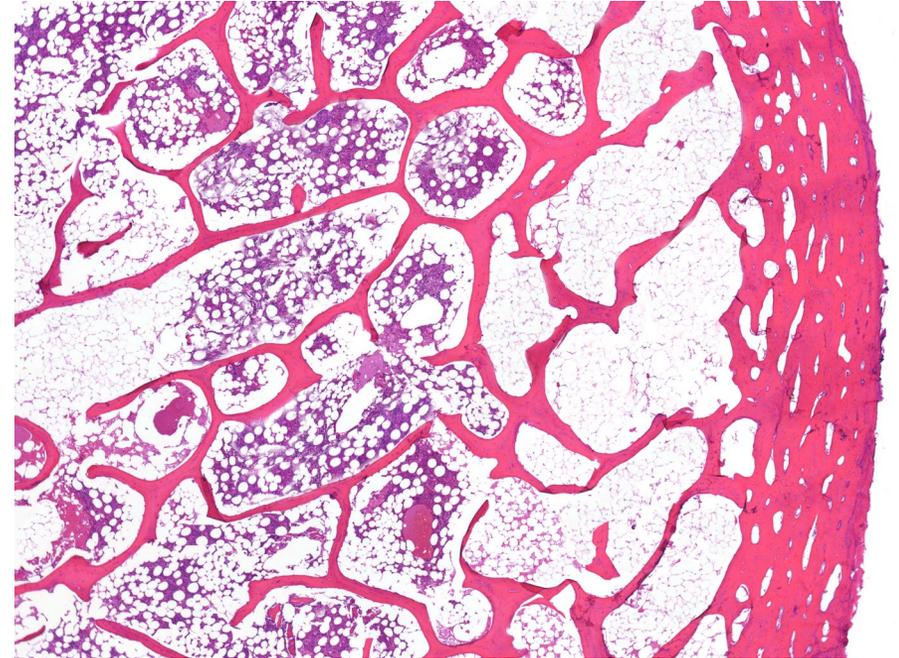
Types of bone

- **Lamellar**

- Collagen fibers are arranged in parallel layers
- Normal adult bone
 - Cortical
 - Cancellous

- **Woven**

- Collagen fibers are oriented randomly
- Seen in remodeling bone or ligament/tendon insertion
- Pathological conditions

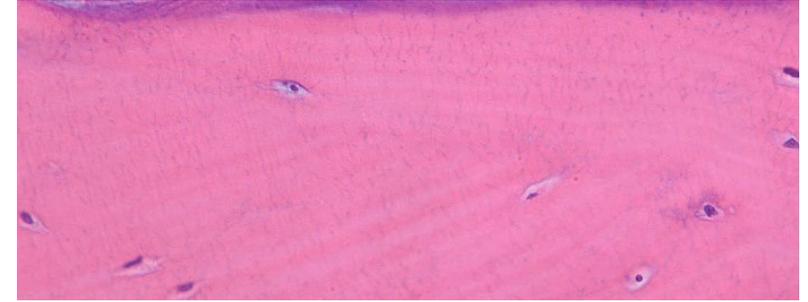


Cancellous Bone

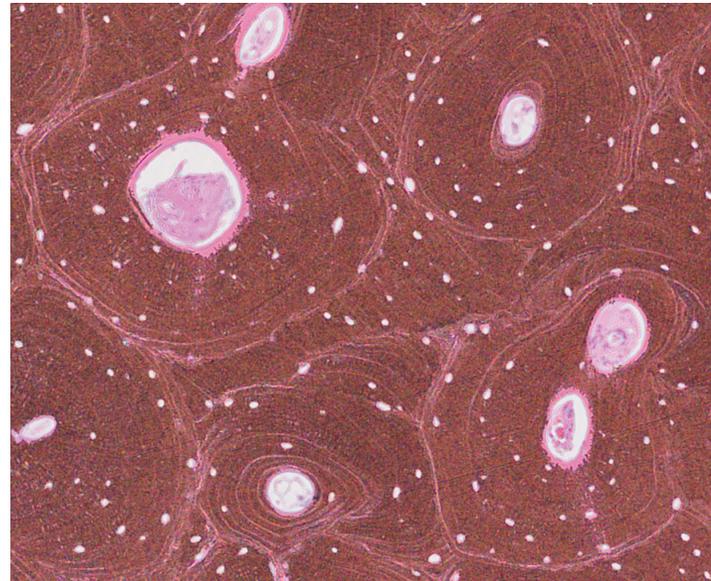
Cortical Bone

Courtesy of Andrew Rosenberg, MD

Lamellar Bone



- Stress Oriented formation – highly organized
- Consists of Osteons and Interstitial lamellae (fibrils between osteons)
- Osteons communicate through Volkmann’s canals
- Cortical Bone
 - Constitutes 80 % of bone
 - Slow turnover rate
- Cancellous Bone
 - Spongy or Trabecular bone
 - Higher turnover rate
 - Less dense than cortical bone



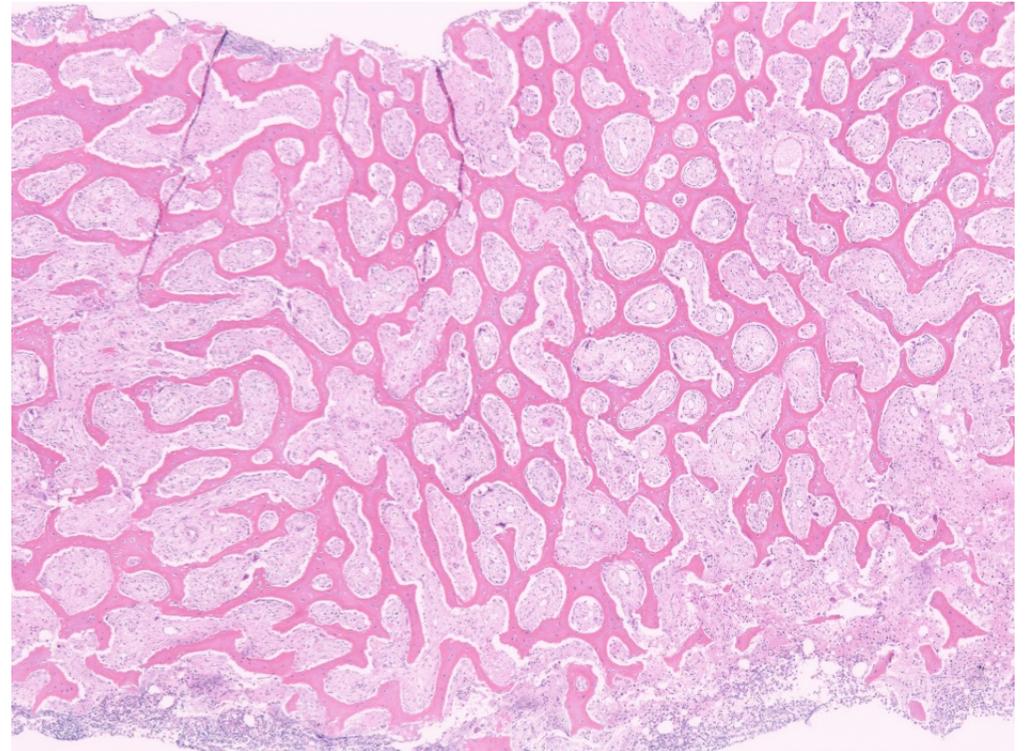
Above- Lamellar bone with osteocytes.

Left-Osteons

Courtesy of Andrew Rosenberg, MD

Woven Bone

- Immature or Pathologic Bone
- Random orientation of collagen
- Has more osteocytes
- Not stress oriented
- Weaker



Fracture with Reactive Woven Bone

Courtesy of Andrew Rosenberg, MD

Mechanism of Bone Formation

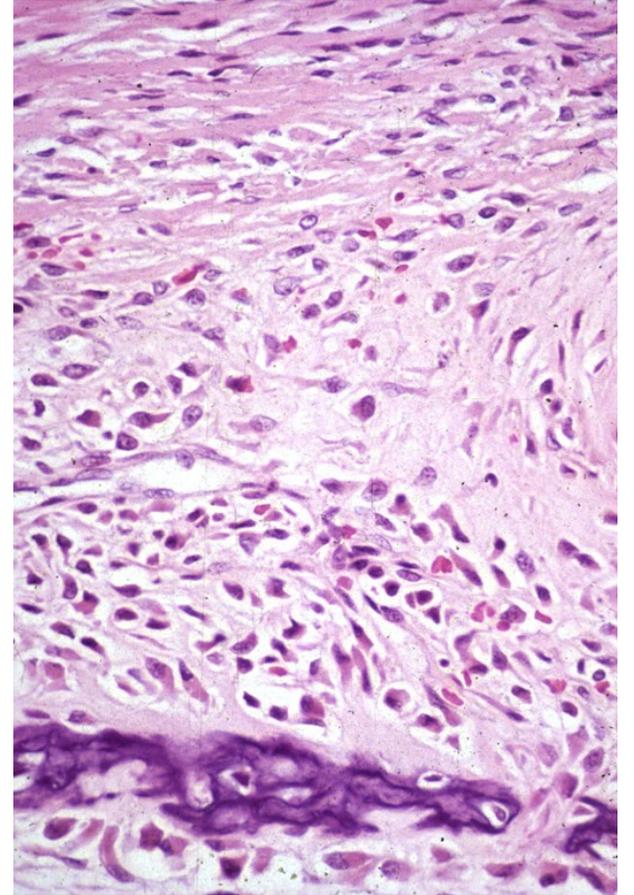
- Bone Remodeling
 - Wolff's Law
 - Bone will adapt according to the stress or load it endures
 - Longitudinal Load will increase density of bone
 - Compressive forces inhibit growth
 - Tensile forces stimulates growth
- Types of Bone Formation
 - Appositional
 - Intramembranous (Periosteal) Bone Formation
 - Endochondral Bone Formation

Appositional Ossification

- Increase in diameter of bone by osteon formation on existing bone
- Osteoblasts align on existing bone surface and lay down new bone
- Periosteal bone increases in width
- Bone formation phase of bone remodeling
- Seen as bone grows in diameter and strength secondary to stress
 - Remodeling due to forces on the bone

Intramembranous Bone Formation

- Mostly seen in flat bones like cranium and clavicle
- Osteoblasts differentiate directly from preosteoblasts and lay down osteoid
- There is no cartilage precursor
- Direct bone healing



Courtesy of Andrew Rosenberg, MD

Endochondral Bone Formation

- Seen in embryonic bone formation, growth plates, and fracture callus
- Cartilaginous matrix is laid down → osteoprogenitor cells come to the area through vascular system → Osteoclasts resorb the cartilage → Osteoblasts make bone
- The Chondrocytes hypertrophy, degenerate and calcify
- Vascular Invasion of the cartilage occurs followed by ossification
- **Cartilage is not converted to bone**
- Bone Grows in Length
- Indirect bone healing

Endochondral Bone Formation

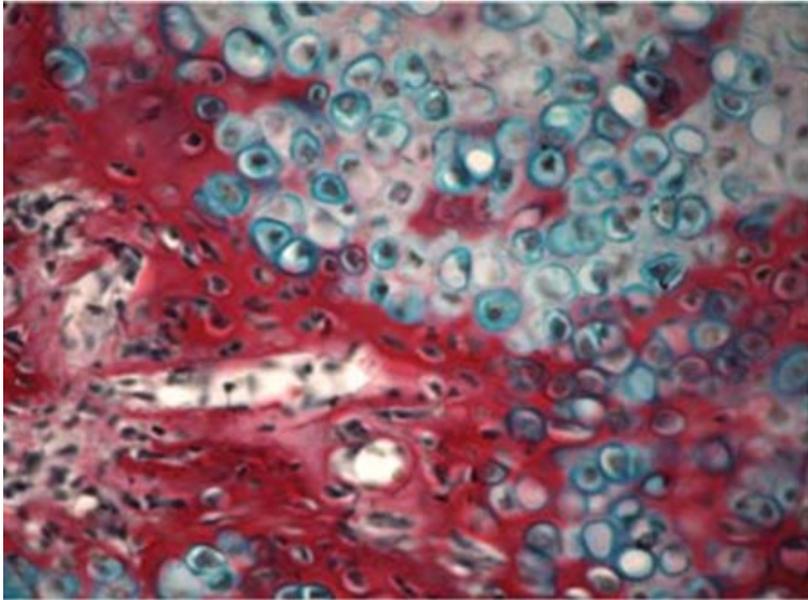
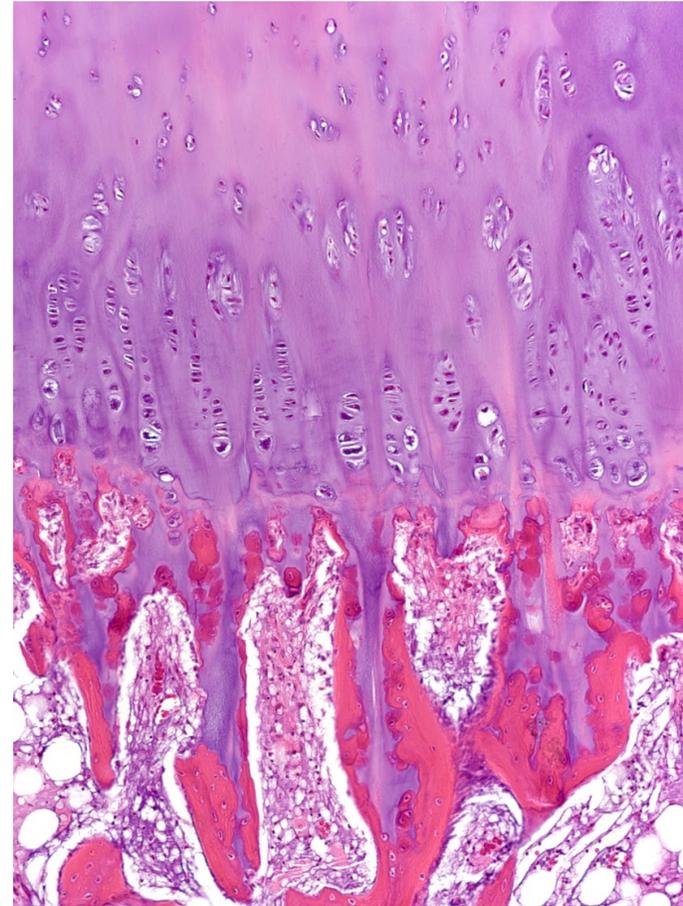


Fig 4-1. Ossification of the cartilage scaffold in endochondral ossification.

Image from Rockwood and Green's Fracture's In Adults.



Resting Zone

Proliferative Zone

Hypertrophic Zone

Calcification Zone

Ossification Zone

Normal Growth plate.

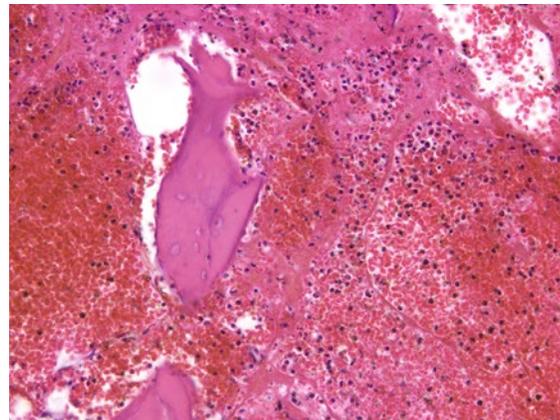
Courtesy of Andrew Rosenberg, MD

Stages of Fracture Healing

- Inflammatory Phase
- Repair
 - Early Callus Phase
 - Mature Callus Phase
- Remodeling Phase

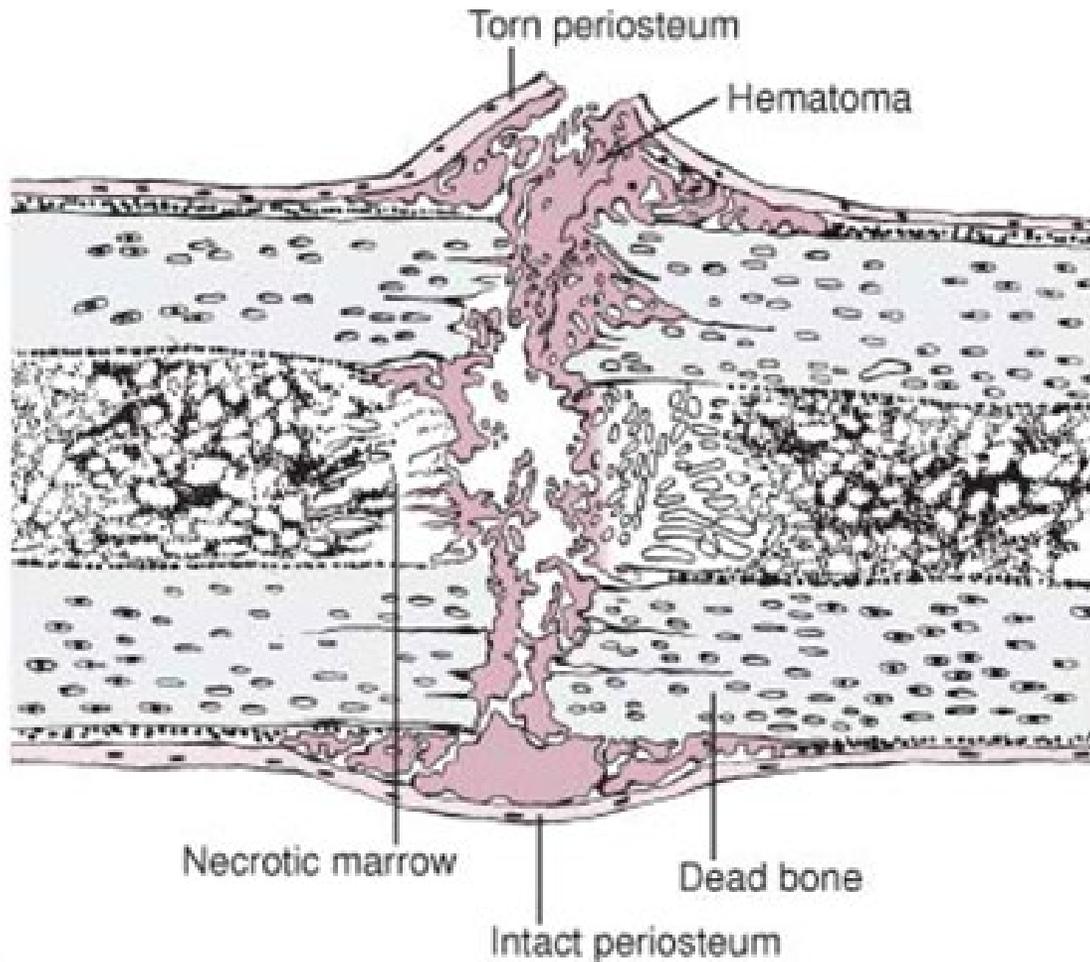
Inflammatory Phase

- Begins as soon as fracture occurs when a hematoma forms
- It lasts about 3-4 days
- Proinflammatory markers are released into the area
 - IL-1, IL-6, TNF alpha
- This attracts cells like fibroblasts, mesenchymal cells and osteoprogenitor cells

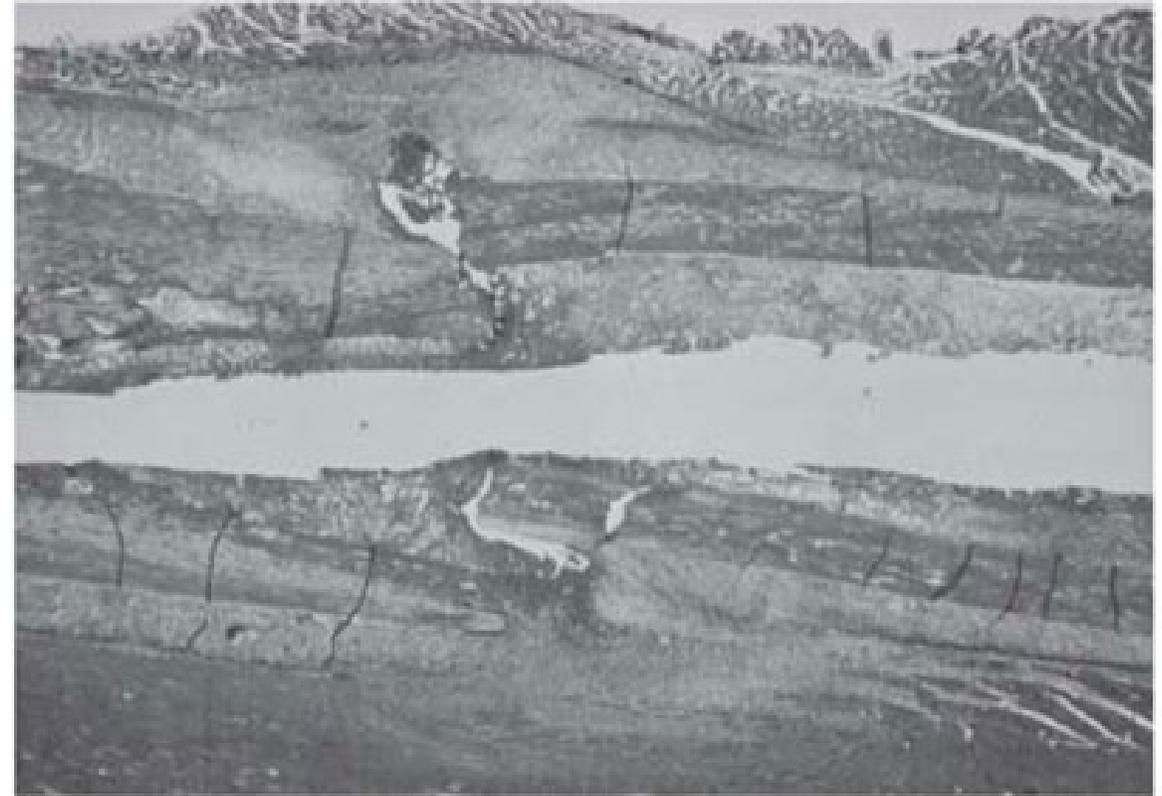


Fracture with hematoma.
Courtesy of Andrew Rosenberg, MD

Inflammatory Phase



A



B

Image from Rockwood and Green's Fractures in Adults. Fig 4-2

Early Callus Phase

- Starts a few days after fracture and lasts weeks
- Vascularization into the area takes place
- Mesenchymal Cells in the area differentiate into Chondrocytes
- Cartilage Callus is formed and provides initial mechanical stability

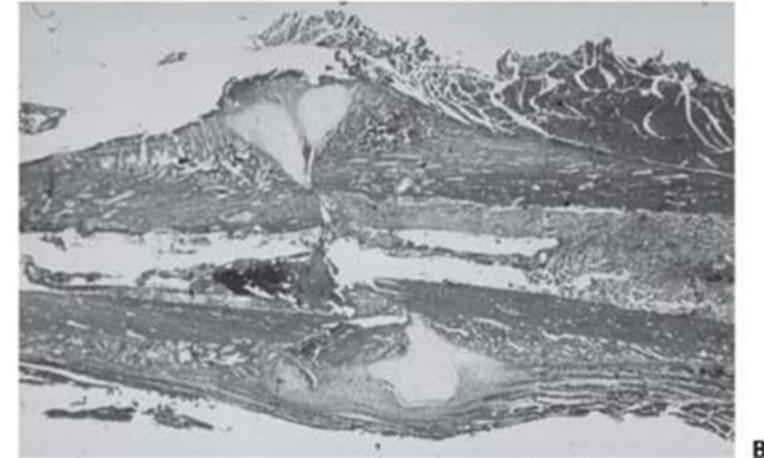
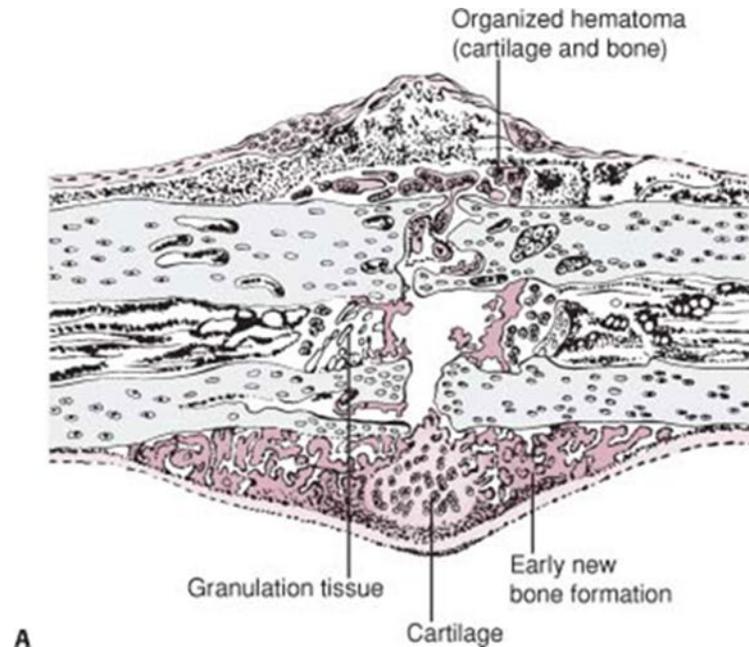


Image from Rockwood and Green's Fractures in Adults. Fig 4-3

Mature Callus Phase

- Cartilaginous Matrix is mineralized
- Cartilage is degraded
- Bone is laid down as woven bone through endochondral ossification
- Fracture is considered healed in this stage

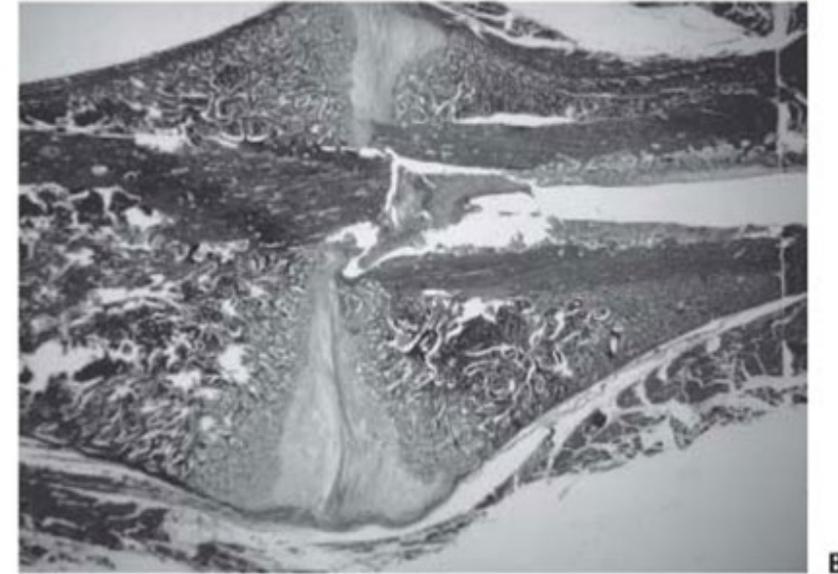
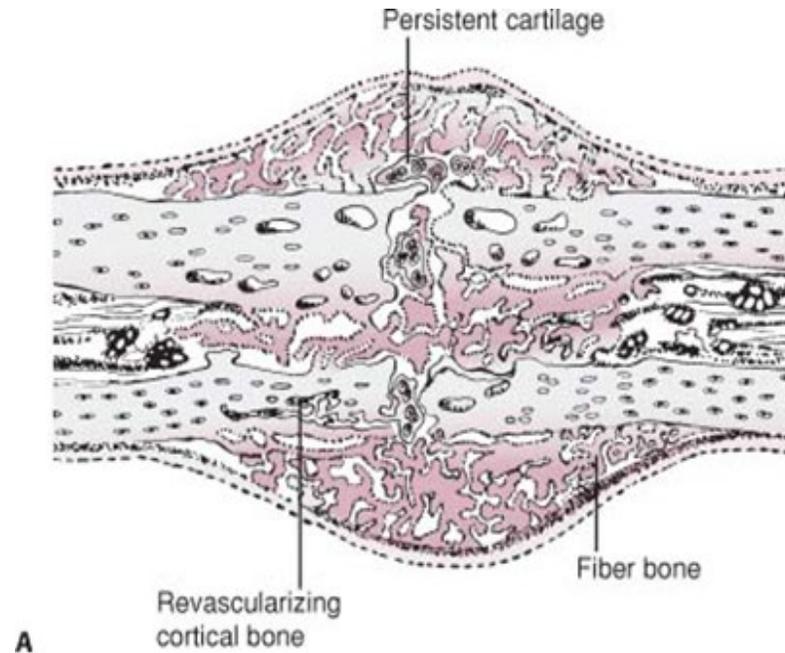


Image from Rockwood and Green's Fractures in Adults. Fig 4-4.

Remodeling Phase

- Happens several months after fracture
- Woven Bone becomes Lamellar bone
- Previous shape of bone begins to be formed through Wolff's Law
- This phase can continue for a year or more
- Fracture healing is complete when marrow space is reconstituted

Cutting Cones

- Primary method of bone remodeling
- Osteoclasts are in the front of the cone and remove the disorganized woven bone
- Osteoblasts trail behind to lay down new bone
- Blood vessel is in the center of the core

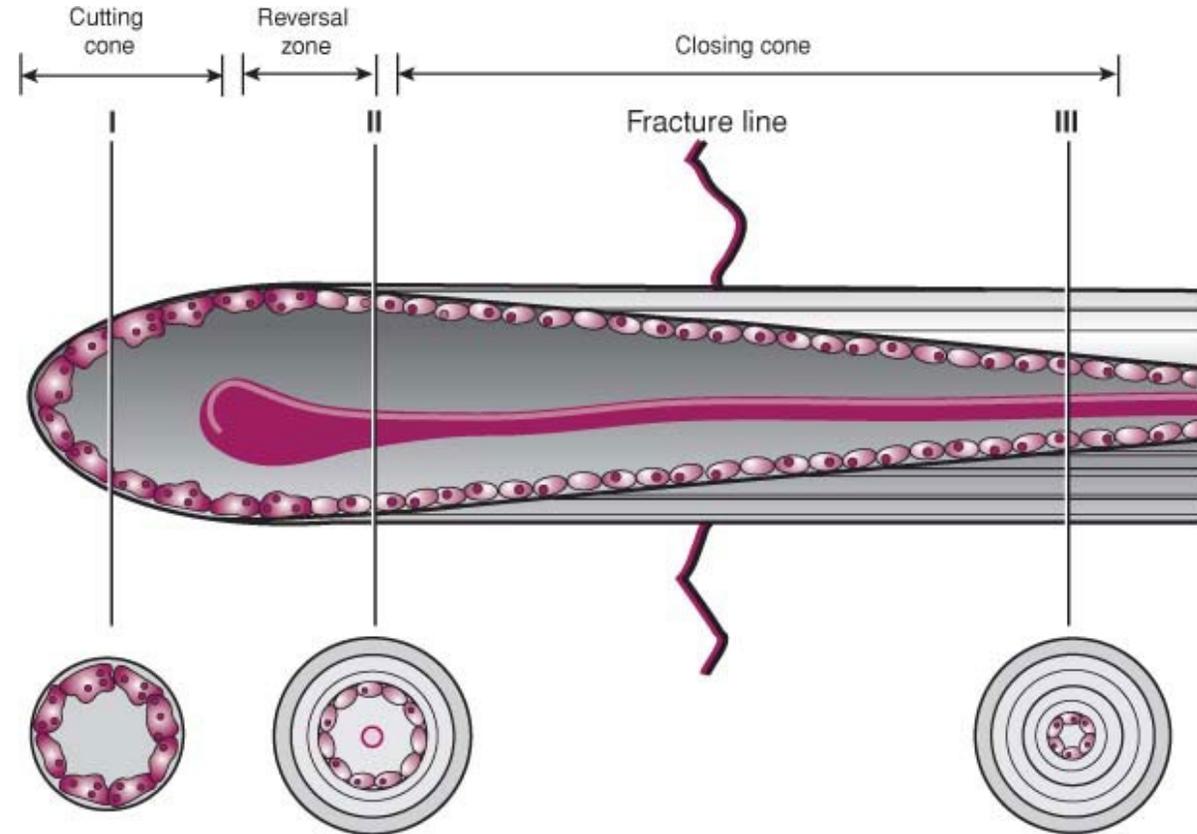


Image from Rockwood and Green's Fractures in Adults Fig 4-6.

Table 4-1 The Essential Components of Fracture Healing

| | Components | Importance in Fracture Healing |
|---------------------|--|--|
| Cells | Inflammatory cells Progenitor cells Chondrocytes Osteoblast Osteoclast Muscle cells | Debride necrotic tissues Signal for upregulation of synthetic functions Form repair tissues Remodel healed bone for optimal strength/weight ratio |
| Scaffold | Hematoma Collagen Noncollagenous proteins | Support cellular function Inflammatory cell chemotaxis Scaffold for mineralization |
| Blood Supply | Blood vessels Supporting cells (pericytes) | Supply inflammatory cells to injury site Deliver building blocks of repair tissues Reverse hypoxic environment |
| Molecules | Matrix-embedded proteins Locally produced factors Systemic hormones | Regulate cellular function and proliferation during fracture healing process |

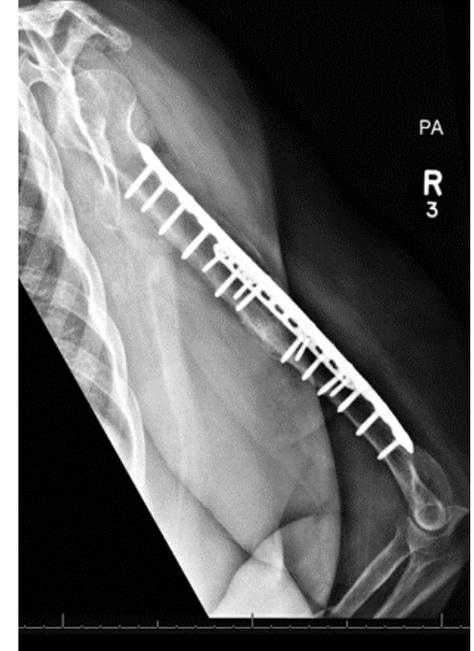
Rockwood & Green's
Fractures in Adults

Clinical Fracture Healing

- Direct (Primary) Bone Healing
 - Cutting Cones
 - Absolute Stability
 - Rigid Fixation
 - No callus formation
- Indirect (Secondary) Bone Healing
 - Endochondral Ossification
 - Relative Stability
 - Comminution
 - Callus Formation



A.



B.

A. Patient treated with fracture brace using secondary bone healing
B. Patient with Compression plating and primary bone healing.

Direct (Primary) Bone Healing

- There is no motion at the fracture site
- Cutting cone crosses the fracture site
- Contact healing- there is direct contact between the two fracture ends which allows for healing to start with lamellar bone formation
- Gap Healing- if $< 200-500$ microns woven bone that is formed can be remodeled into lamellar bone
- Examples: Compression Plating, lag screws and neutralization plate



Indirect (Secondary) Bone Healing

- Some motion at the fracture site
- Relative Stability
- Endochondral Ossification
- Large fracture gaps
- Comminution
- Example: Intramedullary nail, Casting/bracing, Bridge plating



Right femoral shaft fracture treated with IMN.
Post OP 1 month, 8 months, 12 months.

Strain

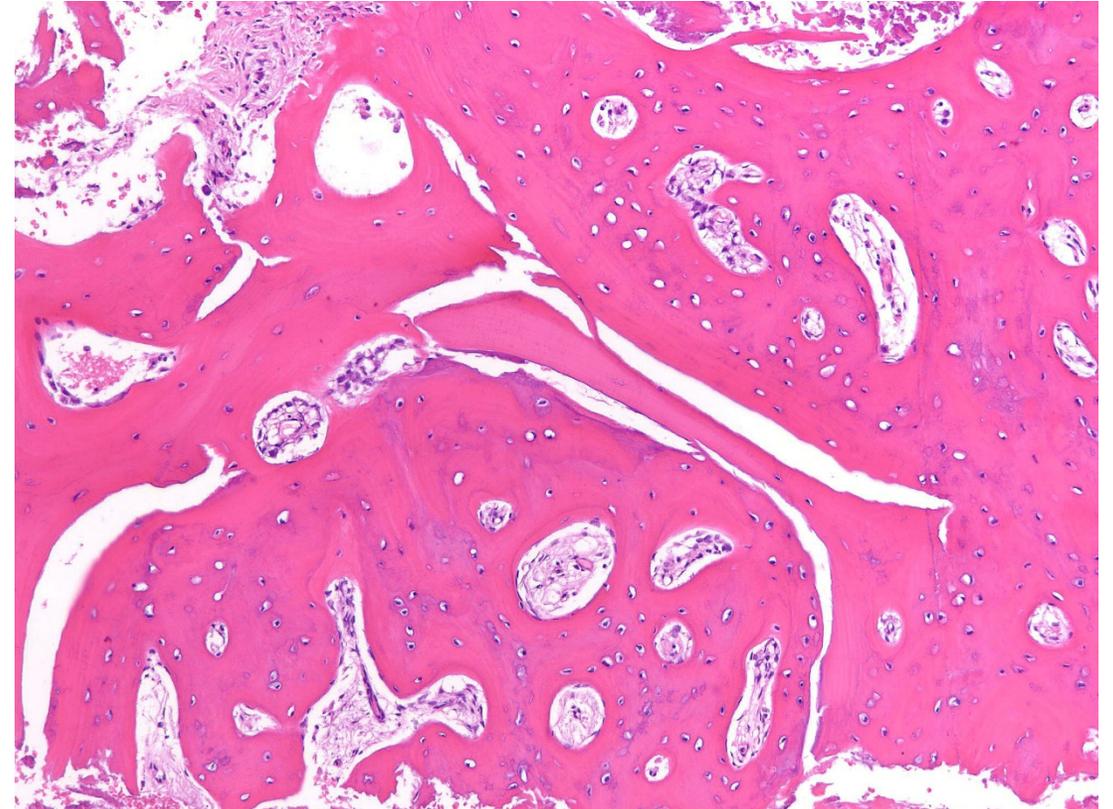
- Strain= change in fracture gap length/ length of fracture gap
- Strain < 2% promotes primary bone healing
- Strain 2-10% promotes secondary bone healing
- Multifragmentary fractures share strain
- Fracture creates mechanical instability and decreased oxygenation. To promote healing the instability needs to be decreased.

Vascularity and Strain

- Vascularity helps create the scaffold for bone formation
- Strain and Vascularity have the most influence in type of bone healing
- Pericytes are stem cells that differentiate into osteoblasts or chondroblasts. They come from the vasculature of the periosteum and endosteum.
- Pericytes become osteoblasts in low strain and high oxygen environment and become chondrocytes in moderate strain and moderate vascularity
- When strain is reduced at the fracture site by stabilization of soft callus formation, then endothelial cells migrate there in response to VEGF
- VEGF is released by chondrocytes and osteoblasts

Direct (Primary) Bone Healing

- Bone healing with compression
- Bone formation with no cartilage cells.
- Osteoblasts and Osteoclasts working to create new bone

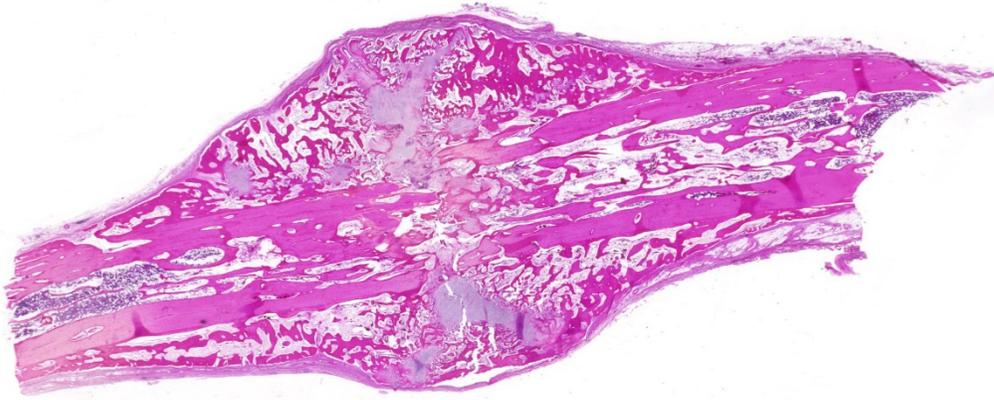


Lamellar Bone formation in fracture site.

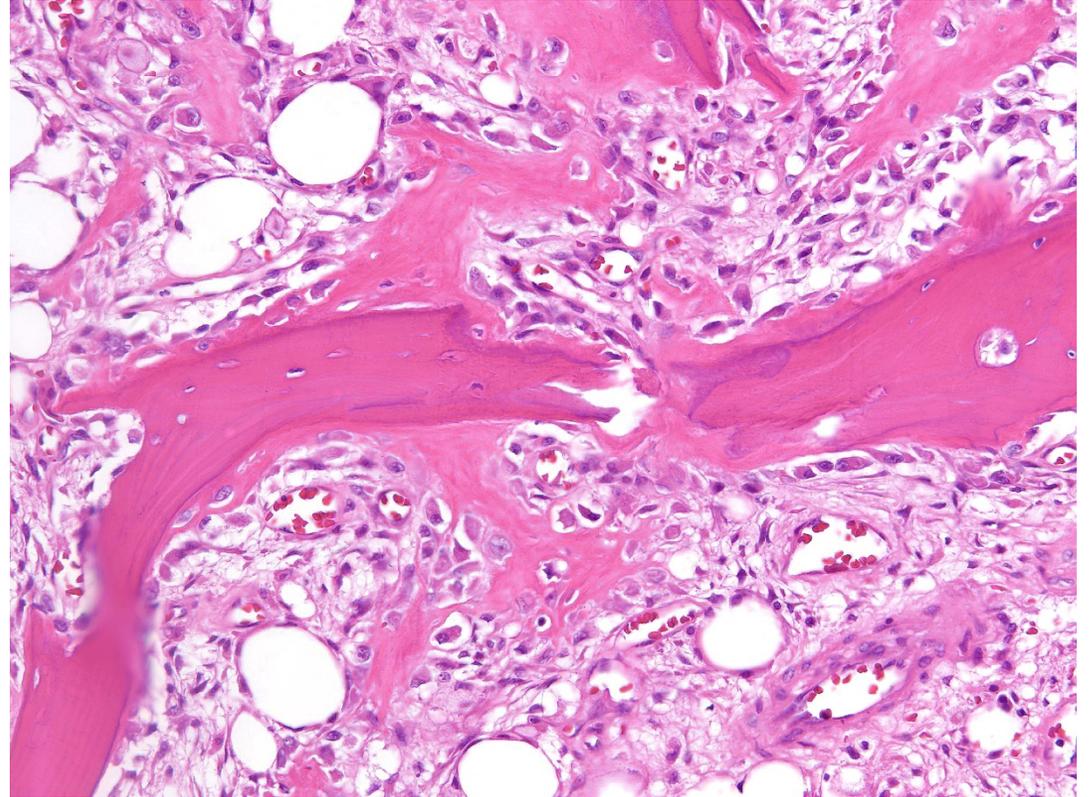
Courtesy of Andrew Rosenberg, MD

Indirect (Secondary) Bone Healing

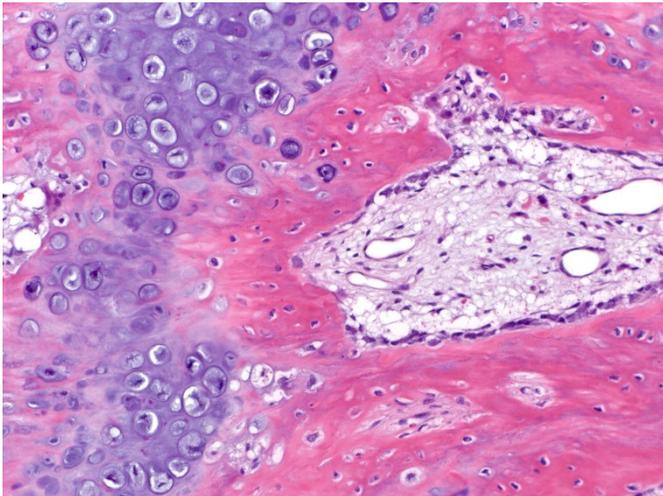
A.



B.



C.



- A. Fracture with Callus
- B. High power view of fracture
- C. Endochondral Ossification

Courtesy of Andrew Rosenberg, MD

Factors affecting Healing

Biological

- Comorbidities
- Nutritional Status
- Cigarette Smoking
- Hormones
- Growth Factors
- NSAIDs

Mechanical

- Soft Tissue Attachments
- Stability
- High vs low energy mechanism
- Extent of bone loss

Biological Factors: Comorbidities/Behavioral

- Comorbidities
 - Diabetes- associated with collagen defects
 - Vascular Disease- decreased blood flow to fracture site
- Nutritional Status
 - Poor protein intake/ Albumin and prealbumin
 - Vit D deficiency
- Cigarette Smoking
 - Inhibits osteoclasts
 - Causes Vasoconstriction decreasing blood flow to fracture site

Biological Factors:

Hormones

- Growth Hormone: Increases gut absorption of calcium, Increases callus volume
- Calcitonin: Secreted from parafollicular cells in thyroid, Inhibits osteoclasts, decreases serum calcium levels
- PTH: Chief cells of parathyroid gland, stimulates osteoclasts
- Corticosteroids: Decrease gut absorption of calcium, Inhibits collagen synthesis and osteoblast effectiveness

Biological Factors:

Growth Factors

- Bone Morphogenic Proteins (BMP): Stimulates bone formation by increasing differentiation of mesenchymal cells into osteoblasts.
- Transforming growth factor Beta (TGF- β): Stimulates mesenchymal cells to produce type II collagen and proteoglycans, stimulate osteoblasts to make collagen
- Insulin like Growth Factor 2 (IGF-2): Stimulates collagen I formation, cartilage matrix synthesis and bone formation
- Platelet-derived growth factor (PDGF): Attract inflammatory cells to fracture sites

Bone Morphogenetic Proteins

- Osteoinductive proteins initially isolated from demineralized bone matrix
- Noncollagenous glycoproteins that are part of the TGF- β family
- Induce Cell differentiation
 - BMP-3 (osteogenin) is an extremely potent inducer of mesenchymal tissue differentiation into bone
- Promote Endochondral ossification
 - BMP-2 is FDA approved for open tibia fractures
 - BMP 7 is FDA approved only for recalcitrant nonunion of long bones
- Regulate extracellular matrix production

Insulin Growth Factors

- Two Types: IGF -1 and IGF II
 - Synthesized by multiple tissues
 - IGF-1 production in the liver is stimulated by Growth Hormone
- Stimulates bone collagen and matrix synthesis
- Stimulates replication of osteoblasts
- Inhibits bone collagen degradation

Transforming Growth Factors

- Super-Family of growth factors (-34 factors)
- 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

Platelet-Derived Growth Factors

- Large polypeptide that has two chains of amino acids
- Stimulates bone cell growth
- Mitogen for cells of mesenchymal origin
- Increases Type 1 Collagen synthesis by increasing the number of osteoblasts
- PDGF-BB stimulates bone resorption by increasing the number of osteoclasts

Summary of Healing Molecules

Table 4-2 Molecules Important in the Fracture Healing Process

| | Source Cells | Effector Cells | Effect on Fracture Healing |
|--|---|---|---|
| Bone Morphogenetic Protein-2 (BMP-2) | Persistent cambial layer, macrophages, osteoprogenitors, bone, and cartilage matrix | Osteoblast and osteocytes in new woven bone | Stimulates differentiation of chondroprogenitor and osteoprogenitor cells in early fracture healing |
| Receptor Activator of Nuclear Factor-Kappa Beta Ligand (RANKL) | Osteoblast, lymphocytes | Osteoclast Precursors | Induces resorption of calcified cartilage and stimulates osteoclast activity |
| Insulin-like Growth Factor-1 (IGF-1) | Osteoprogenitor, bone matrix | Osteoprogenitor cells | Stimulates osteoblast proliferation and differentiation |
| Platelet-derived Growth Factor (PDGF) | Degranulating platelets, fracture hematoma macrophages | Chondroprogenitor and osteoprogenitor cells | Stimulates migration of progenitor cells and osteoblast |
| Transforming Growth Factor Beta (TGF- β) | Platelets, bone, and cartilage matrix | Chondroprogenitor and osteoprogenitor cells | Stimulates progenitor cell proliferation |
| Fibroblast Growth Factor (FGF) | Macrophages, chondrocytes, and osteoblast | Chondroprogenitor and osteoprogenitor cells | Stimulates progenitor cell proliferation |
| Parathyroid Hormone (PTH) | Parathyroid gland (chief cells) | Chondrocytes | Recruits and activates chondrocytes in the early fracture callus |
| Vascular Endothelial Growth Factor (VEGF) | Platelets, hypertrophic chondrocytes | Macrophages, endothelial cells and granulocytes | Angiogenesis, chemotactic for macrophages and endothelial cells and vasodilation |

Biological Factors:

Non steroidal anti inflammatories (NSAIDs)

- NSAIDS work by binding to COX 1 or COX 1 and COX2 which decreases prostaglandin (PG) production. PGs assist in cell recruitment during fracture healing
- Both selective and non selective NSAIDs have been linked to decreased bone healing and nonunion formation
- Some studies suggest that COX 2 inhibitors do not effect healing as much
- Effects of NSAIDs on PG are reversible and levels return to normal at 1-2 weeks when the drug is stopped.

Mechanical Factors

Soft tissue

- Periosteal Stripping
- Disruption of local blood supply
 - Decrease ability of angiogenesis
 - Decrease formation of soft callus or bone formation
- Interposition of fat or soft tissue in fracture site
 - Increase fracture gap
 - Inability to build upon a scaffold



Mechanical Factors

Energy of injury

- High Energy
 - GSW
 - Crush Injury
 - Motor Cycle or Motor Vehicle Accident
 - More soft tissue injury and greater risk of nonunion
- Low Energy
 - Fall from Standing Height
 - Twisting Injury
 - Less soft tissue damage



Mangled foot and open pilon from a Motorcycle Crash



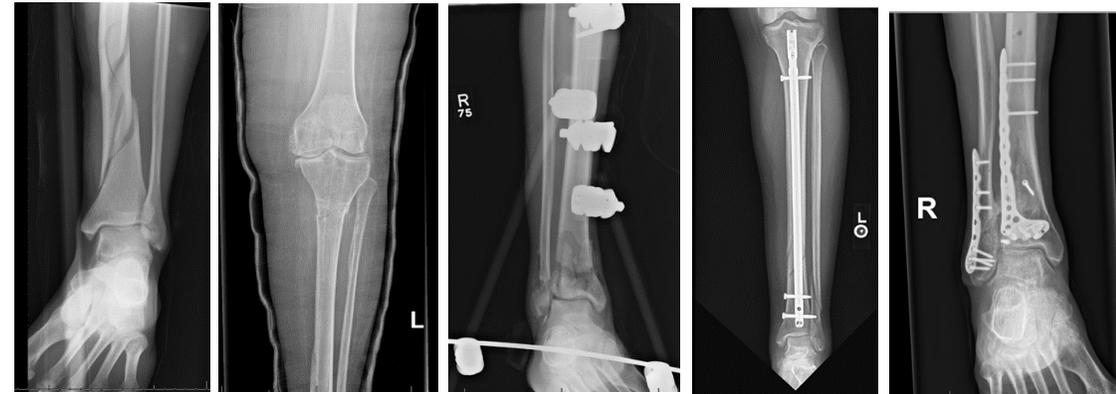
Nondisplaced Lateral Tibial Plateau Fracture

Mechanical Factors

Stability

- Absolute stability
 - No movement between fracture fragments
 - Anatomic Reduction of Fracture
 - Intermembranous Ossification
- Relative Stability
 - Controlled motion between fracture fragments
 - Restoration of length, alignment, and rotation
 - Endochondral Ossification
- Instability
 - Gross movement at the fracture site
 - Cannot make callus or increase stability due to constant motion
 - Leads to nonunion

Stability Spectrum



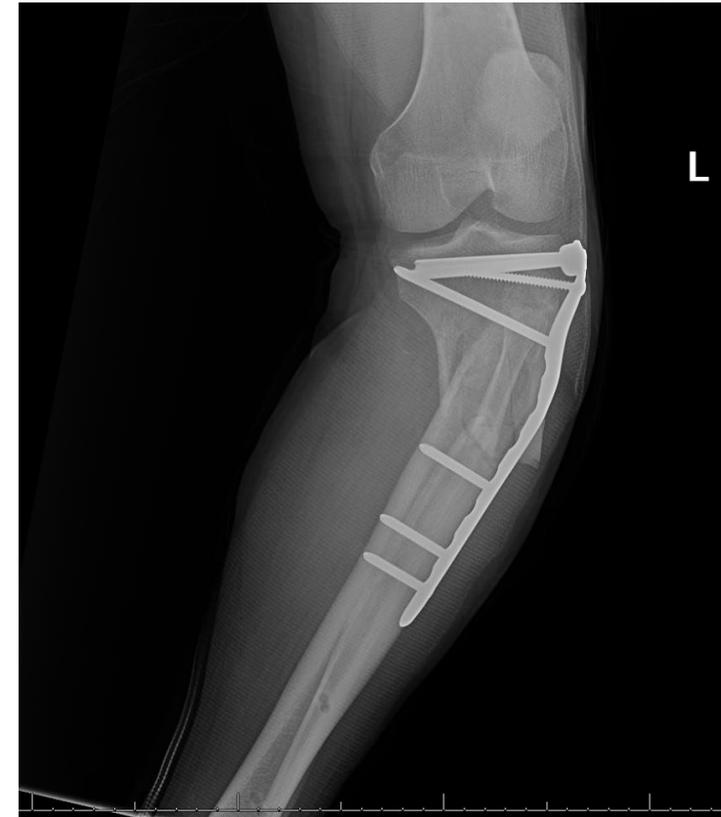
From left to right: Unstable, Casting, External fixation, Intramedullary Nail, and Plate fixation

Failure of Stability

Instability Results in Nonunion

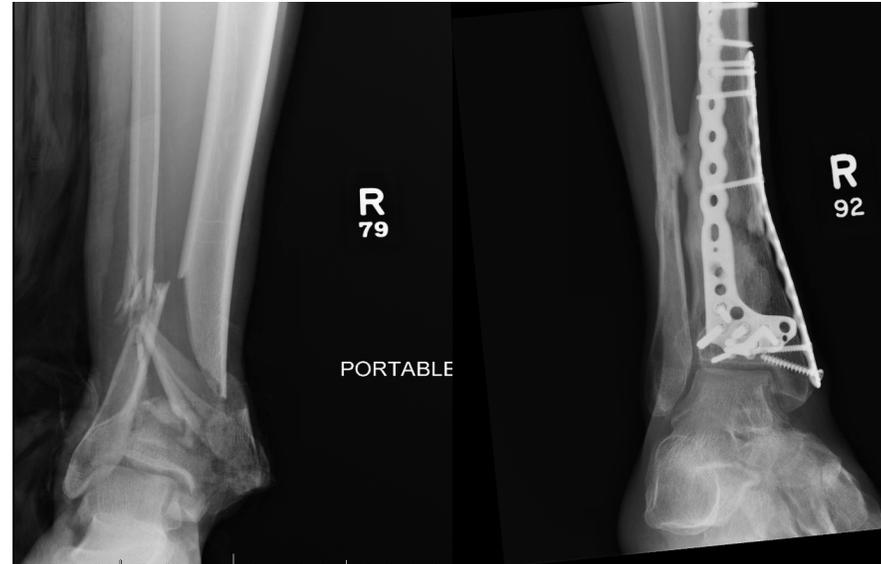


Not Enough Stability Results in Hardware failure and nonunion

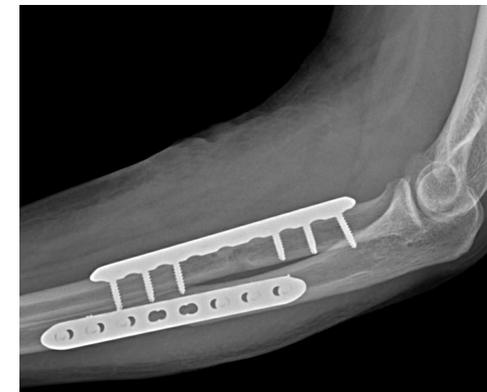


Absolute Stability

- Articular Fractures
 - Pilon
 - Tibial Plateau
 - Distal Humerus
- Anatomic Reductions
 - Fibular Fractures
 - Humeral Shaft
 - Radial and Ulnar Shafts



Injury Xray Open Pilon Fracture and Post Op Xray about 12mo



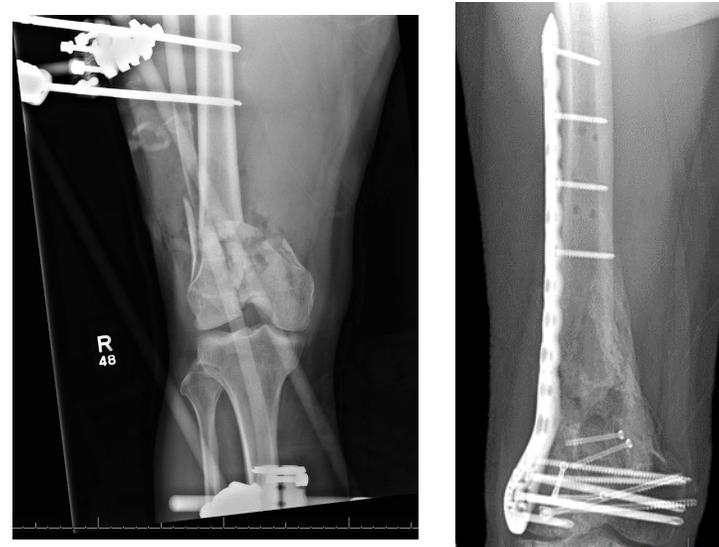
Injury Xray Both Bone Forearm fracture and Post Op Xray 6 mo

Relative Stability

- High Comminution
- Long Bone Fractures
 - Tibia Midshaft Fractures
 - Femur Midshaft Fractures
- Metaphyseal fractures
 - Distal Femur Fractures
 - Proximal Femur fractures



Injury Xray and Post Op 12 months



Injury Xray and Post op 6 months

Summary

- Two main types of bone cells are osteoblasts and osteoclasts
- Two main pathways of bone healing are intramembranous and endochondral ossification
- There are many molecules that play a part and effect bone healing
- Stability of the fracture and blood flow to the region are the most important factors in having a successfully healed fracture