PLEASE CLICK ON THE FOLLOWING LINK TO WATCH THE LECTURE ONLINE:-

https://www.youtube.com/live/knh5CsidDj4?si=Kxw5CTn_o-VvJKbL

Biology of bone healing

Learning objectives

- Explain the different processes of bone healing and review direct and indirect bone healing
- Describe the factors that influence the healing process and those that may lead to delayed union or nonunion
- Recognize the importance of soft tissues for bone healing
- Diamond concept

Bone ?

Bone is a dynamic biological tissue composed of metabolically active cells that are integrated into a rigid framework.

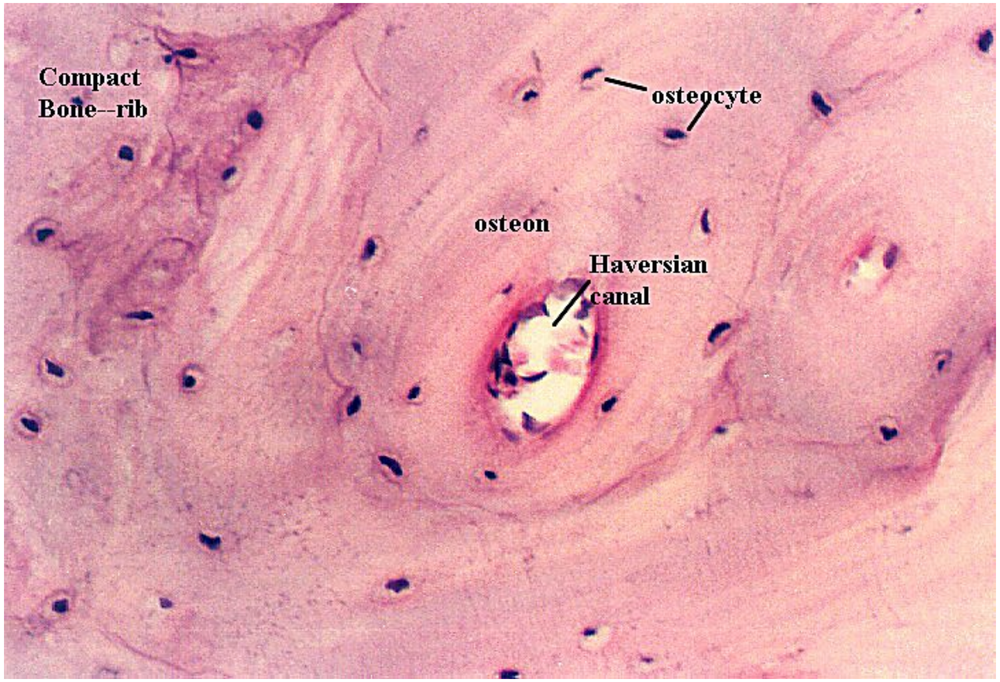
The healing potential of bone, whether in a fracture or fusion model, is influenced by a variety of biochemical, biomechanical, cellular, hormonal, and pathological mechanisms.

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.

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

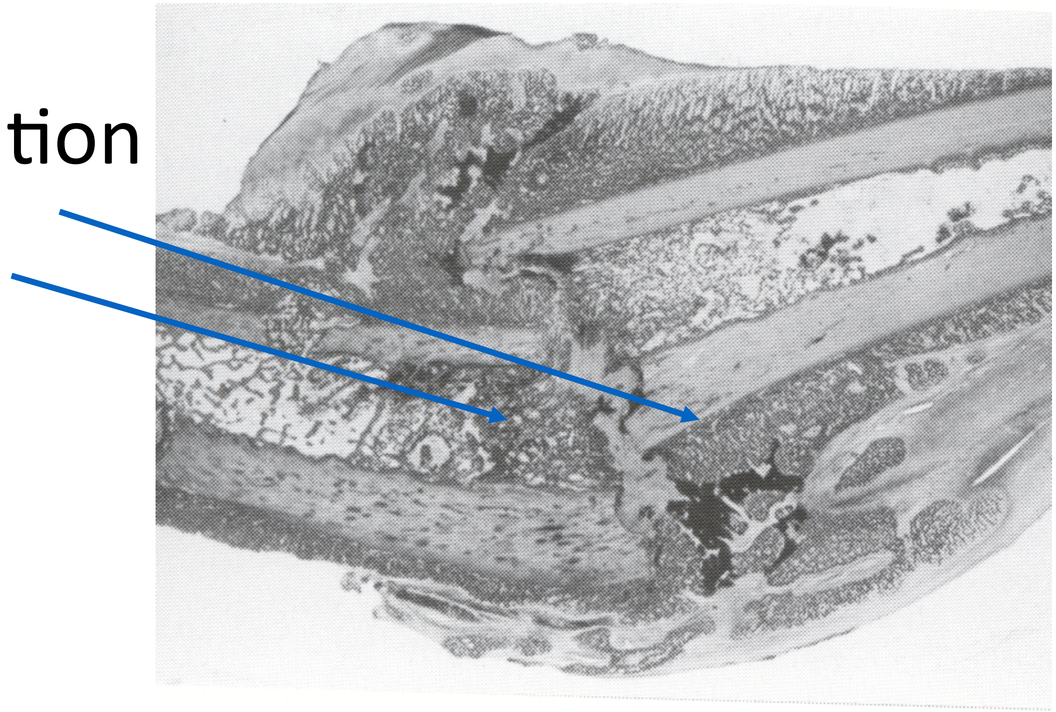


Figure from Rockwood and Green's: Fractures in Adults, 4th ed

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 $Ca_5(PO_4)_3(OH)_2$

Blood Supply

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

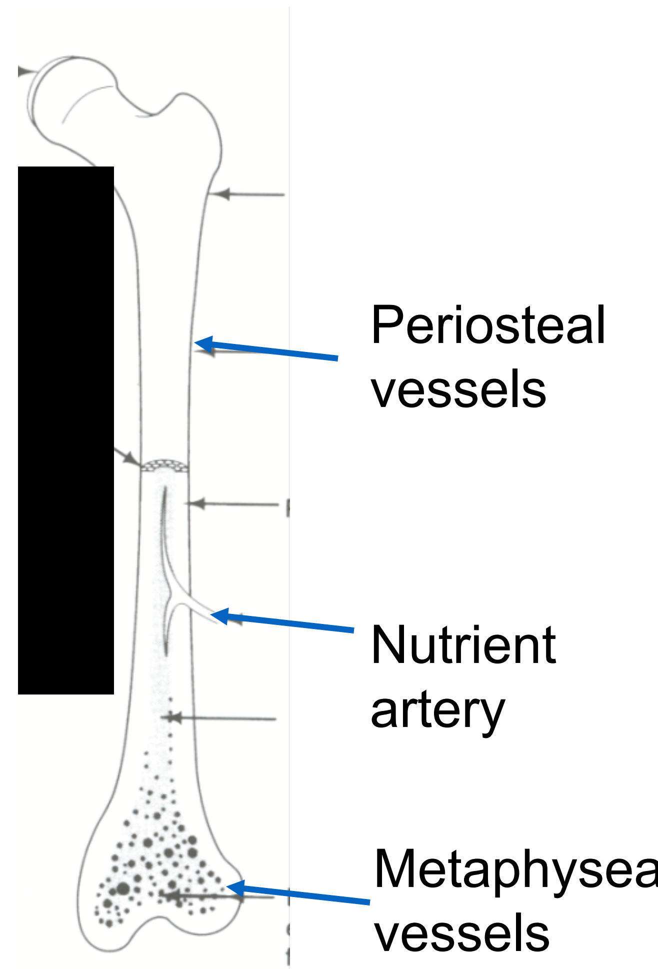


Figure adapted from Rockwood and Green, 5th Ed

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

Requirements for fracture healing

- Intact blood supply to the fractured fragments
- Immobilization by cast, internal fixation or external fixation
- Absence of infection

Different types of bone healing

- Bone healing—definitions :Bone Healing a complex sequential set of events to restore injured bone to pre-fracture condition

- Types of bone healing:

1.**primary bone healing** (strain is < 2%)

intramembranous healing

occurs via Haversian remodeling

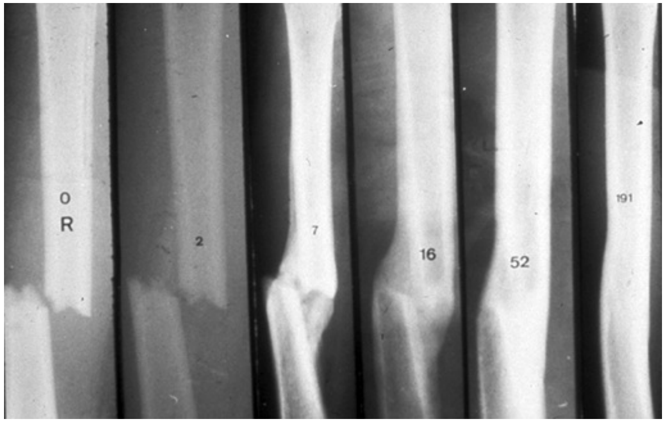
2. **secondary bone healing (strain is between 2%-10%)**

- involves responses in the periosteum and external soft tissues.
- endochondral healing

Radiological

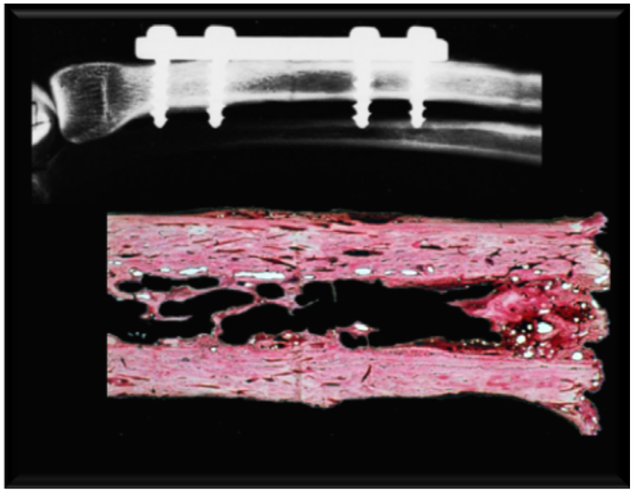
Visible callus formation

Indirect healing



No visible callus formation

Direct healing

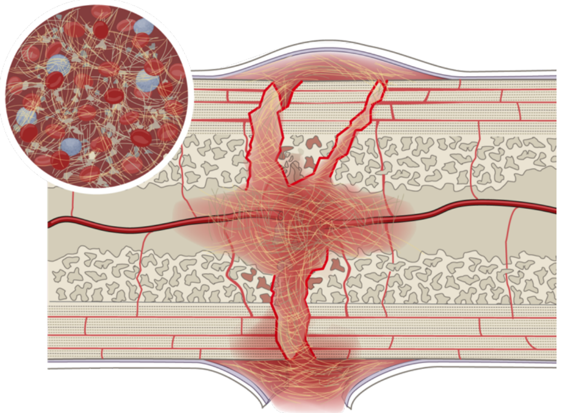


Stages of Fracture Healing

- Inflammation
- Repair
- Remodeling

• Inflammation

- Hematoma forms and provides a source of hematopoietic cells capable of secreting growth factors
- Fibrin fibers stabilize the hematoma (hematoma callus)



The stage of hematoma followed by Inflammation:

- There be a lot of cells in this stage (Macrophages, neutrophils, and platelets release several cytokines)
- this includes PDGF, TNF-Alpha, TGF-Beta, IL-1,6, 10,12

- BMPs, fibroblasts and mesenchymal cells migrate to fracture site and granulation tissue forms around fracture ends

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

Mesenchymal stem cells

- Mesenchymal stem cells are undifferentiated and they differentiate to osteoblasts and chondroblasts

- Important sources of mesenchymal stem cells in fractures include periosteum, bone marrow and surrounding muscles

- Gathered during the inflammation process

Repair

- **Callus** is a temporary bony material, cartilaginous material, fibrous connective tissue and woven bone that replaces the hematoma

Repair

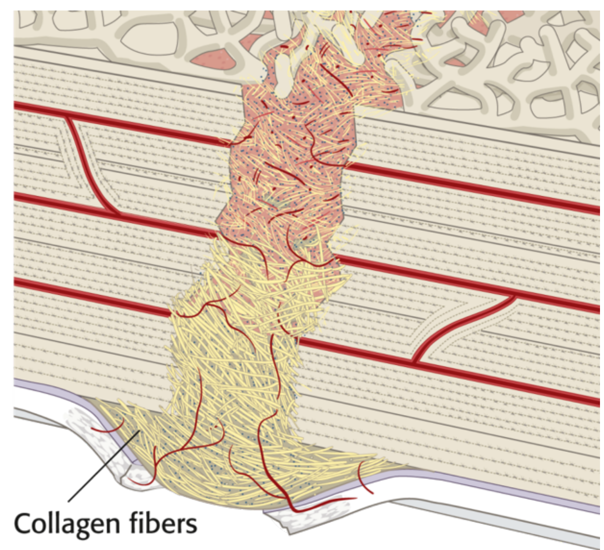
Natural bone healing process begins with soft callus:

•New blood vessels invade the hematoma

•Fibroblasts, derived from the periosteum, colonize the hematoma

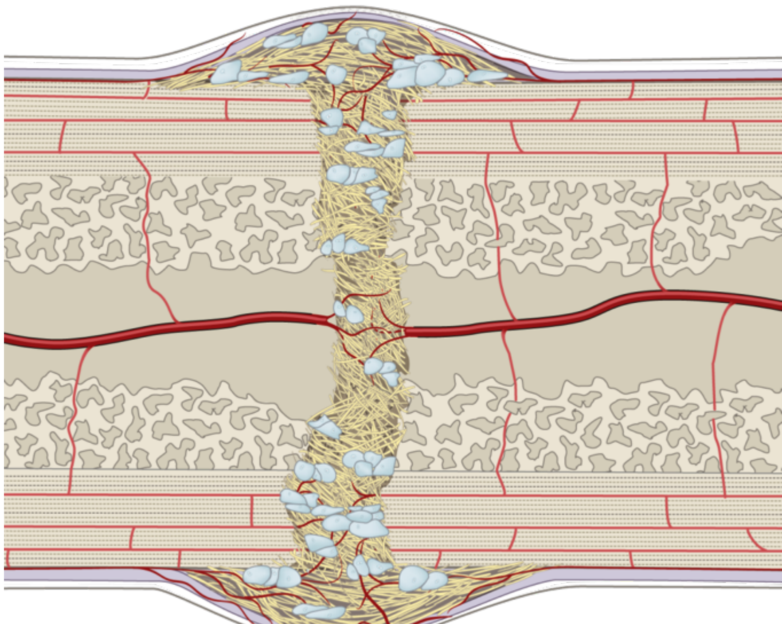
•Fibroblasts produce collagen fibers (granulation tissue)

•Collagen fibers loosely link the bone fragments



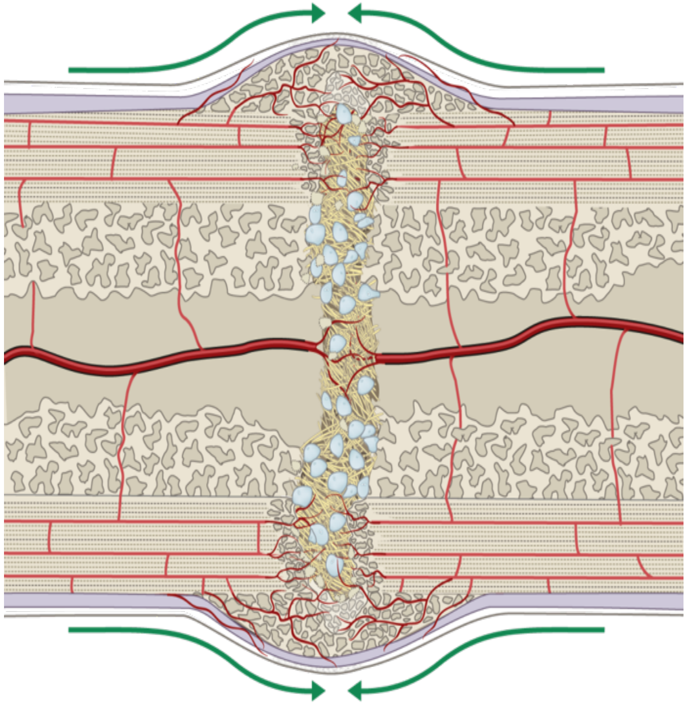
granulation phase, soft callus

- Granulation tissue gradually differentiates into fibrous tissue, and subsequently fibrocartilage
- Within 2 weeks
- During fracture healing granulation tissue tolerates the greatest strain before failure



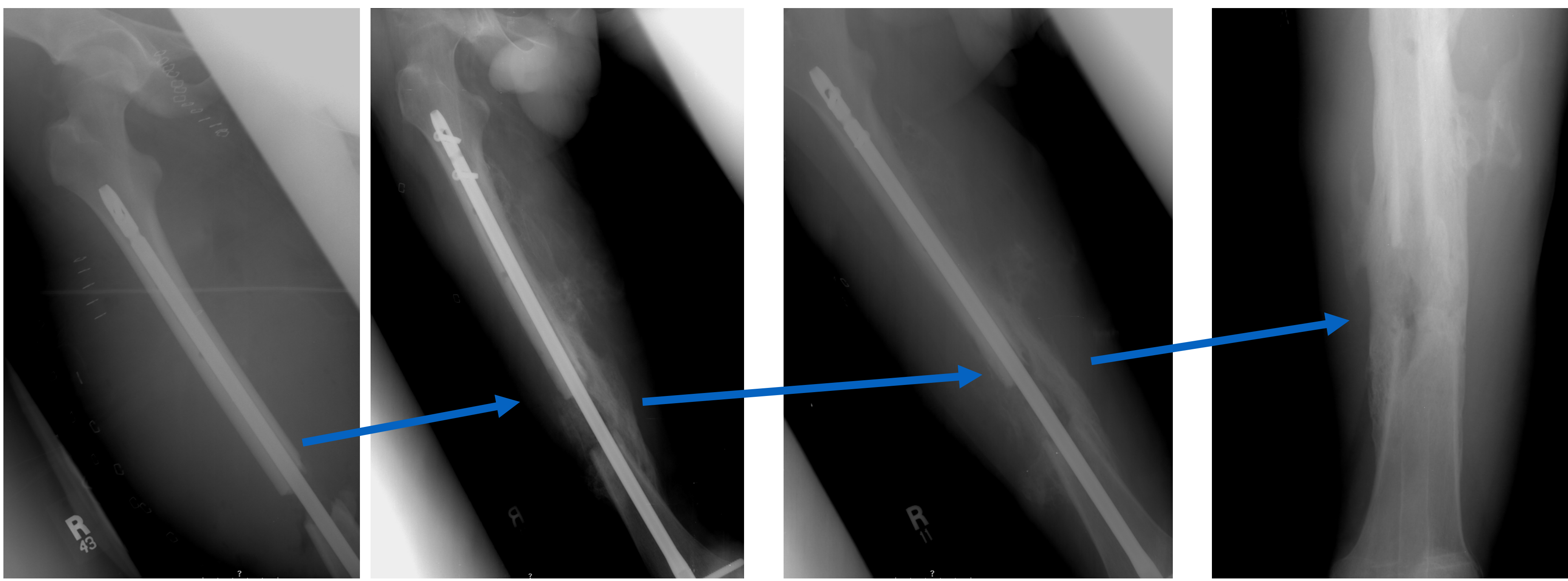
granulation phase, hard callus

- Hard callus stage starts and lasts until the fragments are firmly united by new bone (3–4 months)
- Endochondral ossification forms spindle-shaped bone cuffs
- Starts at the periphery and moves toward the center, further stiffening the healing tissue
- Collagen change from type II to type I
- Type I= bone
- Type II= cartilge



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

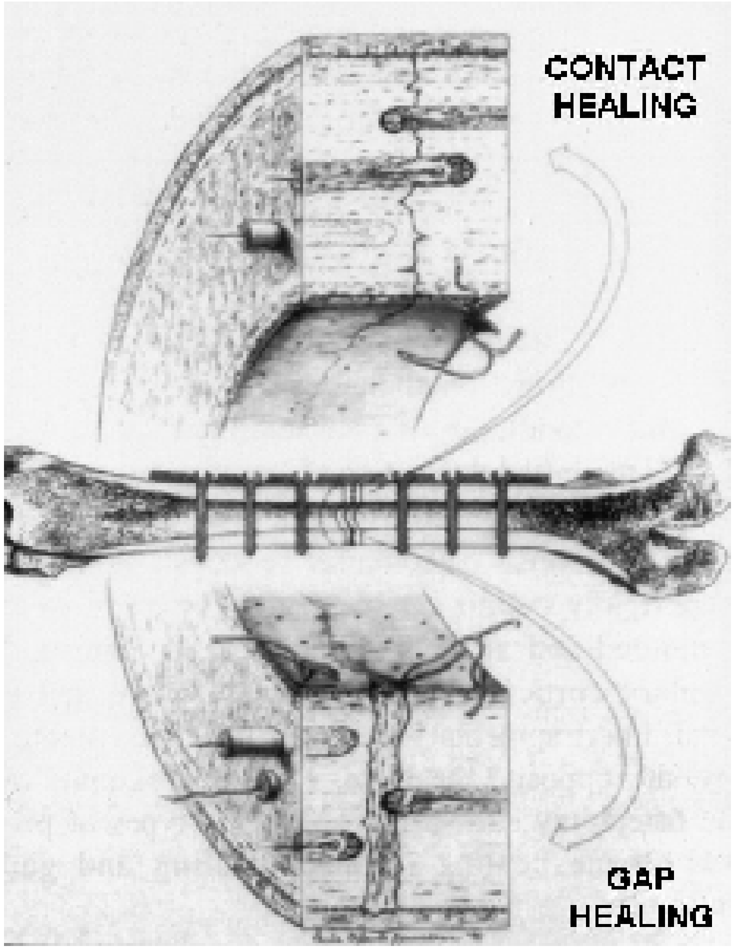
- Bone ends are put in close contact

- When fracture surfaces are in intimate contact (less than 0.1 mm distance) with absolute stability => osteoclasts will form cutting cones that traverse the fracture line => capillaries occupy the newly formed cavities with osteoblasts => form lamellar bone from osteons
- The intermediate stages are skipped (no callus what so ever)
- Primary bone healing process called cutting cone remodeling, Haversian remodeling

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



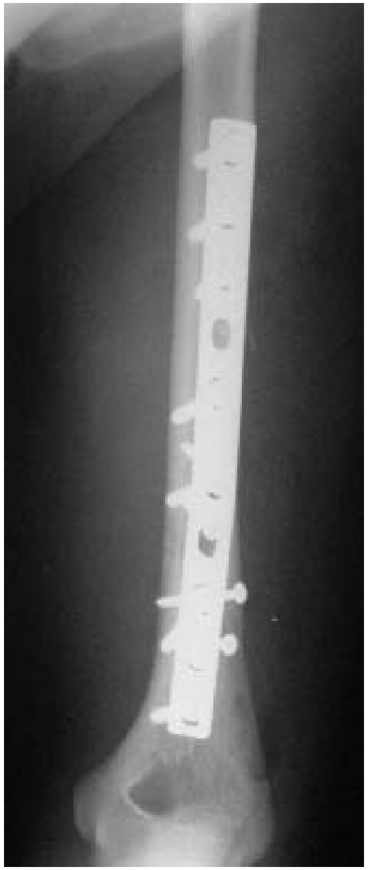
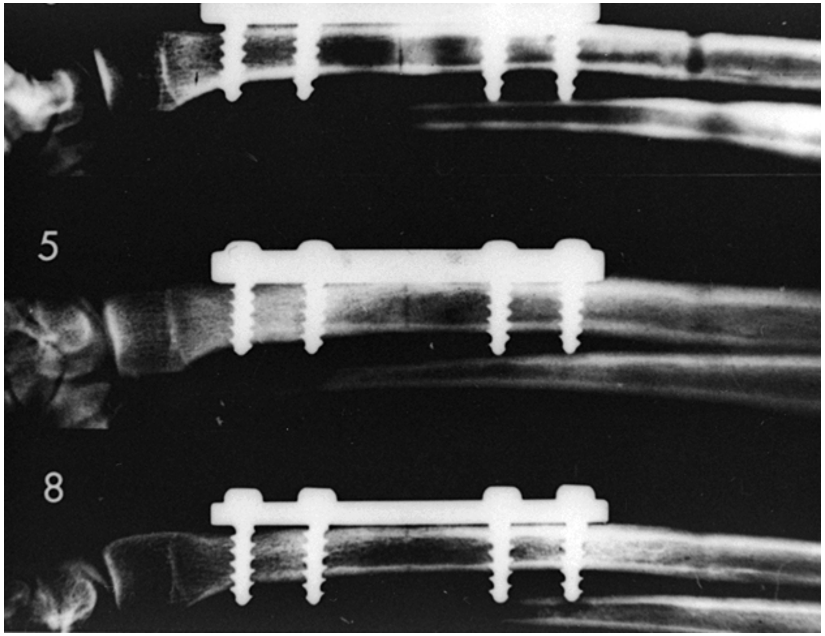


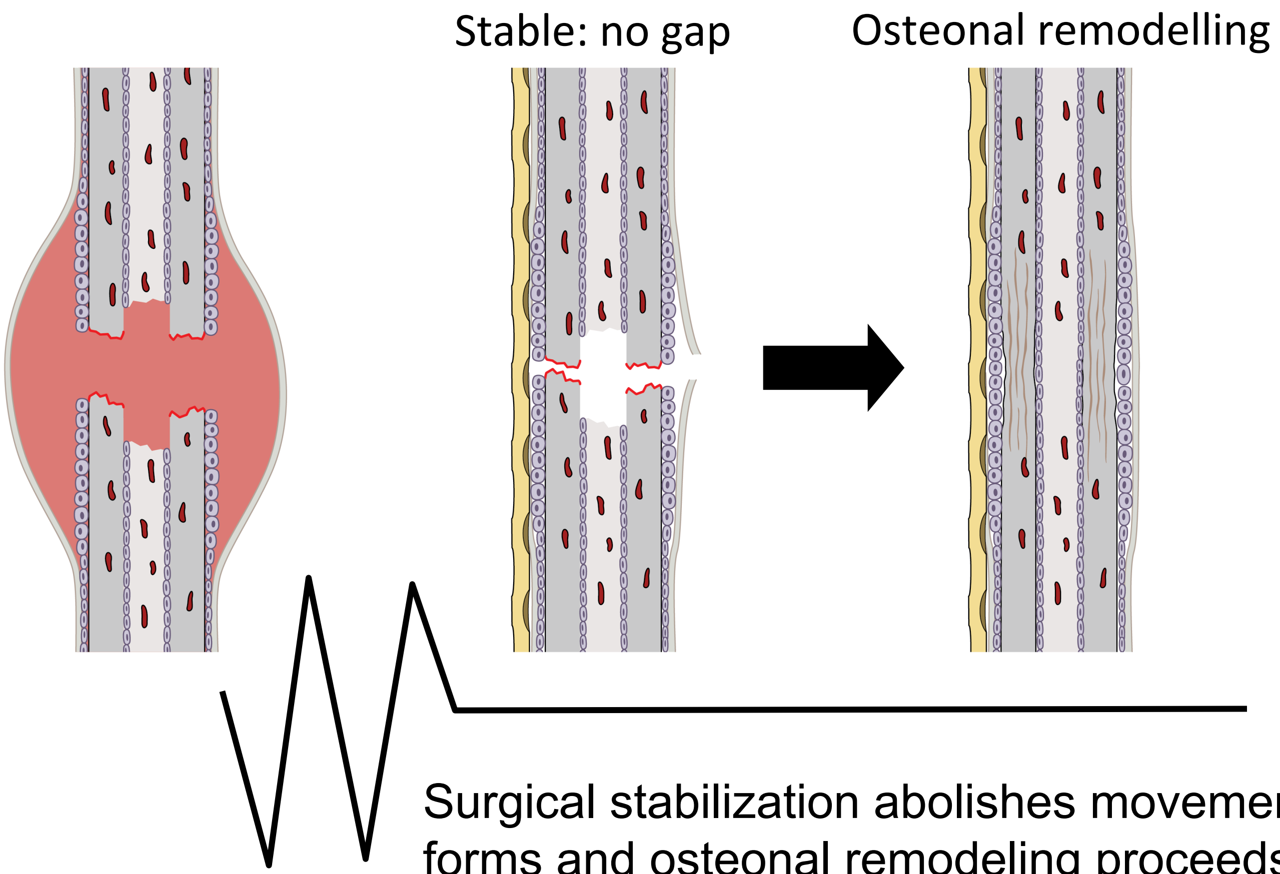
Figure from http://www.vetmed.ufl.edu/sacs/notes

Direct bone healing

- Gap < 2 mm
- No intermediate fibrous tissue
- No movement



Direct bone healing—mechanical effect of internal fixation



Surgical stabilization abolishes movement, so no callus forms and osteonal remodeling proceeds immediately

Indirect Bone Healing

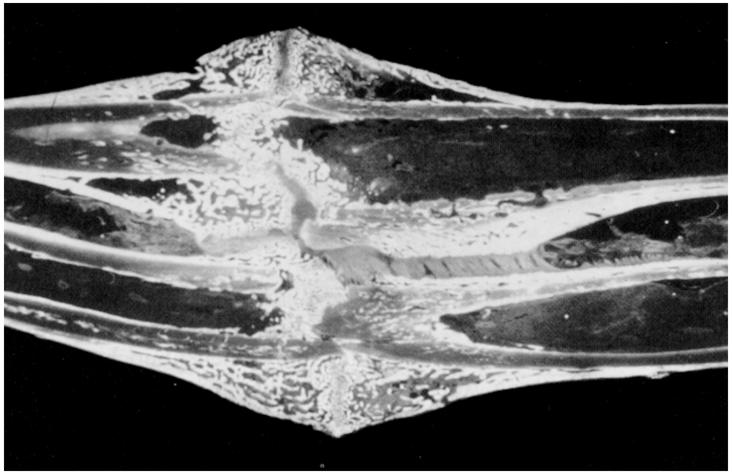
- Mechanism for healing in fractures that have some motion, but not enough to disrupt the healing process.
- Bridging periosteal (soft) callus and medullary (hard) callus re-establish structural continuity
- Callus subsequently undergoes endochondral ossification
- Process fairly rapid - weeks

Indirect bone healing

Gap > 2 mm

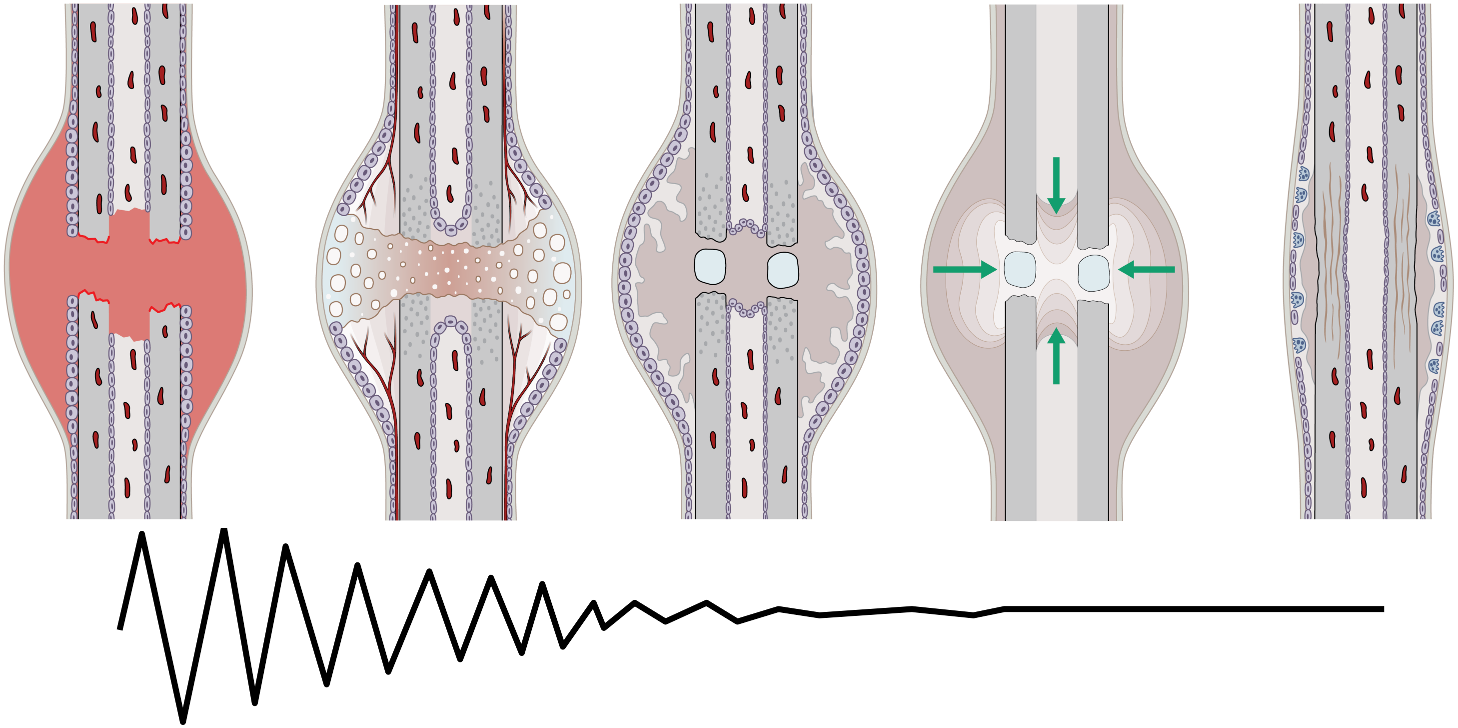
Controlled motion

Living bone



- Granulation tissue
- Ingrowth of vessels
- Fibrocartilage → calcification
- Calcified cartilage → woven bone
- Woven bone → lamellar bone
- Osteonal remodelling

Indirect bone healing—mechanical effect

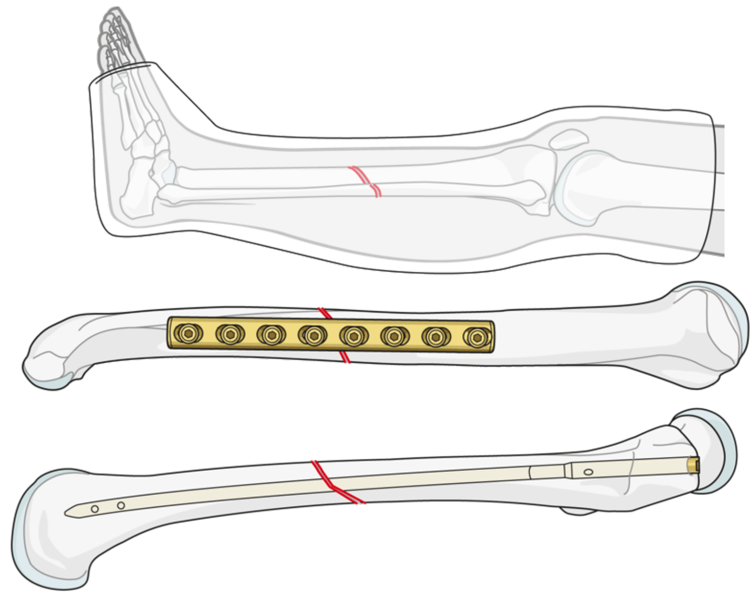


As the callus forms and stiffens, movement is abolished and normal osteonal remodeling can occur

Micromotion—Strain theory

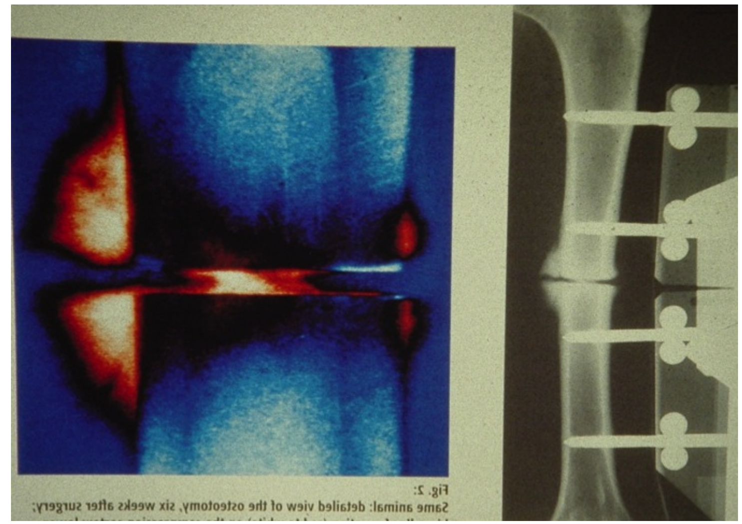
- Load applied to a material produces stress within the material and results in deformation (strain)

- Following a fracture, any motion of one main fragment relative to the other is projected to the fracture zone



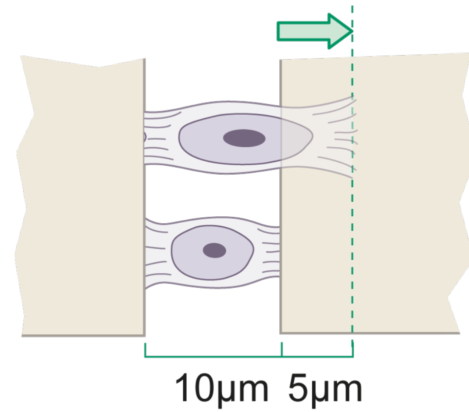
High strain in small gaps

- If only two fragments are involved, the sum of all motion will be projected into the single fracture gap
- Motion amplitudes will limit the capacity of the soft repair tissue (hematoma → collagen → soft callus) to withstand shear and dislocation forces
- If the “strain” on the tissue is too great, tissue integrity is disrupted

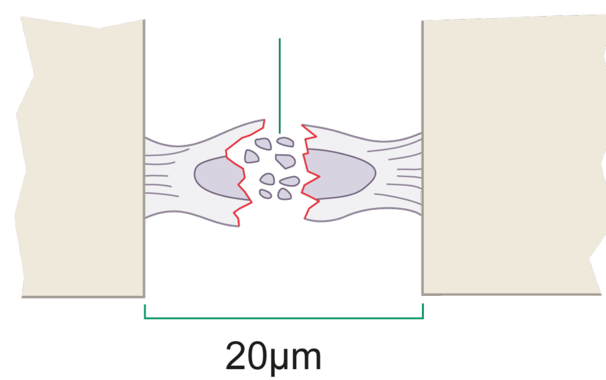


High strain in small gaps

- In a minute gap with only few bridging cells, any micromotion not contained by absolute stability will exceed strain tolerance of the tissues involved and the cell structure is destroyed



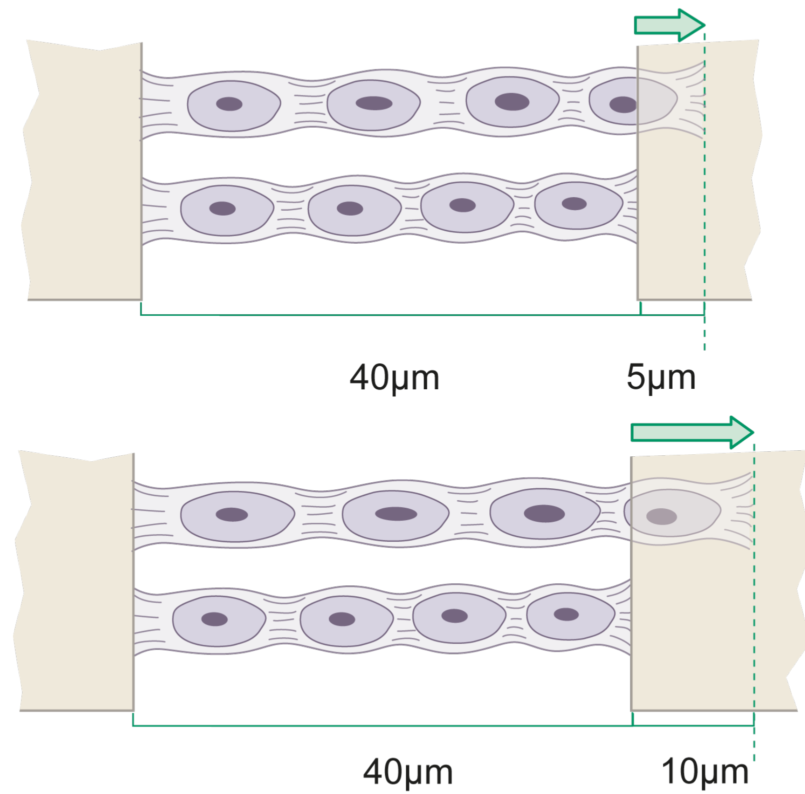
- Tissue specific strain tolerances:
  - Granulation tissue: 100%
  - Lamellar bone: 2%



Low strain in large gaps

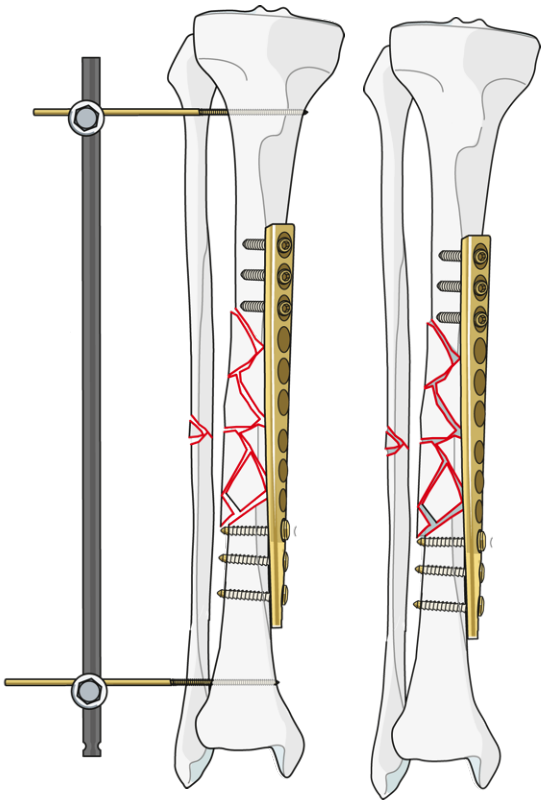
- If the gap is widened (by bone surface resorption), the strain is shared by many more bridging soft-tissue elements and fragment motion does not create an intolerable strain on individual cells

- In larger gaps, **the strain on individual cells is reduced**

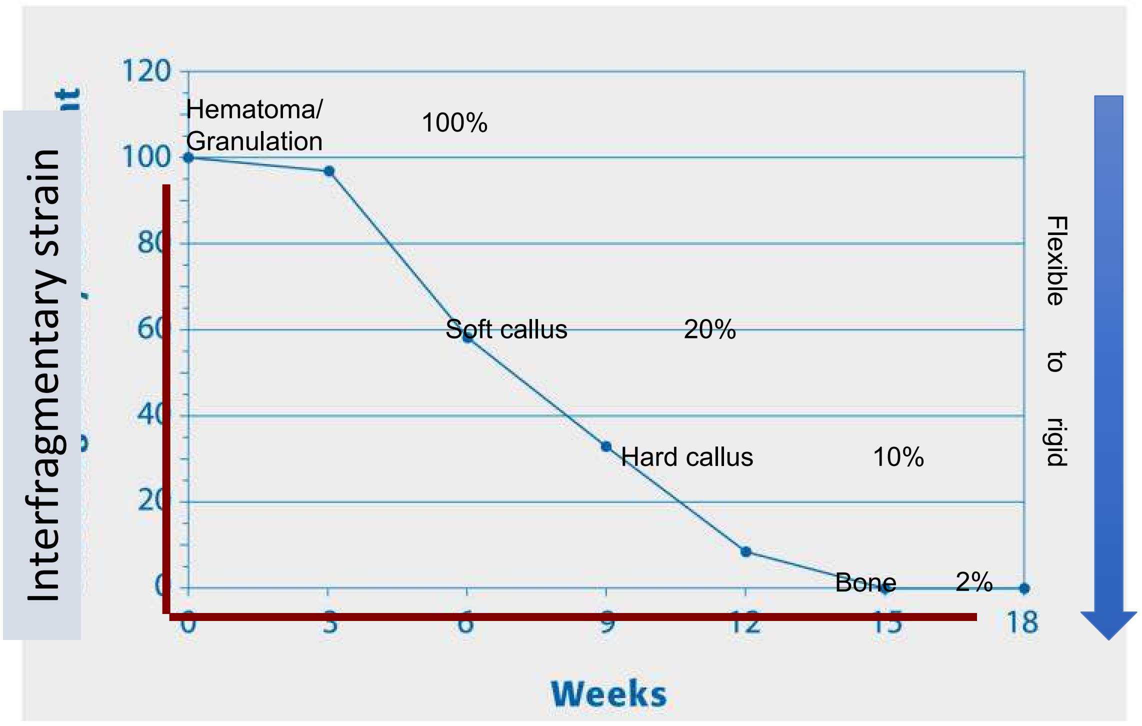


Strain

- This phenomenon explains why strain sharing permits multifragmentary fractures to heal well
- Multiple serial gaps share the overall displacement, and callus induction occurs despite relatively high total motion
- Different strains in different gap sizes also explain why various tissues, ranging from loose connective and fibrocartilage tissue, may exist simultaneously



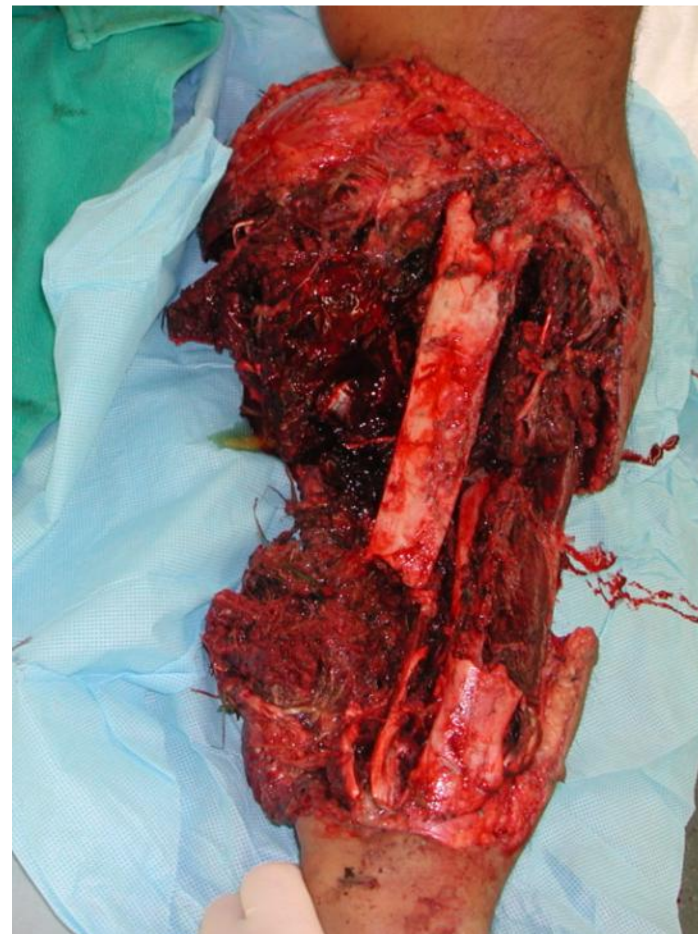
Mechanobiology of bone healing



Indirect bone healing
Direct bone healing

Local Anatomic Factors That Influence Fracture Healing

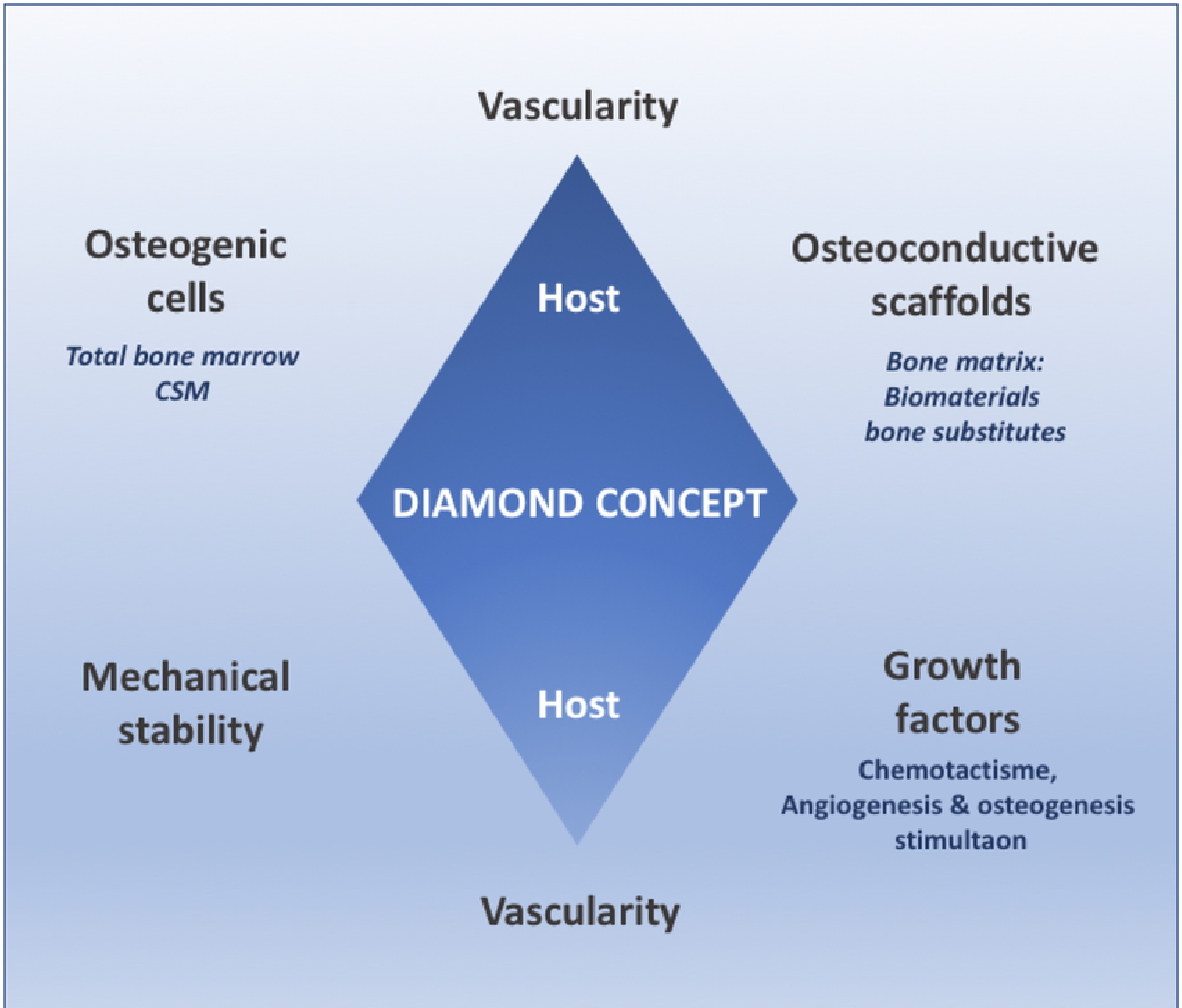
- Soft tissue injury
- Interruption of local blood supply
- Interposition of soft tissue at fracture site
- Bone death caused by radiation, thermal or chemical burns or infection



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

'Diamond concept'



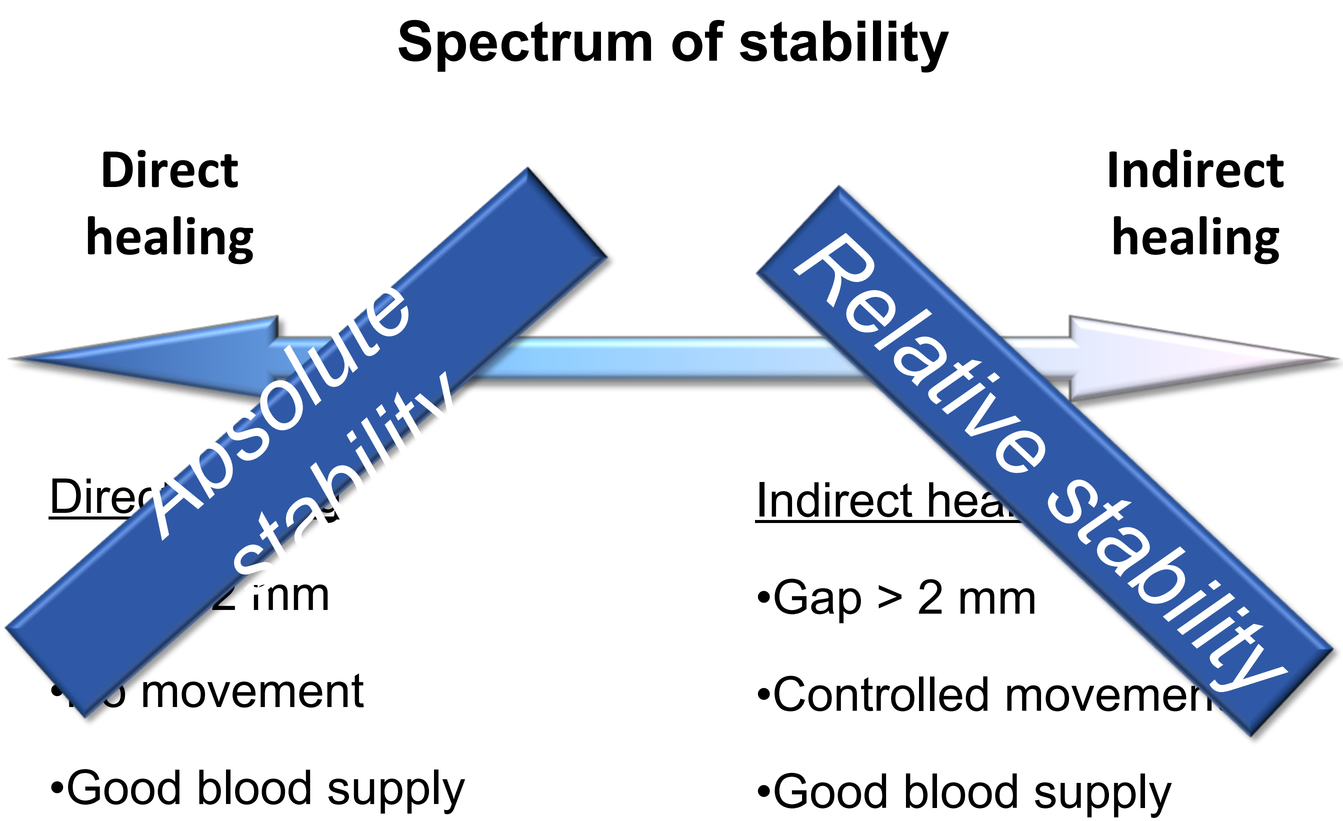
Take-home messages

- Complex structure heals by replication and remodelling
- Bone is programmed to heal:
  - Must be living
  - Controlled movement
- Type of healing varies with mechanical environment

Take-home messages

- Bone healing is a cascade of biological events leading to restoration of the continuity and mechanical properties of the bone
- Healing is dependent on mechanical and biological factors that are closely associated with bone blood supply
- Fracture stability dictates the biologic response:
  - Absolute stability = direct healing
  - Relative stability = callus healing

Take-home messages



Thank you