Paul J. McDermott, Ph.D.
mcdermp@musc.edu
Office: 843 792-3462

HISTOLOGY OF BONE

A. ANATOMICAL BONE STRUCTURE

1. Long Bones
2. Short Bones
3. Flat (Membrane) Bones

B. MAIN COMPONENTS OF BONE TISSUE

1. Cell Types
2. Composition of Bone Matrix

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2. Compact (Dense) Bone

D. COVERINGS OF BONE

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E. STRUCTURE AND FUNCTION OF LAMELLAR BONE

1. Compact Bone
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1. Osteocytes
2. Osteoblasts
3. Osteoprogenitor Cells
4. Osteoclasts

G. HORMONAL REGULATION OF OSTEOCLAST ACTIVITY

1. Parathyroid Hormone (PTH)
2. Calcitonin

H. OSTEOPOROSIS

Suggested Reading:

Junqueira's Basic Histology Text and Atlas, 17th Ed., © 2024, McGraw-Hill, Chapter 8

OBJECTIVES

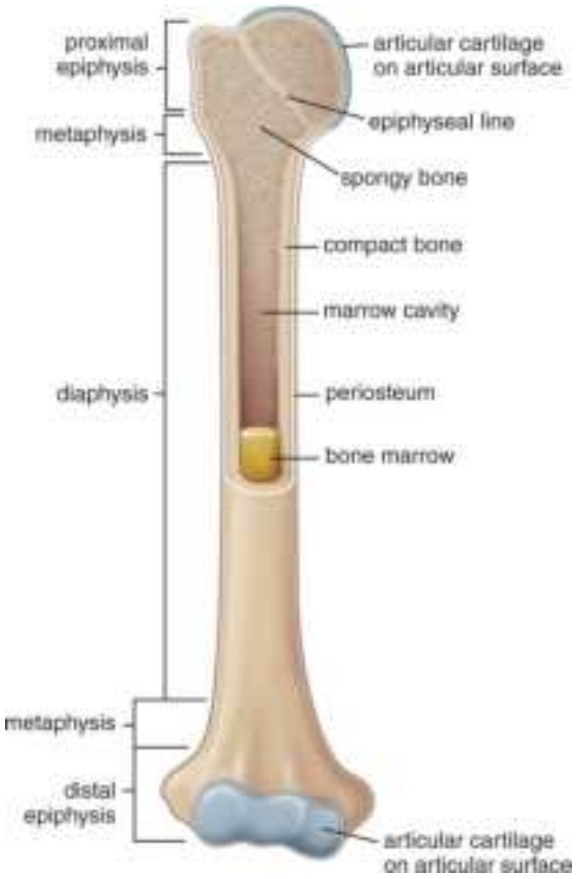
1. Describe the structure and function of the following cell types: osteoblasts, osteocytes, osteoprogenitor cells, osteoclasts.
2. Describe the components of the bone matrix and its functional properties.
3. Specify the proteins secreted by osteoblasts and their role in bone formation.
4. Differentiate between osteoid and mineralized bone matrix.
5. Explain the structural and functional differences between compact and spongy bone.
6. Describe the structural organization of an osteon and the function of the following components: lacunae, Haversian canals, Volkmann canals, canaliculi.
7. Distinguish between interstitial lamellae, external circumferential lamellae and internal circumferential lamellae in compact bone.
8. Describe the structure and function of the periosteum and the endosteum.
9. Describe how osteoblasts regulate the differentiation and activation of osteoclasts
10. Describe the process of bone resorption by osteoclasts.
11. Describe the roles of parathyroid hormone and calcitonin in regulating osteoclast activity.
12. Describe the structural changes in bone associated with osteoporosis.

Illustrations adapted from:

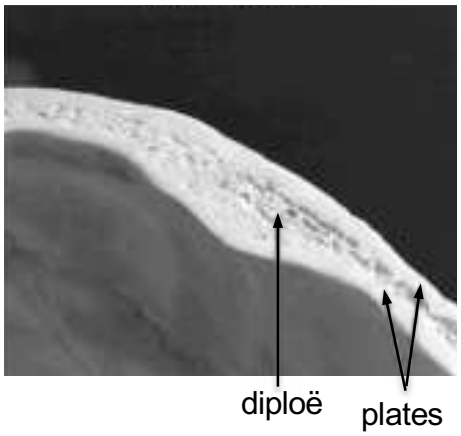
- Histology: A Text and Atlas 7th Ed., © 2016, Wolters Kluwer
- Junqueira's Basic Histology 17th Ed., © 2024, The McGraw-Hill Companies, Inc.

A. ANATOMICAL BONE STRUCTURE

1. **<u>Long Bones</u>** (*e.g.* femur, tibia, humerus) ⟶
   - **a) Epiphysis**
   - **b) Epiphyseal Plate**
   - **c) Metaphysis**
   - **d) Diaphysis**
   - **e) Nutrient Canal**
   - **f) Articular surfaces**
2. **<u>Short Bones</u>** (*e.g.* carpal bones)
   Nearly equal in length and diameter
3. **<u>Flat (Membrane) Bones</u>** (*e.g.* skull)
   - **a) Internal Plate**
   - **b) Diploë**
   - **c) External Plate**



Section through the skull



B. MAIN COMPONENTS OF BONE TISSUE

Bone is a specialized connective tissue that is composed of **5 primary cell types** and an elaborate calcified extracellular matrix, which is referred to as the **bone matrix**.

1. **<u>Cell Types</u>**
   - **a) Osteocytes:** terminally differentiated cells that are located within **lacunae** of lamellar bone. Osteocytes communicate via cytoplasmic processes that extend through canaliculi in the bone matrix to form gap junctions. Osteocytes that cover the **surfaces** of bone (not in lacunae) have cytoplasmic processes that communicate with osteocytes in lacunae through canaliculi.
   - **b) Osteoblasts:** cell type responsible for **synthesis** and **mineralization** of bone matrix during growth and remodeling. They differentiate into osteocytes in lacunae or lining bone surfaces.
   - **c) Osteoprogenitor Cells:** mesenchymal stem cells that **differentiate** into osteoblasts and and osteocytes.
   - **d) Osteoclasts**: multinucleated cells derived from a **monocyte lineage** that are involved in the **resorption** and **remodeling** of bone tissue.
2. **<u>Composition of Bone Matrix</u>**
   - **a) ECM:** mostly **Type I collagen** embedded in ground substance containing: 1) **proteoglycans** with **chondroitin sulfate** and **keratin sulfate glycosaminoglycans**, 2) adhesive glycoproteins such as **dentin matrix protein** (DMP) and **osteopontin**, 3) calcium binding proteins such as **osteocalcin** and **osteonectin**, 4) protein phosphatases, 5) growth factors and cytokines.
   - **b) Inorganic Components:** mineralization of bone tissue occurs by deposition of Ca^{2+} and PO_4^- to form hydroxyapatite crystals [$Ca_{10}(PO_4)_6(OH)_2$].

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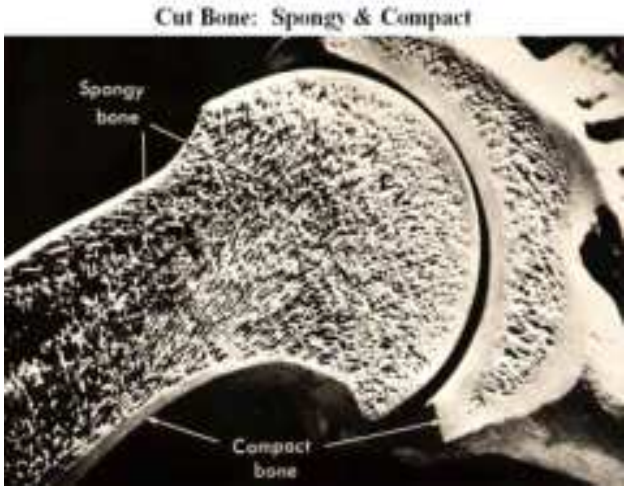
C. TYPES OF BONE TISSUE

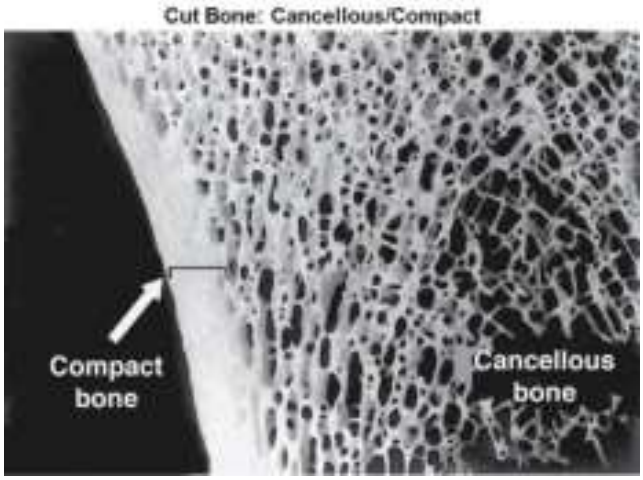
1. Spongy (Cancellous) Bone

Spongy bone is composed of bony spicules or trabeculae that are organized into a lattice-like network. It is found mainly in the epiphyses of long bones and it constitutes the diploë that is located between the outer and inner layers of compact bones in the skull.

2. Compact (Dense) Bone

In long bones, compact bone forms the diaphysis and lines the outer surface of the epiphyses. In the flat bones such as the skull, compact bone forms the inner and outer plates.





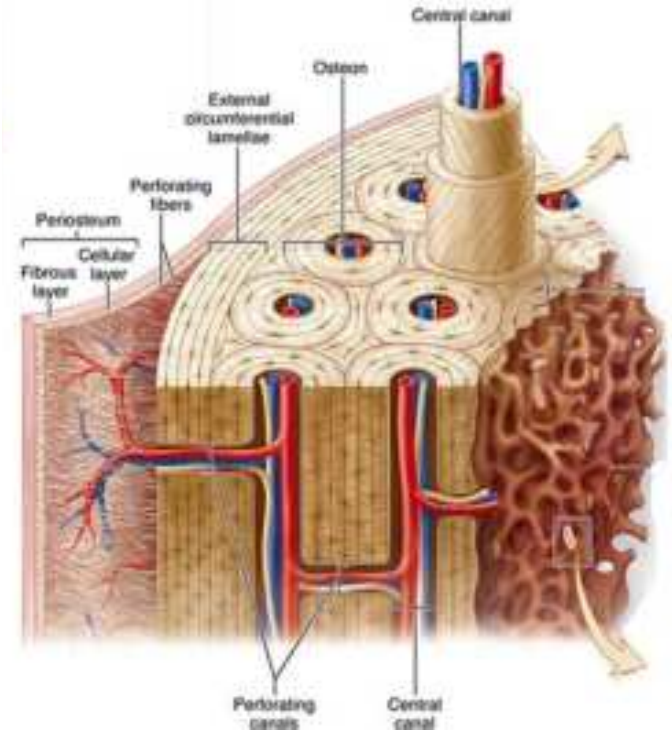
In these images, dense compact bone can be seen covering the inner mass of spongy or cancellous bone. Hematopoietic tissue fills the spaces between the bony trabeculae of the cancellous bone. By limiting the amount of heavy, compact bone, spongy bone reduces overall mass to lighten the bones. Nonetheless, from a biomechanical standpoint, the spongy bone architecture provides for a substantial amount of strength and structural integrity while providing space for production of blood cells in the bone marrow.

D. COVERINGS OF BONE

1. Periosteum: 2 Layers

a) Fibrous Layer: dense, irregular connective tissue containing blood vessels. It gives rise to collagen fibers (**Sharpey** or **perforating fibers**) that extend into the bone matrix and bind it tightly to the underlying bone.

b) Cellular Layer: vascular layer containing **osteoprogenitor** cells that are capable of proliferating and differentiating into osteoblasts and osteocytes.

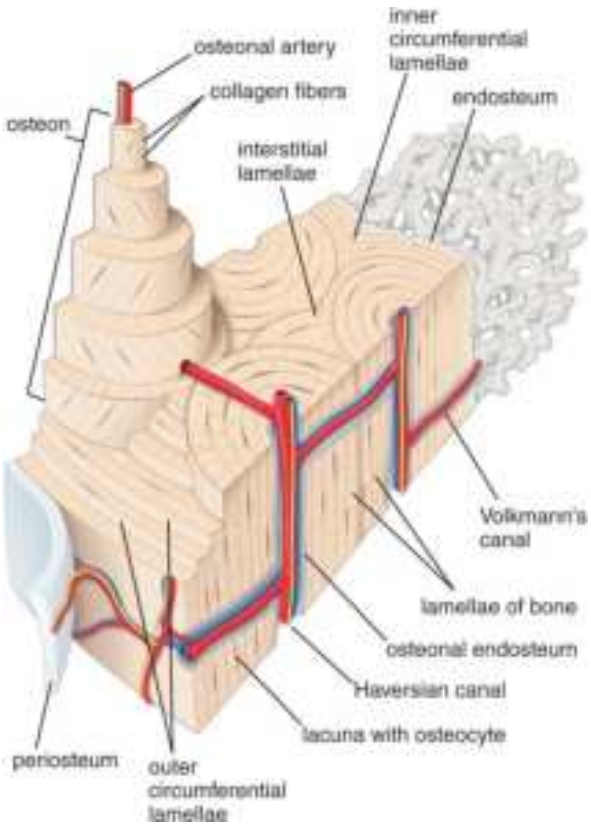


2. Endosteum

Thin cell layer that lines the internal surface of bones including the marrow cavity and the trabeculae of spongy bone. It consists of **osteoprogenitor** cells that can differentiate into osteoblasts and osteocytes.

E. STRUCTURE AND FUNCTION OF LAMELLAR (MATURE) BONE

Histologically, both **compact** and **spongy** bone are organized into multiple layers of calcified bone matrix referred to as **lamellar** bone. Collagen fibers within a layer or lamella of the bone matrix are arranged either in parallel or concentrically around a **central** (Haversian) canal. The direction of the collagen fibers in successive lamellae is oriented at right angles.

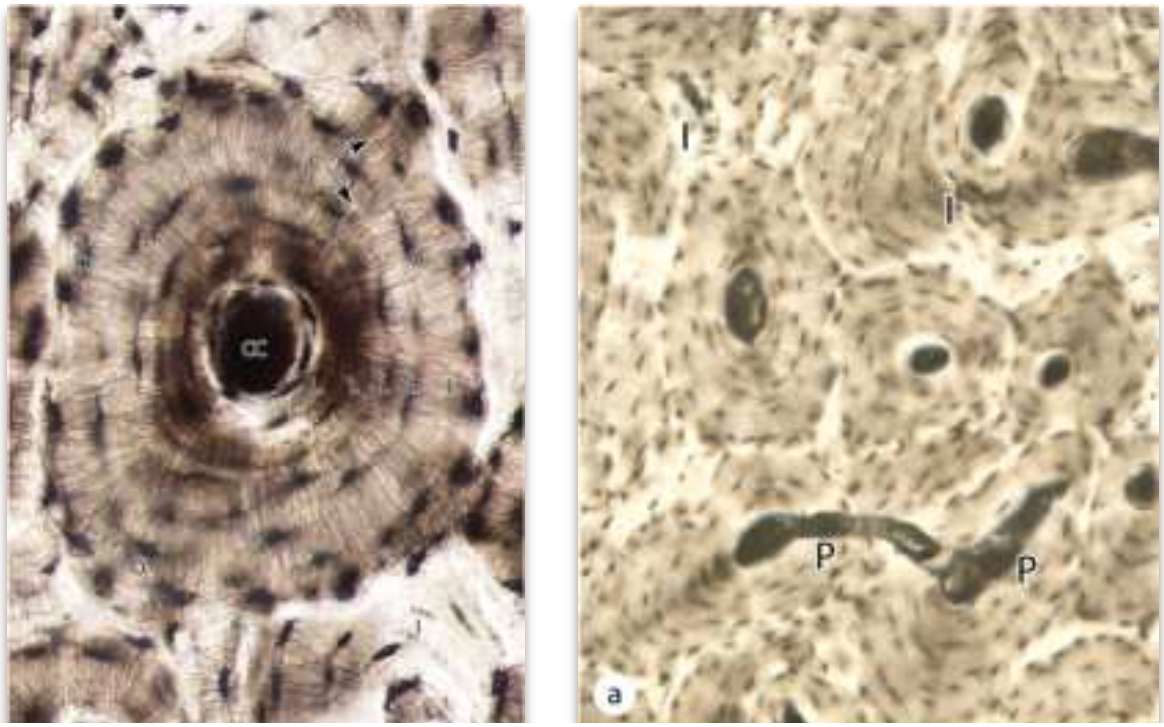


1. <u>Compact Bone</u>

a) Osteons (Haversian system)

The osteon is the primary structural unit of compact bone. It consists of 4-10 concentric lamella around the **Haversian or central canal.** Haversian canals are lined by endosteum and contain blood vessels, nerves and a small amount of connective tissue.

Osteocytes reside in **lacunae** that are located between lamellae. Osteocytes communicate with each other via **gap junctions** by sending out long, slender processes called **filopodia** through **canaliculi** (little canals).

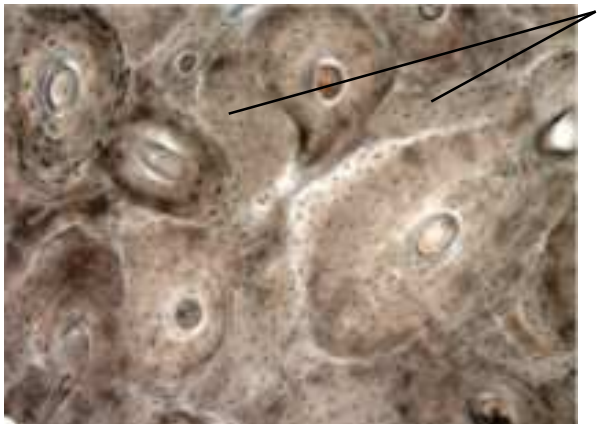


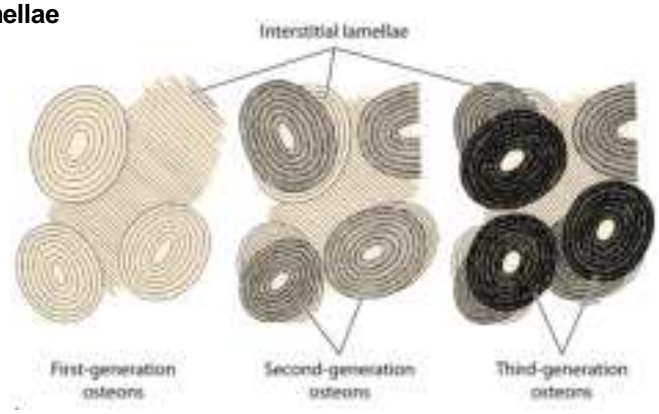
The upper left shows an osteon in a section of ground bone. Note the **concentric lamellae** around the **Haversian canal**, lacunae and the canaliculi. In the upper right, **Perforating** (P) or **Volkmann canals** form a network of channels that traverse through the bone matrix and connect the central canals. They provide a continuous means of communication between Haversian canals, periosteum and the marrow cavity.

E. STRUCTURE AND FUNCTION OF LAMELLAR (MATURE) BONE

1. Compact Bone

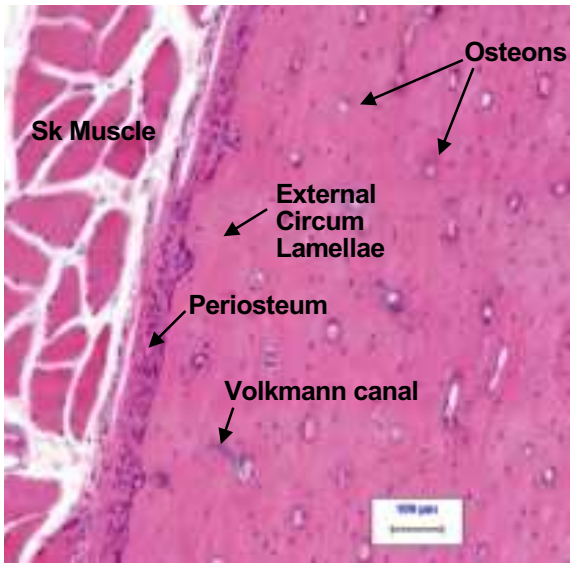
b) Interstitial Lamellae: lamellae situated between osteons in compact bone. They are remnants of osteons that have been partially degraded during bone growth and remodeling.

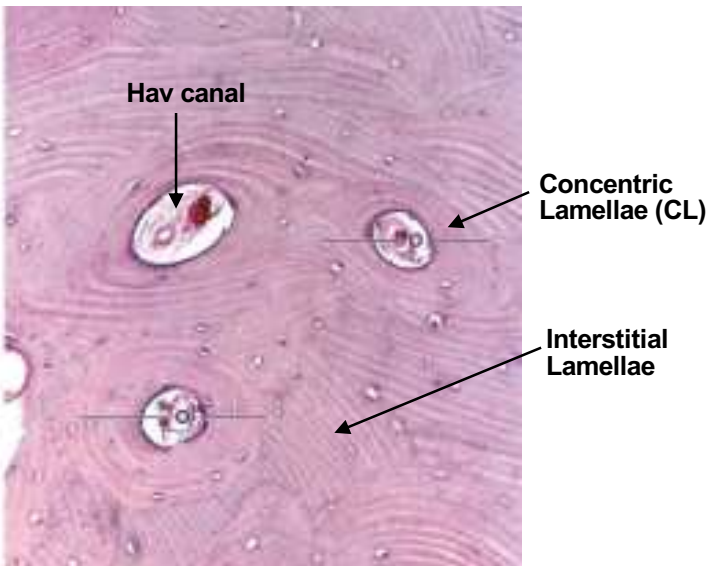




c) External Circumferential Lamellae: lamellae situated deep to the periosteum

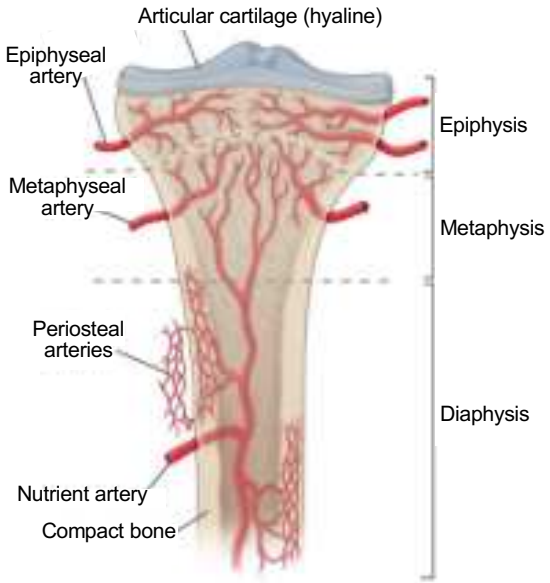
d) Internal Circumferential Lamellae: lamellae that surround the marrow cavity





e) Blood Supply

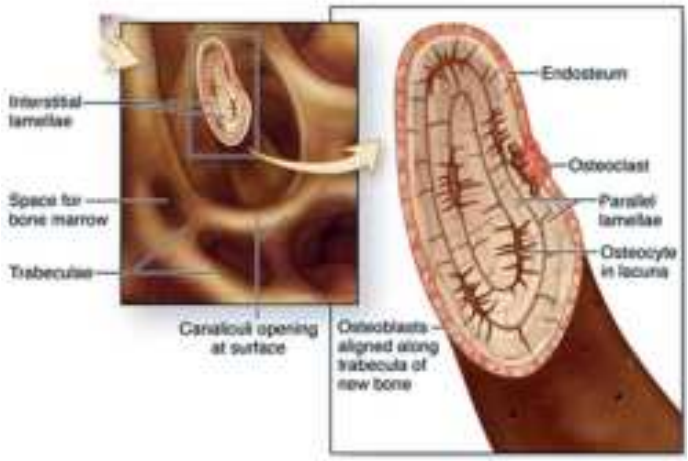
Bone is a **highly vascular tissue**. In long bones, most of the blood supply to the bone tissue is provided by arteries that enter initially into the marrow cavity via **nutrient foramina**. The blood vessels extend outward through Volkmann canals and branch further to supply the Haversian canals. As shown in this schematic, a portion of the blood supply arises from branches of periosteal arteries, which supply the outer part of the bone.



E. STRUCTURE AND FUNCTION OF LAMELLAR (MATURE) BONE

2. Spongy Bone

Spongy (trabecular) bone is also organized into lamellae. Like compact bone, osteocytes of spongy bone reside in lacunae and communicate via canaliculi by forming **gap junctions**. However, spongy bone usually does not have Haversian systems carrying blood vessels because the trabeculae are relatively thin, which allows for effective diffusion of nutrient materials from the marrow cavity.

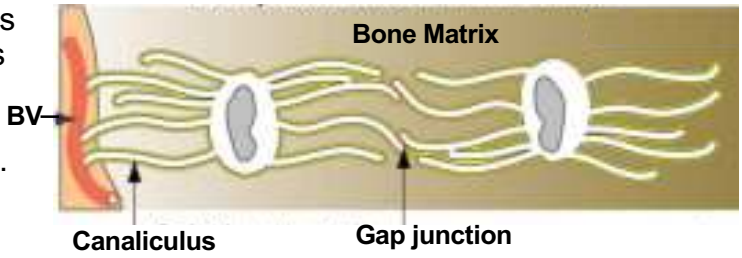


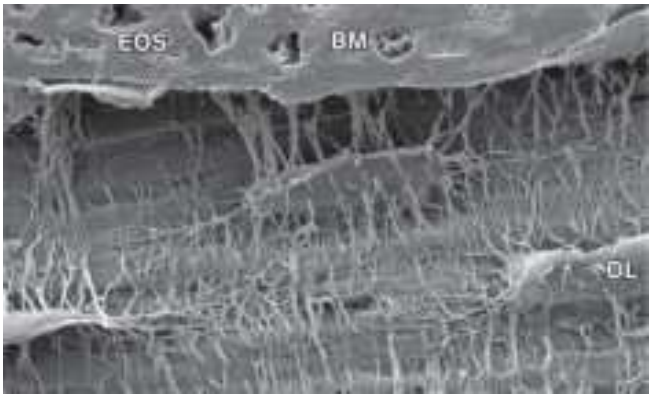
F. CELL TYPES OF BONE TISSUE

1. Osteocytes

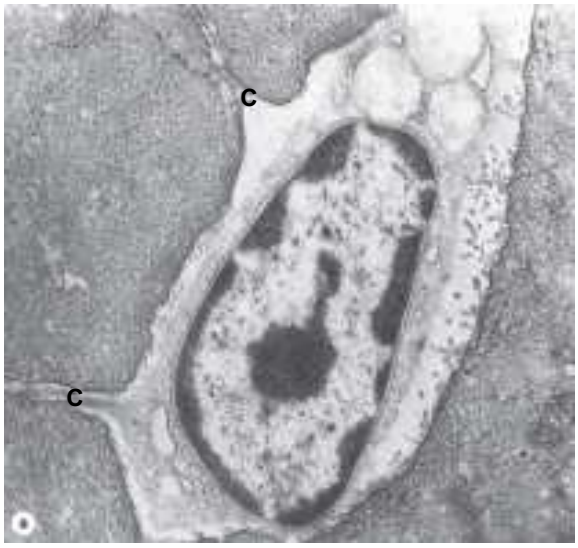
a) Structure: Osteocytes are derived from osteoblasts that have become surrounded by bone matrix. Once matrix is mineralized, an osteocyte occupies a space called a **lacuna**. Osteocytes function as a syncytium by sending out cytoplasmic processes through canaliculi to communicate with other osteocytes via gap junctions. In addition, osteocytes can form gap junctions with osteoblasts. Osteocytes are less active metabolically than osteoblasts.

- This schematic illustrates that osteocytes in lacunae are supplied by blood vessels (**BV**) in the Haversian canal. Nutrients are transported through the cytoplasmic processes away from a Haversian canal. In addition, a small extracellular space within the lumen of canaliculi transports molecules by passive diffusion.





Scanning EM of a resin-embedded bone sample that shows a network of canaliculi interconnecting three osteocyte lacunae (**OL**) and endosteal cells. The upper part of the image shows bone marrow cells (**BM**), which are separated from bone tissue by the endosteum (**EOS**).

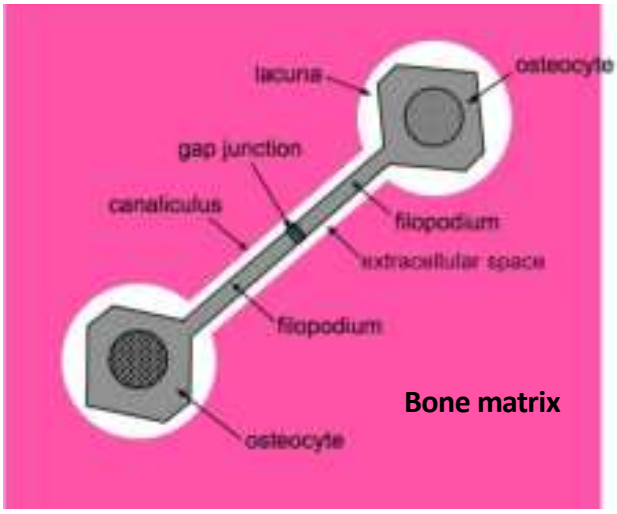


Transmission EM of an osteocyte in a lacuna. Note the cytoplasmic processes (**C**) extending into the canaliculi, the large euchromatic nucleus and RER. This image shows the extracellular space between the cytoplasmic processes of the osteocyte and the surrounding bone matrix of the canaliculi.

F. CELL TYPES OF BONE TISSUE

1. Osteocytes

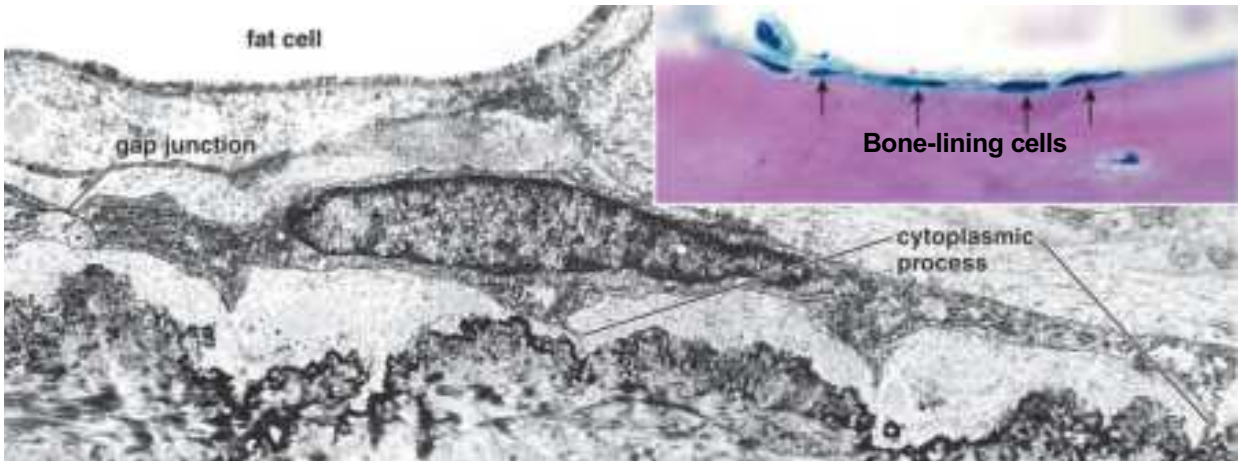
b) Functions



- Osteocytes have a major role in bone metabolism by participating in remodeling of the matrix. The osteocytes can change the volume of lacunae and the extracellular space in the canaliculi by resorption or deposition of their surrounding bone matrix. In fact, total surface area of bone matrix that surrounds the osteocytes in the lacunae and their associated cell processes in the canaliculi is much greater than the surface area of the outer and inner surfaces of the bone itself. Thus, **minute changes** in the amount of bone matrix surrounding individual osteocytes and their cell processes can have **substantial effects** on the levels of Ca^{2+} and PO_4^- in the blood.
- Osteocytes can respond to mechanical forces exerted on a bone to either increase or decrease the surrounding bone matrix. For example, increased mechanical forces associated with weight lifting can stimulate bone formation by osteocytes while a reduction in mechanical forces as a consequence of immobilization or muscle atrophy can decrease bone by resorption. Changes in mechanical forces are sensed within canaliculi by movement of extracellular fluid between osteocyte cell processes and surrounding bone matrix. When mechanical stress is applied, a transient electrical potential is generated by streaming of the extracellular fluid. This results in activation of voltage-gated Ca^2 channels in osteocytes that triggers bone formation. Conversely, a reduction in mechanical force promotes resorption by stimulating osteocytes to secrete matrix metalloproteinases (MMPs).

c) Bone-lining Cells

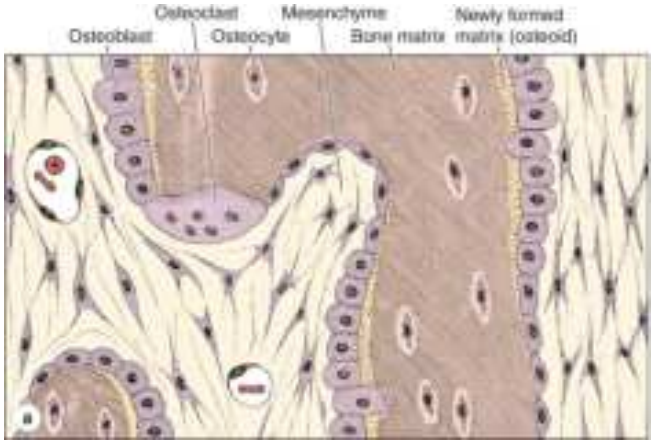
Some sources refer to osteocytes that line the **periosteal** (outer) or **endosteal** (inner) **surfaces** of bone as “bone-lining cells”. These osteocytes do not reside lacunae and appear flattened in shape. As shown in the TEM below, bone-lining cells form gap junctions with each other and communicate with osteocytes in lacunae by extending cytoplasmic processes into the bone matrix to form gap junctions. Bone-lining cells are relatively **inactive or quiescent,** but are able to differentiate into osteoblasts during periods of bone remodeling.

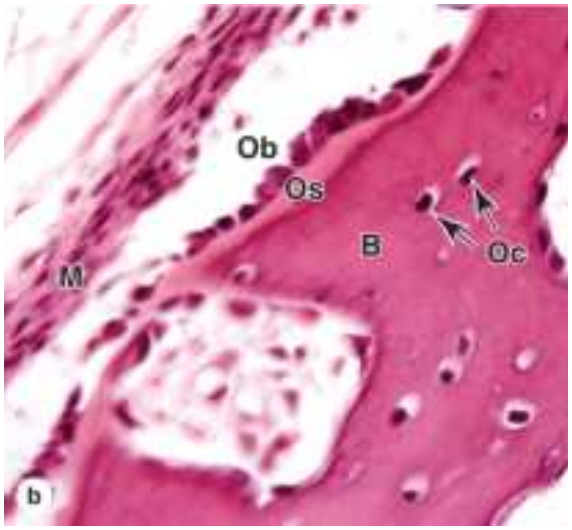


F. CELL TYPES OF BONE TISSUE

2. Osteoblasts

a) Structure and Function: Osteoblasts are secretory cells that synthesize the organic matrix of bone (**osteoid**) and control the **mineralization** of the matrix. Osteoblasts are generated by differentiation of osteoprogenitor cells and retain the ability to proliferate by mitosis. Osteoblasts express receptors for PTH (PTH1R) and vitamin D3 (VDR). Osteoblasts are usually cuboidal in shape and form a single layer on the surface of a forming bone. Osteoblasts have a euchromatic nucleus and basophilic cytoplasm due to an extensive RER involved in protein synthesis and secretion. They can communicate with osteocytes via canaliculi that penetrate the osteoid.





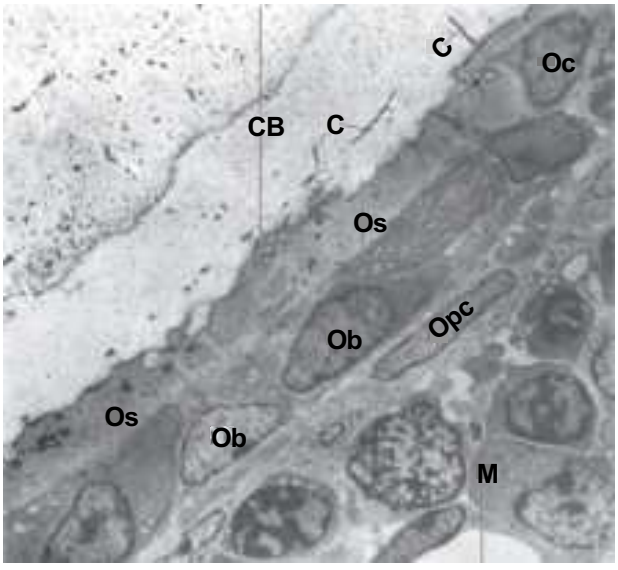
Right: LM showing osteoblasts (**Ob**) laying down osteoid (**Os**), which becomes mineralized to form bone matrix (**B**). Note osteocytes (**Oc**) in lacunae.

b) Major Secretory Products of Osteoblasts (Osteoid)

- Type I collagen – constitutes 90-95% of the organic bone matrix
- Proteoglycans
- Osteocalcin
- Osteonectin
- Osteopontin
- Alkaline phosphatase

c) Mineralization of Bone

Mineralization involves calcification of osteoid to produce hardened bone matrix. Osteoblasts secrete alkaline phosphatase to increase PO_4^-, which in combination with Ca^{2+} binding proteins osteocalcin & osteonectin and the adhesive protein osteopontin, leads to formation of **hydroxyapatite crystals** that embed collagen and proteoglycans in bone matrix.



Right: TEM of bone formation. Osteoblasts (**Ob**) are laying down osteoid (**Os**). Note the osteocyte (**Oc**) that is surrounded by matrix and the canaliculi (**C**). Calcified bone (**CB**) is produced by mineralization of the osteoid. Osteoprogenitor cells (**Opc**) are flattened cells lining the marrow cavity (**M**) and the osteoblasts.

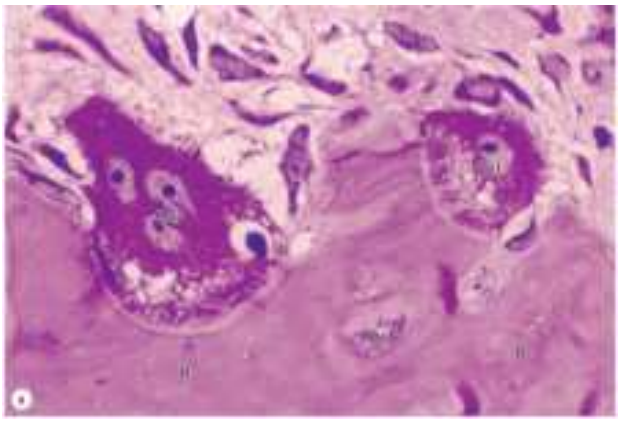
F. CELL TYPES OF BONE TISSUE

3. Osteoprogenitor Cells

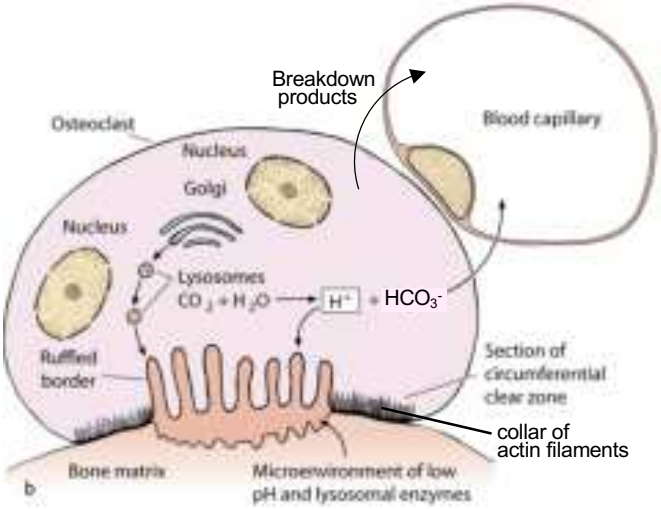
Osteoprogenitor cells are flattened cells found on the inner and outer surfaces of bone. These cells are difficult to identify in routine sections. They are derived from mesenchymal stem cells that originated in bone marrow and serve as the precursor cell for osteoblasts and osteocytes.

4. Osteoclasts

a) Structure and Function: Osteoclasts are large, multinucleated cells generated by fusion of a **monocyte-derived lineage** that originates in the bone marrow. Osteoclasts function in bone resorption during growth and remodeling of the matrix. They are usually located in cavities or depressions called **Howship's lacunae,** which are active sites of bone resorption. Osteoclasts stain eosinophilic due to abundant mitochondria.



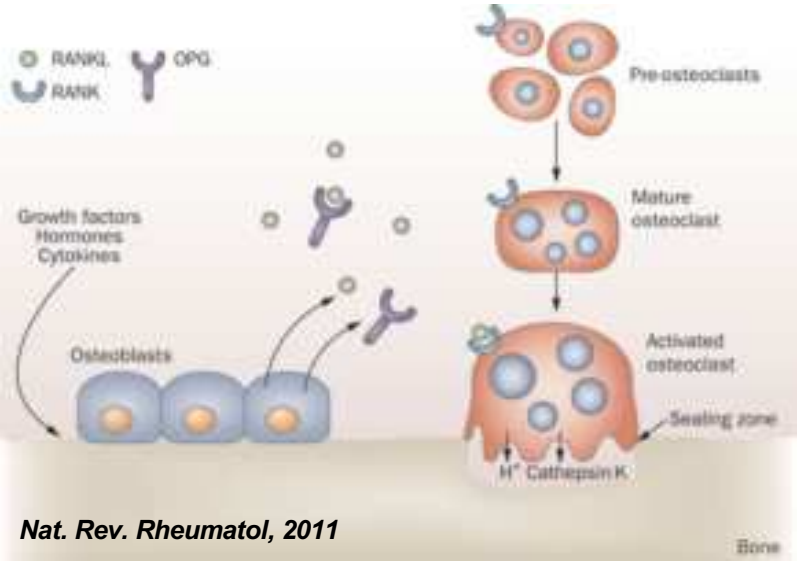
- Osteoclasts attach to the bone surface via integrins and form a ruffled border in contact with bone matrix. This **contact zone** becomes surrounded by a cytoplasmic ring or collar of actin filaments that seals off the underlying extracellular space with a low pH microenvironment.
- Osteoclasts contain the enzyme **carbonic anhydrase II** that catalyzes the following reaction: $H_2O + CO_2 \rightarrow H_2CO_3$, which dissociates into $H^+ + HCO_3^-$. Next, H^+ is actively transported by proton pumps into the extracellular space created by the osteoclast while Cl^- is transported via chloride channels. An acidic microenvironment (pH 4.5) is created that enables activation of acid phosphatases and lysosomal enzymes such as cathepsin K and collagenase. As bone is resorbed in this extracellular space, a cavity called a Howship's lacuna is formed.
- **Lysosomal enzymes** degrade the organic part of the bone (osteoid), and the low pH facilitates release and redistribution of Ca^{2+} by dissolving the hydroxyapatite crystals.
- The breakdown products of lysosomal digestion are released into the extracellular space and taken up in the bloodstream.



F. CELL TYPES OF BONE TISSUE

4. Osteoclasts

b) Mechanism of Osteoclast Differentiation and Activation



Nat. Rev. Rheumatol, 2011

Pre-osteoclasts: A monocyte lineage derived from progenitor cells (CFU-GM) of hematopoiesis in the bone marrow

Mature Osteoclast: Fusion of pre-osteoclasts

RANK: Receptor Activator of Nuclear Factor κB

RANKL: Receptor Activator of Nuclear Factor κB Ligand

OPG: Osteoprotegerin

- RANKL expressed by osteoblasts binds to RANK on the surface of pre-osteoclasts, which promotes **fusion** to form mature osteoclasts. RANKL also is expressed by other types of stromal cells in the bone marrow.
- RANKL binds to RANK expressed by the mature osteoclasts to promote activation.
- OPG is a "decoy receptor" secreted by osteoblasts that binds to RANKL, which prevents binding of RANKL to RANK. This **inhibits** osteoclast formation and subsequent activation.
- The **balance** between RANKL and OPG regulates formation and activity of osteoclasts.

G. HORMONAL REGULATION OF OSTEOCLAST ACTIVITY

1. Parathyroid Hormone (PTH)

a) Mechanism of Action: PTH binds to G protein-coupled receptors (PTH1R) expressed in osteoblasts, which triggers secretion of RANKL. Ligand binding to PTH1R also decreases secretion of OPG by the osteoblasts. Consequently, RANKL binds to RANK expressed by pre-osteoclasts to stimulate generation of osteoclasts and to RANK expressed by mature osteoclasts to stimulate their activity.

b) Effects: PTH **increases calcium levels** in blood by **promoting bone resorption**, which increases efflux of calcium and phosphate to the extracellular fluid. Other hormones, growth factors and cytokines are involved also in regulating osteoclast activity by controlling the balance of RANKL and OPG secreted by osteoblasts.

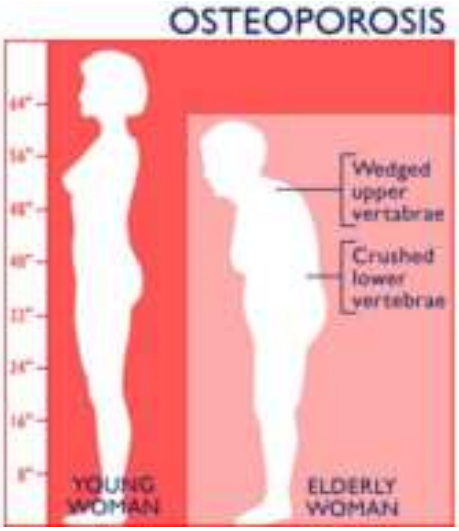
2. Calcitonin

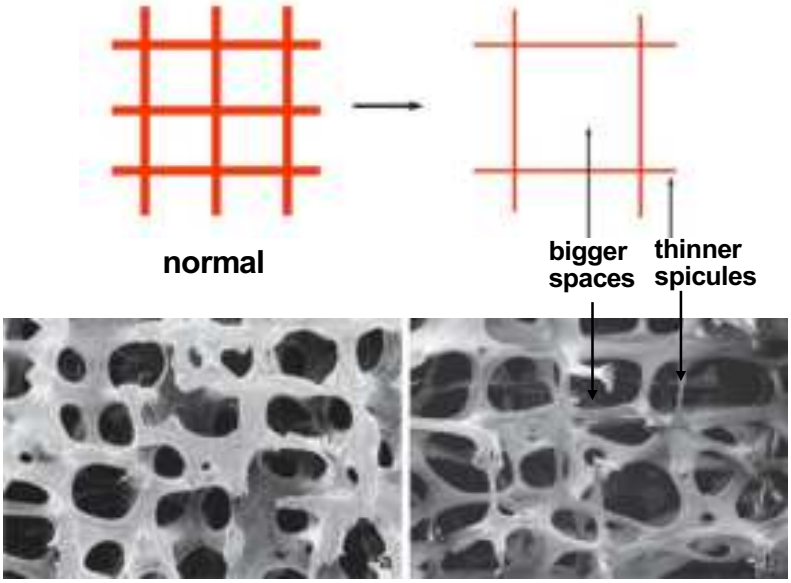
a) Mechanism of Action: Calcitonin binds to receptors (hCTR1) expressed by osteoclasts to cause two main effects: **1**) decrease in number of osteoclasts and recruitment to bone sites, and **2**) inhibition of secretory and resorptive activity of osteoclasts.

b) Effects: Calcitonin **decreases calcium levels** in blood by **inhibiting bone resorption** by osteoclasts.

H. OSTEOPOROSIS

Osteoporosis is a disease that occurs when bone resorption is greater than bone formation. This results in bone loss and reduced bone mineral density. It is common in postmenopausal women and patients who are chronically immobilized. In spongy bone, two major structural changes in architecture lead to weakened bones: **thinner spicules** (trabeculae) and **bigger spaces** in the scaffolding of osteoporotic tissue.





These images show the major differences between normal bone and osteoporotic bone. Note the thinner spicules (trabeculae) and the bigger holes in the framework. Dr. Bruner will cover osteoporosis in much more detail.