Doctor 022

Histology

Sheet no.

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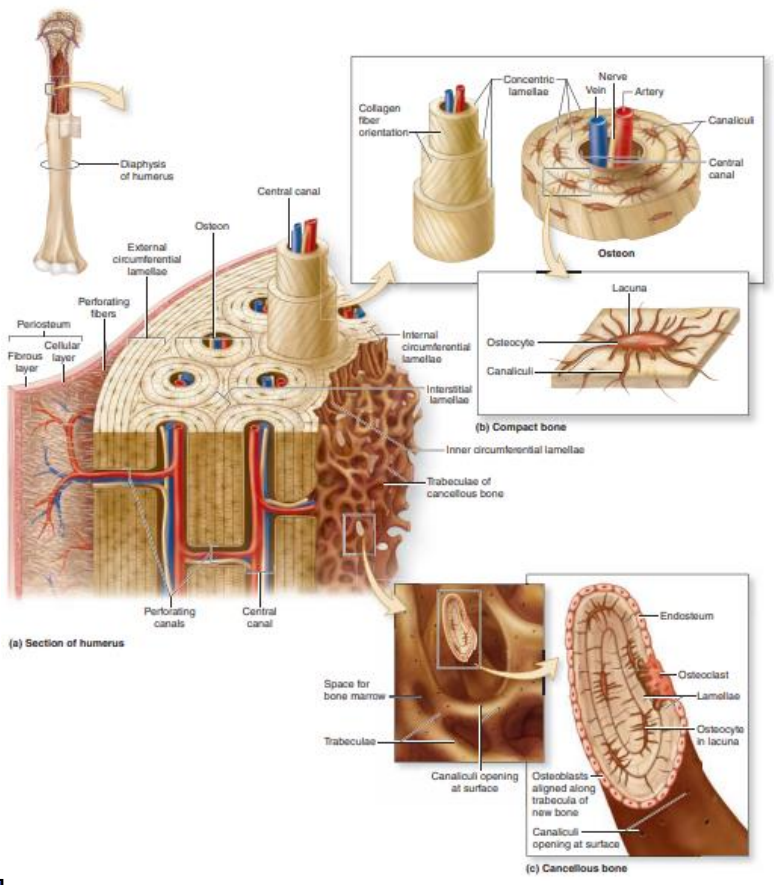
BONE TISSUE

- The bone tissue is the hardest tissue in our body

Functions

1. **Main constituent of the adult skeleton**
   - EXPLANATION: Our skeleton is made up of bones and other associated structures such as: cartilage, ligaments and capsules (which cover the joints); however, the bone is the main component
2. **Provides solid support for the body, protects vital organs such as those in the cranial and thoracic cavities**
   - EXPLANATION: Bones can make cavities which house other organs like the cranial cavity within the skull and the thoracic cavity
3. **Encloses internal (medullary) cavities containing bone marrow**
   - EXPLANATION: There are two types of bone:
     - **Compact bone (cortical bone)**
     - **Cancellous bone (trabecular bone):** has spaces which house the bone marrow
4. **Bone tissue also serves as a reservoir of calcium, phosphate and other ions**
   - EXPLANATION: Calcium is essential for a variety of bodily processes, including muscle contraction and this is especially important for cardiac muscles, as they are responsible for the heart pumping which keeps the body alive. Therefore, maintaining normal calcium levels is crucial for the proper functioning of the body. So when the calcium level in the body drops below normal, the body must correct it. One way it does this is by releasing calcium from the bones using **osteoclasts**.



Histology of the bone

Bones can be classified based on the shape into:

1. Long bones:
   - Ex: humerus, femur, tibia, fibula, phalanges and metacarpals
2. Short bones
3. Flat bones
4. Irregular bones
5. Sesamoid bones

1. Periosteum

Look at the cross-section of a long bone and notice that:

1. It has a dense connective tissue wrap called the **periosteum**
   - The periosteum is similar to perichondrium of the cartilage
   - It is composed of two layers:
     i. **Outer fibrous** layer of **dense irregular connective tissue** containing bundled type 1 collagen and fibroblasts
     ii. **Inner cellular** layer includes osteoblasts, bone lining cells and osteoprogenitor cells (mesenchymal stem cells)
   - It is perforated by the neurovascular bundle (vein, artery and nerve), which makes the bone an innervated vascular tissue. This vascular and neural supply positively impacts the bone tissue's ability to regenerate and repair itself. In contrast, cartilage, which lacks this innervated vascular nature, has limited regenerative capacity

UNDERSTAND: When a bone is broken, the healing process begins immediately. **The bone responds by increasing the number of osteoblasts at the site of fracture,** where they synthesize matrix and start bone formation. At first, the bone that is formed is a **woven bone**, which is not perfect in terms of structure. However, over time, the woven bone becomes internally organized, resulting in a structure that closely resembles the original bone

This regenerative ability is made possible by the bone's good **blood supply**

Starting from the outside of the bone and moving inwards:

- We first encounter the periosteum, which is composed of two layers: the outer fibrous layer and the inner cellular layer
- Deeper still, we encounter the outermost layer of the bone, which is compact in nature
- As we continue towards the center of the bone, we come across spongy bone (trabecular or cancellous bone). This type of bone is characterized by bone trabeculae and spaces that are usually filled with bone marrow that produces blood cells (usually spaces are filled with red bone marrow but as a person ages, it is gradually replaced by yellow bone marrow, which is rich with adipocytes)

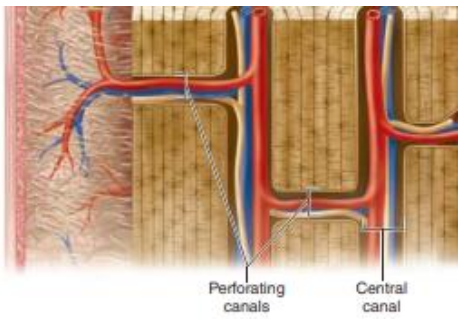
(V2)

2. Compact bone

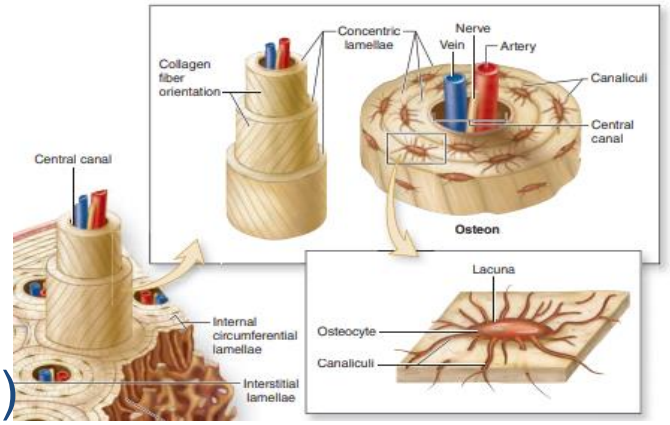
- We can see whitish structures with black structures in between
- The bone is a complex tissue composed of cells and calcified extracellular material called the **bone matrix**. The matrix is organized in layers that are referred to as **lamellae**
- The cells in between are mature semi-retired cells known as **osteocytes**
- Lamellae are found in 4 different locations and forms:
  - The lamellae near the periosteum are called **external circumferential lamellae**
  - Those near the spongy bone are called **internal circumferential lamellae**
  - In between we can see rounded circle structures called **osteons (Haversian systems)** which are composed of **central canals (Haversian canals)** surrounded by **concentric lamellae**
  - The neurovascular bundles are located in the central canals
  - Scattered among the intact osteons you can find **interstitial lamellae**

2. Compact bone Continued

- The central canals, which are parallel to the longitudinal axis of the bone, communicate with each other through another type of canals called **perforating canals (Volkmann's canals),** which are parallel to the transverse axis of the bone



Osteons



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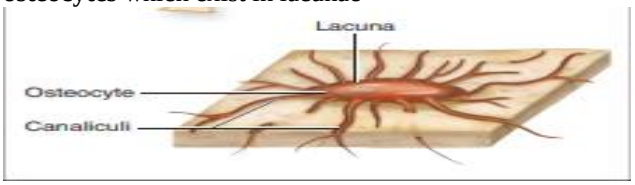
- **Osteons (Haversian systems)** are the organizational units inside the bone and are complexes of concentric lamellae
- Osteons are typically 100-250 µm in diameter, surrounding a central canal that contains small blood vessels, nerves, and **endosteum**
- Between the lamellae you can see the old bone cells; the **osteocytes** which exist in lacunae



- Osteocytes have cytoplasmic processes, unlike chondrocytes

WHY??

Osteocytes have processes which allow for the exchange of nutrients, unlike chondrocytes. This is due to the hard and tough matrix of the bone, which makes diffusion of nutrients difficult. In contrast, chondrocytes are able to diffuse nutrients at slow rates due to their less rigid matrix. As a result, osteocytes have adapted by sending out processes to reach neighbor cells and facilitate nutrient exchange

- Due to the hard bone matrix, these processes (measuring 250-300 nm in diameter) should pass through tiny canals called **canaliculi**

Components of the bone tissue

- Having described the bone tissue, we will now examine the components of the bone tissue
- The components of the bone tissue:
  a) Bone matrix:
     - Organic materials
     - Inorganic materials
  b) Cells:
     - Osteoblasts
     - Osteocytes
     - Osteoclasts

a) Bone matrix

- The bone matrix is different from other tissue matrices as it undergoes modification
- The bone matrix is composed of both inorganic and organic materials:
  - Inorganic materials:
    a) Give the bone its hardness and differentiates the bone from other tissues
    b) Include: **Calcium Hydroxyapatite (calcium phosphate** in its crystalline form and it is the most abundant**), citrate**, **magnesium** and **noncrystalline calcium phosphate**

(V2)

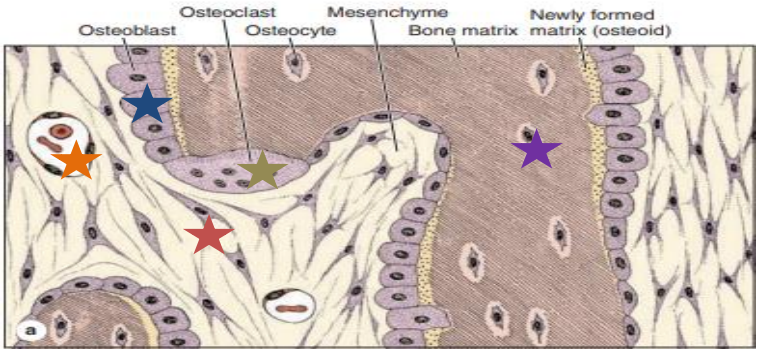
  - Organic materials:
    Include:
    1. Collagen:- 90% of the organic material is collagen type 1
       - Collagen plays an equally important role as calcium hydroxyapatite in giving the bone tissue its strength
       - Without collagen, bone becomes brittle
       - Collagen, **particularly type 1**, binds every element of the bone together
    2. Proteoglycans
    3. Glycoproteins

Why is the bone tissue tough while the cartilage is thick gelatinous, although they both contain collagen?

- Because bone matrix has inorganic materials while cartilage does not, even though hyaline is subject to calcification (but only with aging)

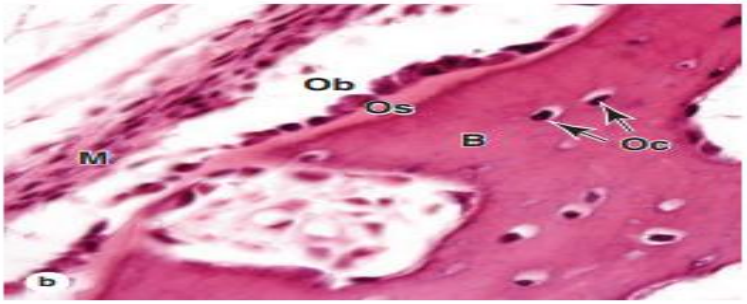
لأنه الbone فيها inorganic materials بينما الcartilage ما فيها، مع إنه حكينا إنه الhyaline بصير فيه calcification بس هاي بتصير مع تقدم العمر فقط

b) Bone cells



- We will examine the 3 types of cells during bone formation (happens in the embryo)
- Remember that the connective tissue in general originates from mesenchymal stem cells
- Notice the **mesenchymal stem cells** ★, and between these cells there is mainly ground substance and fibers
- Mesenchymal stem cells have a specific pathway that promotes bone formation, leading to the development of **osteoblasts** that are always located on the surface ★
- Osteoblasts resemble cuboidal epithelial cells and are polarized (have basal and apical surfaces, and different areas of the cell have different structures)
- Osteoblasts are responsible for synthesizing the bone matrix and mineralization
- Some osteoblasts will differentiate into **osteocytes** that are trapped within the bone matrix ★
- NOTICE that the number of osteoblasts is greater than that of osteocytes
- **Osteoclasts** are multinucleated giant cells that are also found on the surface and play an important role in bone resorption and bone remodeling ★
- Notice the blood vessel: we need good blood supply during bone formation ★

b) Bone cells Continued



- This picture and the previous picture are related
- Bone (B) is the pink structure where you can find osteocytes (Oc)
- Osteoblasts (Ob) are always found on the surface
- You can also find mesenchyme (M)
- **Due to the high collagen (type 1) content in the bone matrix, the bone matrix is usually acidophilic**
- The bone cannot be processed with its full structure due to its tough nature, and it is required to undergo **decalcification** before proceeding with other steps of sample preparation, and then we can stain with H&E
- Accordingly, we can rewrite the previous sentence as follows: **Due to the high collagen (type 1) content in the bone matrix, the <u>decalcified</u> bone matrix is usually acidophilic**
- **NOTE:** If a bone is decalcified by a histologist, its shape is preserved but it becomes soft and pliable like other connective tissues.
- It is possible to observe the bone in its fully mineralized state without decalcifying it. This can be achieved by embedding the hard bone in a strong resin (plastic) material that is equally or more solid than the bone, instead of wax. The embedded bone can then be sectioned for observation. And can be seen as the following pictures:

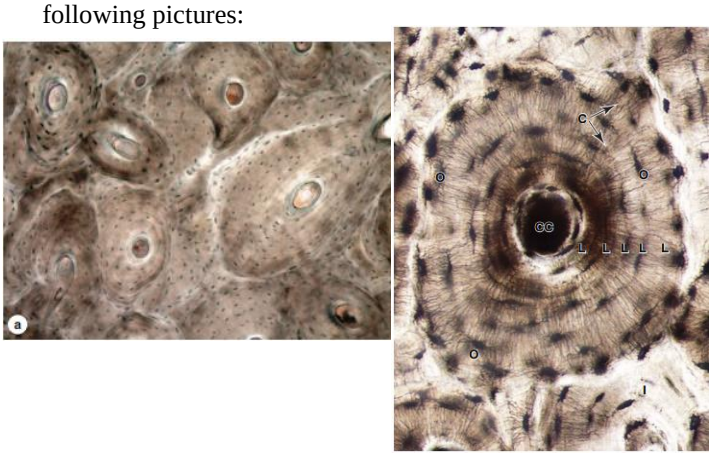


FIGURE: AN OSTEON

(1) Osteoblasts:

a. Are growing cells that originate from mesenchymal stem cells
b. Synthesize and secrete the organic components of the bone matrix (young and active)
c. Osteoblasts, especially active ones, are located exclusively at the surfaces of bone matrix, to which they are bound by integrins
d. When their synthetic activity is completed:
   - Some osteoblasts differentiate as **osteocytes** entrapped in matrix-bound lacunae
   - Some flatten and cover the matrix as **bone lining cells**
   - The majority undergo **apoptosis** (programmed death of cells)

NOTE: The toughness of bone is due to its matrix and components. Therefore, if the number of cells increases, the matrix decreases, resulting in weaker bone tissue. As a result many osteoblasts undergo apoptosis.

(2) Osteocytes:

a. Some osteoblasts become surrounded by the material they secrete and then differentiate as **osteocytes**
b. Are found in cavities **(lacunae)** between bone matrix layers **(lamellae)**
c. Have cytoplasmic processes in small **canaliculi** that extend into the matrix (250-300 nm in diameter)
d. Semi-retired cells
e. Osteocytes communicate with one another and ultimately with nearby osteoblasts and bone lining cells via **gap junctions** at the ends of their processes
f. Osteocytes are **the most abundant cells** in bone
g. Osteocytes exhibit less RER, smaller Golgi complexes, and more condensed nuclear chromatin than osteoblasts
h. Osteocytes maintain the calcified matrix, and their death is followed by rapid matrix resorption

(V2)

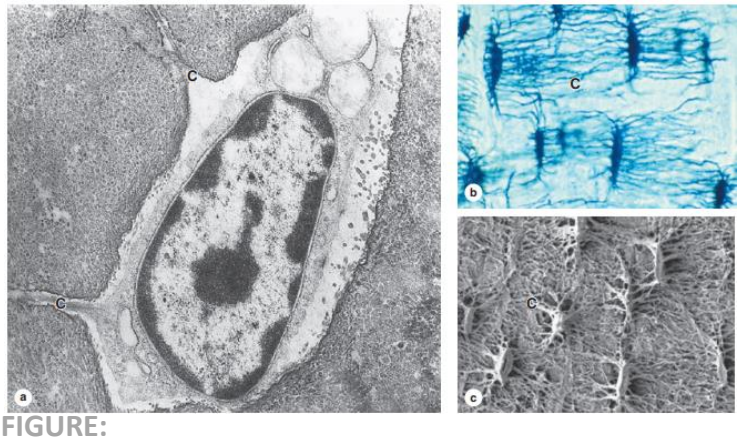
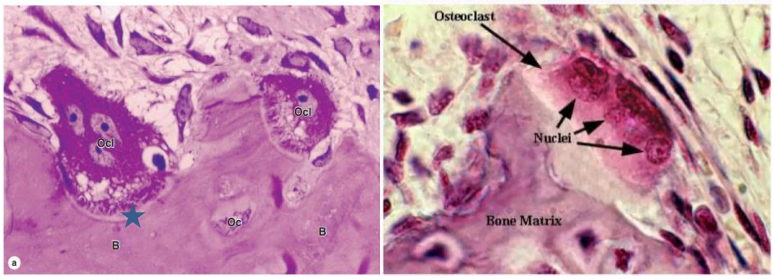


FIGURE:
a) TEM showing an osteocyte in a lacuna and two dendritic processes in canaliculi (C) surrounded by bony matrix
b) Photomicrograph of bone, not decalcified or sectioned, but ground very thin to demonstrate lacunae and canaliculi. The lacunae and canaliculi (C) appear dark
c) SEM of non-decalcified, sectioned, and acid-etched bone showing lacunae and canaliculi (C)

(3) Osteoclasts:

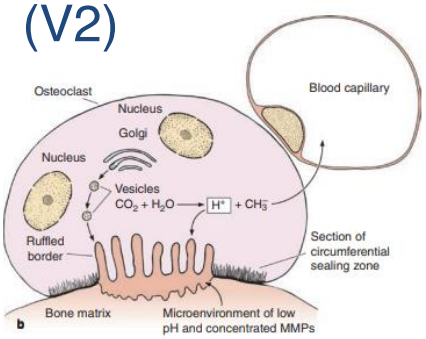
a. Are giant, multinucleated, motile cells that are formed through the fusion of multiple monocytes that migrated to the bone
b. Involved in removing calcified bone matrix and remodeling bone tissue
c. In areas of bone undergoing resorption, osteoclasts on the bone surface lie within enzymatically etched depressions or cavities in the matrix known as **resorption lacunae (resorption cavities = Howship lacunae)**
d. In an active osteoclast the membrane domain that contacts the bone forms a circular **sealing zone** where integrins bind the cell tightly to the bone matrix
e. The circumferential sealing zone surrounds an area of microvilli and other cytoplasmic surface projections close to this matrix , called the **ruffled border**

(V2)



- Osteoclasts are always located on the surface of the bone matrix
- In picture (a) above you can observe:
  - The space just beneath the osteoclast appears less eosinophilic compared to the cell itself
  - The name given to that area is **Howship lacunae**, which is the active site for the osteoclast function ★

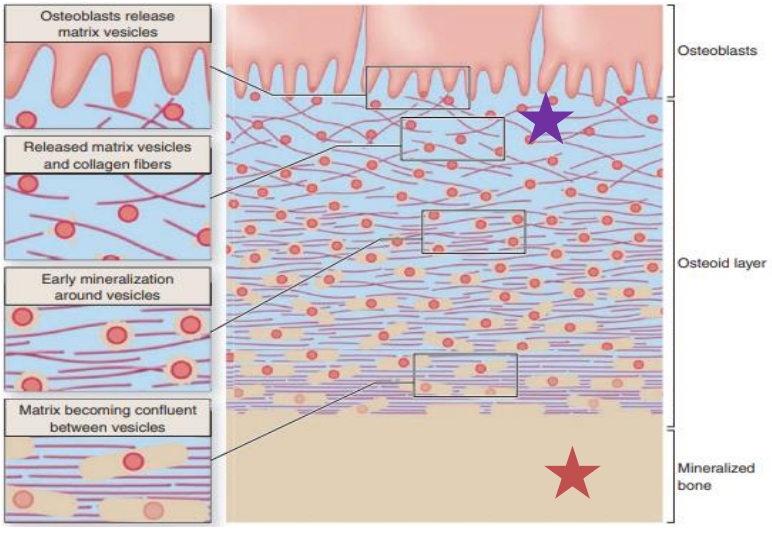
(V2)



- Look at picture (b):
  - In order for the osteoclast to resorb the matrix, this space is acidified to ~pH 4.5 by proton pumps in the ruffled part of the cell membrane
  - A **sealing zone** is required in a region with low acidity, to provide a framework for the reactions, and prevent leakage of enzymes during the digestion (resorption) of the bone matrix
  - The region referred to as the **ruffled border** is where the actual release of inorganic materials take place
  - Look at the capillary beside the osteoclast: Liberated Ca^{+2} and other ions are released directly and taken up by the blood
- Look at picture (c):
  - SEM of bone, shows a slowly migrating osteoclast and the etched area where the osteoclast has resorbed the bone matrix and released inorganic materials
  - The osteoclast moves to the next region and completes the process, as its activity is limited to a certain depth (osteoclasts cannot demineralize the entire thickness of the lamellae)

PLEASE NOTE that the previous information about osteoclasts (written in blue) was only discussed briefly in approximately 3 minutes. Therefore, if you are having trouble comprehending the provided information, it is recommended that you refer to pages 143 & 144 in the textbook for a more detailed understanding

Mineralization in bone matrix



- **Osteoblasts** secrete matrix (organic) components at the cell surface in contact with existing bone matrix, producing a layer between the osteoblast layer and the preexisting bone surface called **osteoid** (soft tissue of the bone)
- Osteoid is rich in:
  - **Collagen I**
  - **Glycoproteins**
  - **Proteoglycans**

- This process of **bone appositional growth** is completed by **mineralization** (deposition of salts in the newly formed matrix)
- The process of mineralization involves depositing two primary minerals, namely **calcium and phosphate (in the form of hydroxyapatite)**, into the bone matrix
- Calcium and phosphate are deposited in different ways into the bone matrix
- **Osteocalcin**, a glycoprotein present in osteoid (osteocalcin is a vitamin K-dependent polypeptide), along with other glycoproteins, bind to Ca^{+2} ions and concentrate this mineral in the matrix
- At the same time, **osteoblasts** release **matrix vesicles** from their basal surfaces, which subsequently disperse within the osteoid
- These matrix vesicles are rich in **alkaline phosphatase** whose activity raises the local concentration of phosphate PO_4^{-3} ions
- When both calcium and phosphate ions are highly concentrated, matrix vesicles serve as foci (centers) for the formation of hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ crystals

- In the previous figure, observe the white structures surrounding the vesicles which reflects the presence of hydroxyapatite → the amount of hydroxyapatite gradually increases over time, indicating that the youngest vesicles possess the least amount of hydroxyapatite (at the top near osteoblasts) and vice versa. Thus, as we move downwards, the osteoid become older and more mineralized

★ Represents the fully mineralized bones

★ Represents the youngest part of the osteoid

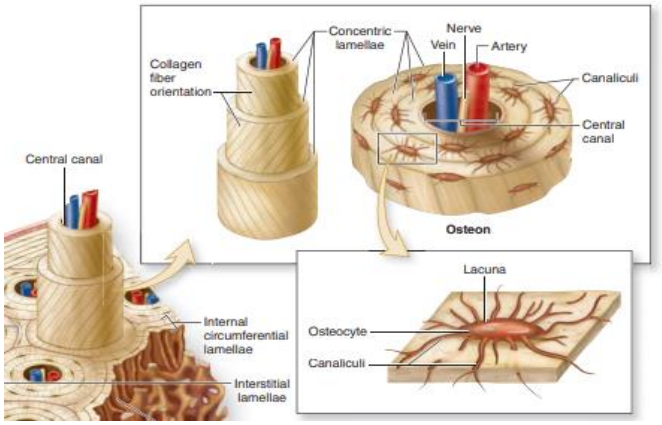
- These hydroxyapatite crystals surrounding the matrix vesicles rapidly increase in size by adding more minerals and eventually produce a confluent mass of calcified material embedding the collagen fibers and proteoglycans
-NOTE: Osteocalcin and matrix vesicles are quite important in mineralization. Any issues with these substances could lead to improper mineralization. For instance, if a genetic disorder affects osteocalcin, it may lead to the formation of hypomineralized bone (low in calcium)

Periosteum & Endosteum

- External and internal surfaces of all bones are covered by connective tissue of the periosteum and the endosteum
- **Periosteum:**
  - Is a dense connective tissue, containing mostly bundled type I collagen, but also fibroblasts and blood vessels
  - Periosteum contains mainly type 1 collagen but also contains other fibrillar collagens (types II and III)
- Bone is vascularized by small vessels that penetrate the matrix from the periosteum
- **Endosteum:**
  - Covers all trabeculae that project into the marrow cavities
  - The endosteum is not as thick as the periosteum
  - Contains osteoprogenitor cells, osteoblasts, and bone lining cells, but within a sparse, delicate matrix of collagen fibers

(V2)

- Osteoprogenitor cells are present in both the periosteum and the endosteum, which allows for regeneration and repair to come from both of these sources



- Consider the organization of the lamellae in osteons:
  - The striations in the lamellae are not random, but rather are arranged in such a way as to allow you to visualize the collagen fibers running in very specific orientations. The **collagen fibers** are almost always arranged perpendicular to each other, resulting in bone matrix with maximum strength

(V2)

(V3)

The information that has been recently added is colored in blue (V2)

The collagen fibers are almost always arranged perpendicular to each other, instead of: **The lamellae** are almost always arranged perpendicular to each other (V3)