Dr. Ali Mohsin

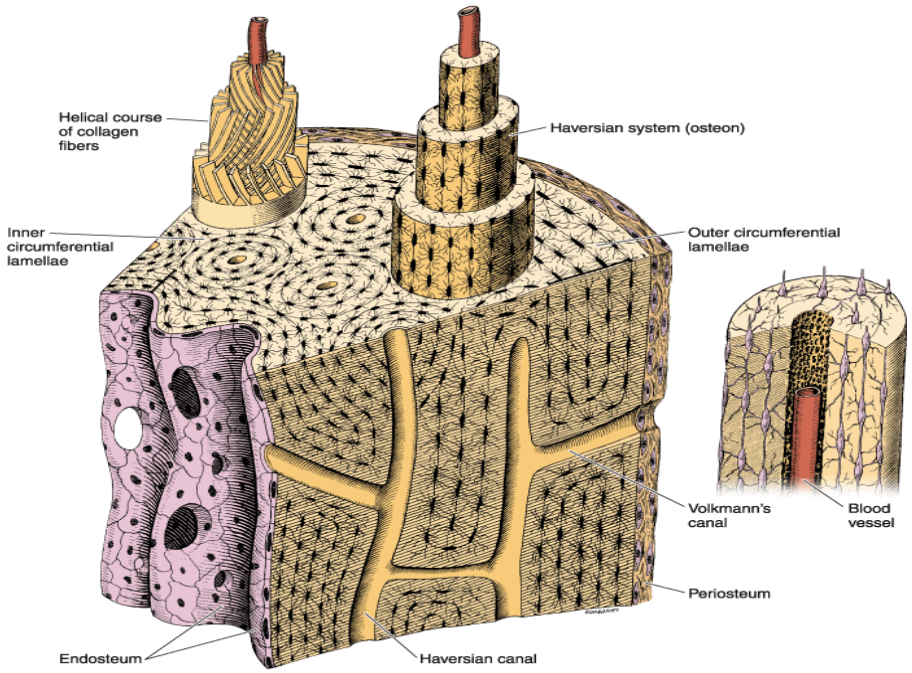
Bone Histology -1

Bone is the main constituent of the skeleton, it (1) supports fleshy structures, (2) protects vital organs, (3) mediates movement, (4) harbors the bone marrow (where blood cells are formed), & (5) serves as a reservoir of calcium, phosphate, and other ions that can be released or stored in a controlled fashion. Bone tissue is highly vascularized and metabolically very active.

Bone is a specialized (supporting) connective tissue composed of intercellular calcified material, the bone matrix, and three cell types: osteocytes, which are found in cavities (lacunae) within the matrix; osteoblasts, which synthesize the organic components of the matrix; and osteoclasts, which are multinucleated giant cells involved in the resorption and remodeling of bone tissue.

Unlike in cartilage, metabolites are unable to diffuse through the calcified matrix of bone, the exchanges between osteocytes and blood capillaries depend on communication through the **canaliculi**, which are thin, cylindrical spaces that perforate the matrix.

All bones are lined on both internal and external surfaces by layers of tissue containing osteogenic cells: endosteum on the internal surface and periosteum on the external surface.



Bone Cells

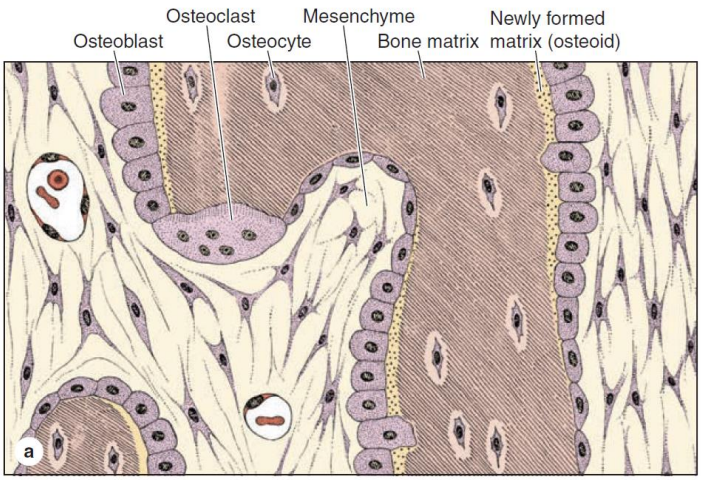
Osteoblasts (bone forming cells):

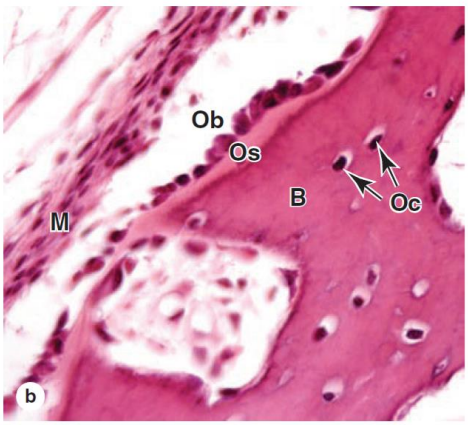
Osteoblasts originate from mesenchymal stem cells, they are responsible for the synthesis of the organic components of bone matrix (type I collagen, proteoglycans, and glycoproteins like osteonectins). Osteoblasts are exclusively located at the surfaces of bone tissue in a way similar to simple epithelium. When they are active (in matrix synthesis), osteoblasts have a cuboidal to columnar shape and basophilic cytoplasm

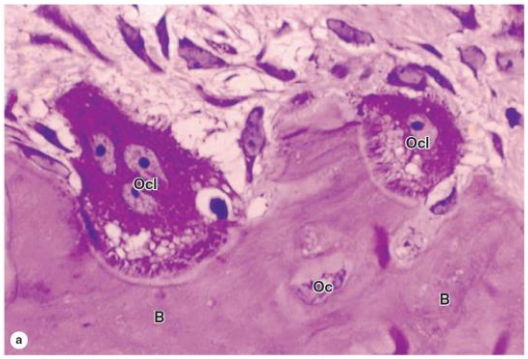
(resulting from rER & ribosomes). Osteoblasts are polarized cells. Matrix components are secreted at the basal surface of the cell, which is in contact with older bone matrix, producing a layer of new (but not yet calcified) matrix, called osteoid, between the osteoblast layer and the previously formed (calcified) bone. Subsequently deposition of calcium salts into the osteoid occurs. When their synthetic activity is completed, some osteoblasts differentiate as osteocytes entrapped in lacunae, some flatten and cover the matrix surface as bone lining cells, and the majority undergo apoptosis.

Osteocytes (bone maintaining cells):
Some osteoblasts are gradually surrounded by newly formed matrix and become osteocytes. Osteocytes lie in the lacunae (small cavities containing the osteocytes) situated between lamellae (layers) of bone matrix. Only one osteocyte is found in each lacuna, with small amount of extracellular non-calcified substance. There are many tiny canaliculi connecting lacunae to each other. They house cytoplasmic processes of osteocytes, that make contact via gap junctions, so molecules are passed from cell to cell.
Osteocytes are flat, almond-shaped cells with a significantly less rough endoplasmic reticulum and Golgi complex and more condensed nuclear chromatin than osteoblasts. These cells are actively involved in the maintenance of the bony matrix, and their death is followed by resorption of this matrix. Osteocytes are long-living cells.

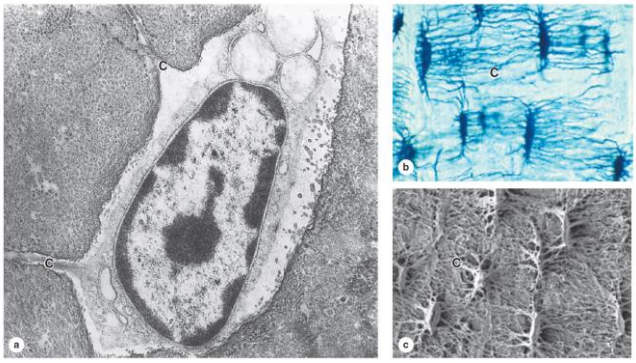
Osteoclasts (bone resorbing cells):
Osteoclasts are very large, branched motile cells. The cell body contain multiple nuclei (because they originate from the fusion of multiple bone marrow-derived monocytes). In areas of bone undergoing resorption, osteoclasts lie within depressions in the matrix (Howship's lacunae) dug by osteoclast's enzymes.
In active osteoclasts, the surface facing the bone matrix is folded, forming a **ruffled border.** Below this border, the bone matrix is depressed, allowing a small pocket between them. Surrounding the ruffled border is a cytoplasmic zone (the **sealing zone**) that is rich in actin filaments, providing strong adhesion of the osteoclast to the bone matrix. The osteoclast secretes matrix metalloproteinases and other hydrolytic enzymes, and pumps protons (i.e: acid) into the subcellular pocket, promoting the localized digestion of collagen and dissolving calcium salt crystals. Osteoclast activity is controlled by cytokines (small signaling proteins) and hormones.



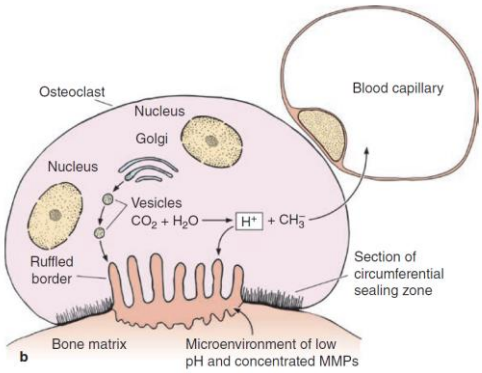


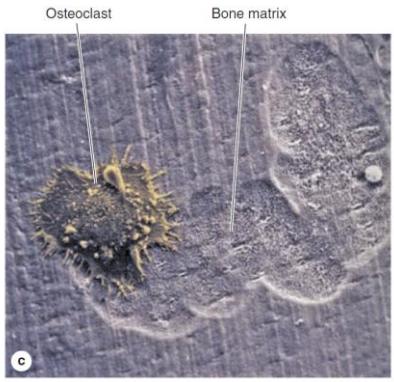


Osteocytet



Osteoclast





Bone Matrix

Bone matrix is made by organic (50%) & non-organic (50%) components.

The organic matter embedded in the calcified matrix is 90% type I collagen, but also includes small proteoglycans and glycoproteins such as osteonectin. Calcium-binding proteins, like osteocalcin, and the phosphatases promote calcification of the matrix.

Non-organic components are minerals. Calcium hydroxyapatite (containing calcium & phosphate: $Ca_{10}(PO_4)_6(OH)_2$) is most abundant, but bicarbonate, citrate, magnesium, potassium, and sodium ions are also found. Significant quantities of noncrystalline calcium phosphate are also present.

The association of minerals with collagen fibers is responsible for the hardness and resistance of bones. If a bone is decalcified, its shape is preserved, but it becomes flexible. If a bone is devoid of collagen, its shape is also preserved, but it becomes fragile.

Periosteum & Endosteum

External and internal surfaces of bone are covered by connective tissue called periosteum and endosteum, respectively.

The ***periosteum*** consists of an outer layer of collagen fibers and fibroblasts. Bundles of periosteal collagen fibers, called Sharpey's fibers, penetrate the bone matrix, binding the periosteum to bone. Periosteal blood vessels branch and penetrate the bone, carrying metabolites to and from bone cells. The periosteum's inner layer is more cellular and includes osteoblasts, bone lining cells, and mesenchymal stem cells (called osteoprogenitor cells). Osteoprogenitor cells can proliferate extensively and produce new osteoblasts, so they play a prominent role in bone growth and repair.

Internally the very thin ***endosteum*** lines the bone & covers small trabeculae of cancellous bone that project into the marrow cavities. The endosteum also contains osteoprogenitor cells, osteoblasts, and bone lining cells, but within a sparse, delicate matrix of collagen fibers.

The principal functions of periosteum and endosteum are nutrition of osseous tissue and provision of a continuous supply of new osteoblasts for repair or growth of bone.

Types of Bone

- Anatomical classification of **bones**: long, short, flat, & irregular bones.
- Macroscopic classification of **bone tissue**: compact bone & cancellous bone (microscopically they are almost identical).
- Developmental classification of bone tissue: primary & secondary.

Compact bone:

Here, bone tissue forms a solid mass of bone with no cavities. In a long bone, compact bone tissue forms the bone shaft (diaphysis) around the bone marrow cavity, & the outer layer of the metaphysis & epiphysis.

Under microscope, compact bone matrix is made of bone layers (lamellae) having small cavities (lacunae) between them that contain osteocytes.

In compact bone, lamellae are arranged as follows:

- Outer circumferential lamellae: large in number, occupy the outer zone, beneath & parallel to the periosteum.
- Inner circumferential lamellae: less in number, occupy the inner zone parallel to the endosteum.
- Concentric lamellae: Osteons (Haversian systems) are longitudinal cylinders of bone made of concentric lamellae with a central narrow canal "***the central (Haversian) canal***" filled with loose connective tissue. Osteons fill the zone between the outer & inner circumferential lamellae & are parallel to the long axis of the bone. Central canals are connected to each other by several transverse or oblique canals "***perforating (Volkmann's) canals***", that also connect central canals to the outer & inner surfaces of the bone.
- Interstitial lamellae: short parallel lamellae that fill the small triangular spaces between osteons.

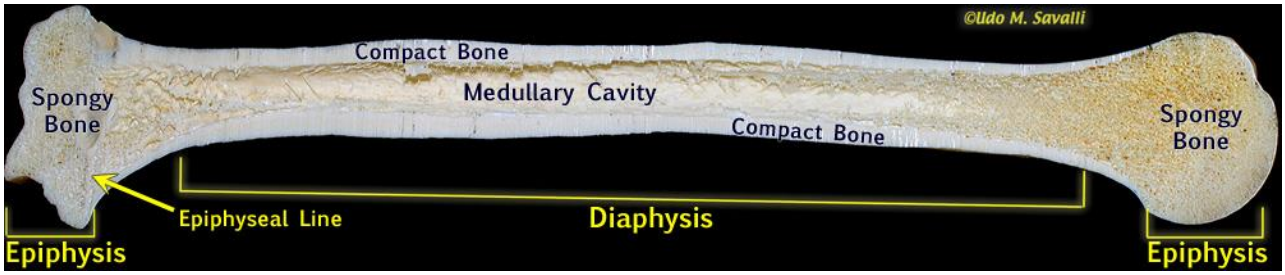
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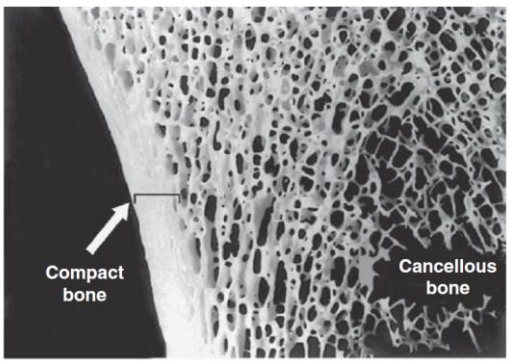
- In each lamella, collagen fibers are arranged spirally, in a direction opposite to that of the collagen fibers of the adjacent lamella.
- Osteons are in a continuous renewal process, therefore, they differ in diameter & number of lamellae.
- Perforating canals are not surrounded by concentric lamellae, they perforate the lamellae of the osteons. Perforating canals are lined with endosteum & filled with loose connective tissue.
- Blood vessels & nerves enter the bone via foramina & are distributed through all central & perforating canals.

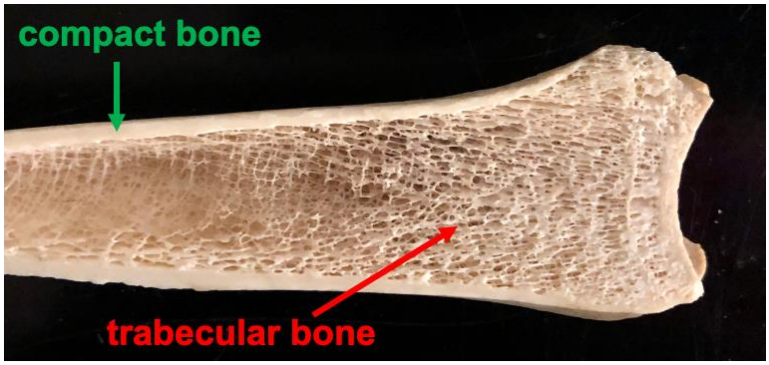
Cancellous bone:

Cancellous (spongy, trabecular) bone fills the epiphysis & metaphysis of long bones & form a thin zone on the inner surface of diaphysis around the marrow cavity. In short & irregular bones, cancellous bone forms the core & compact bone makes the outer layer of the bone. In flat bones, 2 plates of compact bone sandwich a thin zone of cancellous bone called the diploe.

Cancellous bone is made of bony trabeculae separated by extensively interconnected cavities, just like a sponge. Histologically, it is similar to compact bone in being made by concentric lamellae with lacunae containing osteocytes, but the lamellae are not perforated by central or perforating canals. On the surface of the bony trabeculae, osteoblasts are present, together with fewer osteoclasts, all surrounded by thin endosteum.

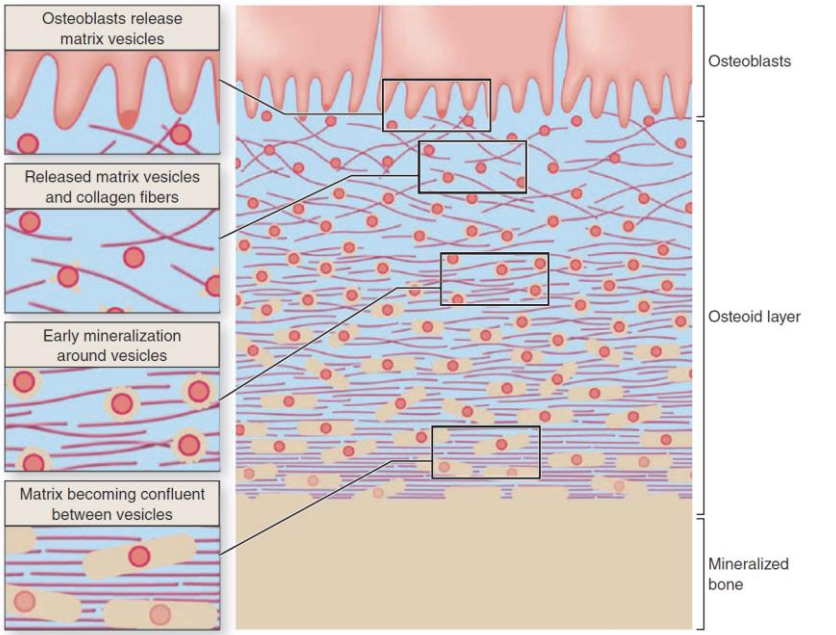


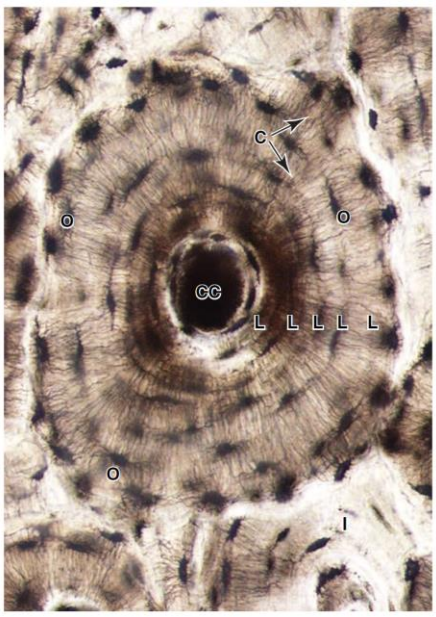


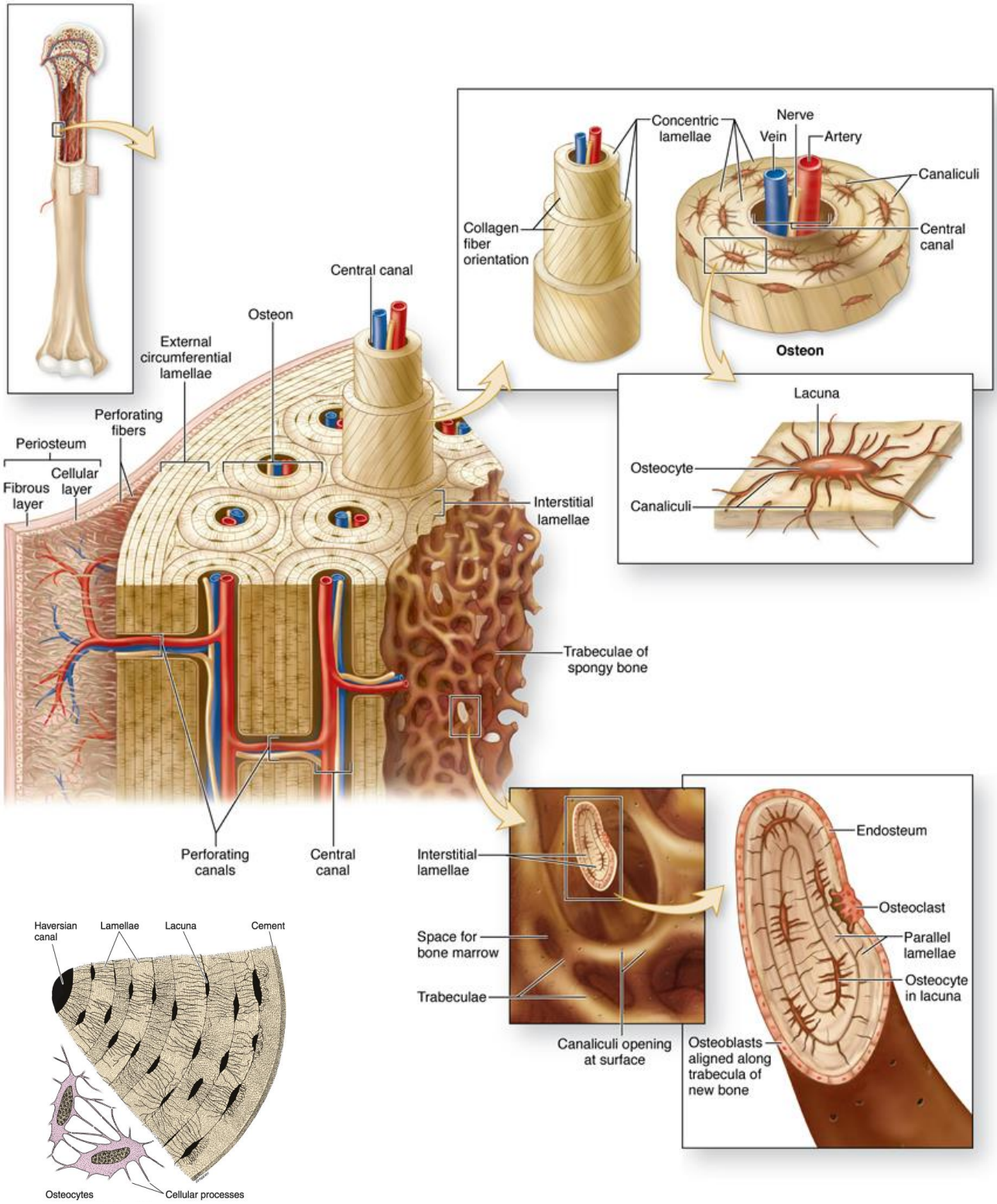


Primary bone tissue:
Primary (immature, woven) bone is the first bone tissue to appear in embryonic development and in fracture repair and other repair processes. It is characterized by random disposition of fine collagen fibers, a lower mineral content and a higher proportion of osteocytes than in secondary bone tissue. Primary bone tissue is usually temporary, it is replaced in adults by secondary bone tissue except in few places (near the sutures of the flat bones of the skull, in tooth sockets, and in the insertions of some tendons).

Secondary bone tissue:
Secondary (mature, lamellar) bone tissue is usually found in adults. It shows collagen fibers arranged in lamellae forming compact or cancellous bone tissue.







Type of Bone	Histological Features	Major Locations	Synonyms
Woven bone, newly calcified	Irregular and random arrangement of cells and collagen; lightly calcified	Developing and growing bones; hard callus of bone fractures	Immature bone; primary bone; bundle bone
Lamellar bone, remodeled from woven bone	Parallel bundles of collagen in thin layers (lamellae), with regularly spaced cells between; heavily calcified	All normal regions of adult bone	Mature bone; secondary bone
Compact bone, ~80% of all lamellar bone	Parallel lamellae or densely packed osteons, with interstitial lamellae	Thick, outer region (beneath periosteum) of bones	Cortical bone
Cancellous bone, ~20% of all lamellar bone	Interconnected thin spicules or trabeculae covered by endosteum	Inner region of bones, adjacent to marrow cavities	Spongy bone; trabecular bone; medullary bone