BONE TISSUE

- **Bone** is a specialized form of connective tissue that, like other connective tissues, consists of cells and extracellular matrix. The feature that distinguishes bone from other connective tissues is the mineralization of its matrix, which produces an extremely hard tissue capable of providing support and protection. The mineral is calcium phosphate in the form of **hydroxyapatite crystals**. By virtue of its mineral content, bone also serves as a storage site for calcium and phosphate. Both calcium and phosphate can be mobilized from the bone matrix and taken up by the blood as needed to maintain appropriate levels throughout
- the body. Thus, in addition to support and protection, bone plays an important secondary role in the homeostatic regulation of blood calcium levels.
- **Bone matrix contains mainly type I collagen along with other matrix (noncollagenous) proteins.**

- The major structural component of **bone matrix** is **type I collagen** and, to a lesser extent, **type V collagen.**

- Trace amounts of other types such as type III, XI, and XIII collagens have also been found in the matrix. All collagen molecules constitute about 90% of the total weight of the bone matrix proteins. The matrix also contains other matrix (noncollagenous) proteins that constitute the **ground substance** of bone.
- As a minor component of bone, constituting only 10% of the total weight of bone matrix proteins, they are essential to bone development, growth, remodeling, and repair. Both the collagen and the ground substance become mineralized to form bone tissue. The four main groups of noncollagenous proteins found in the bone matrix are the following:

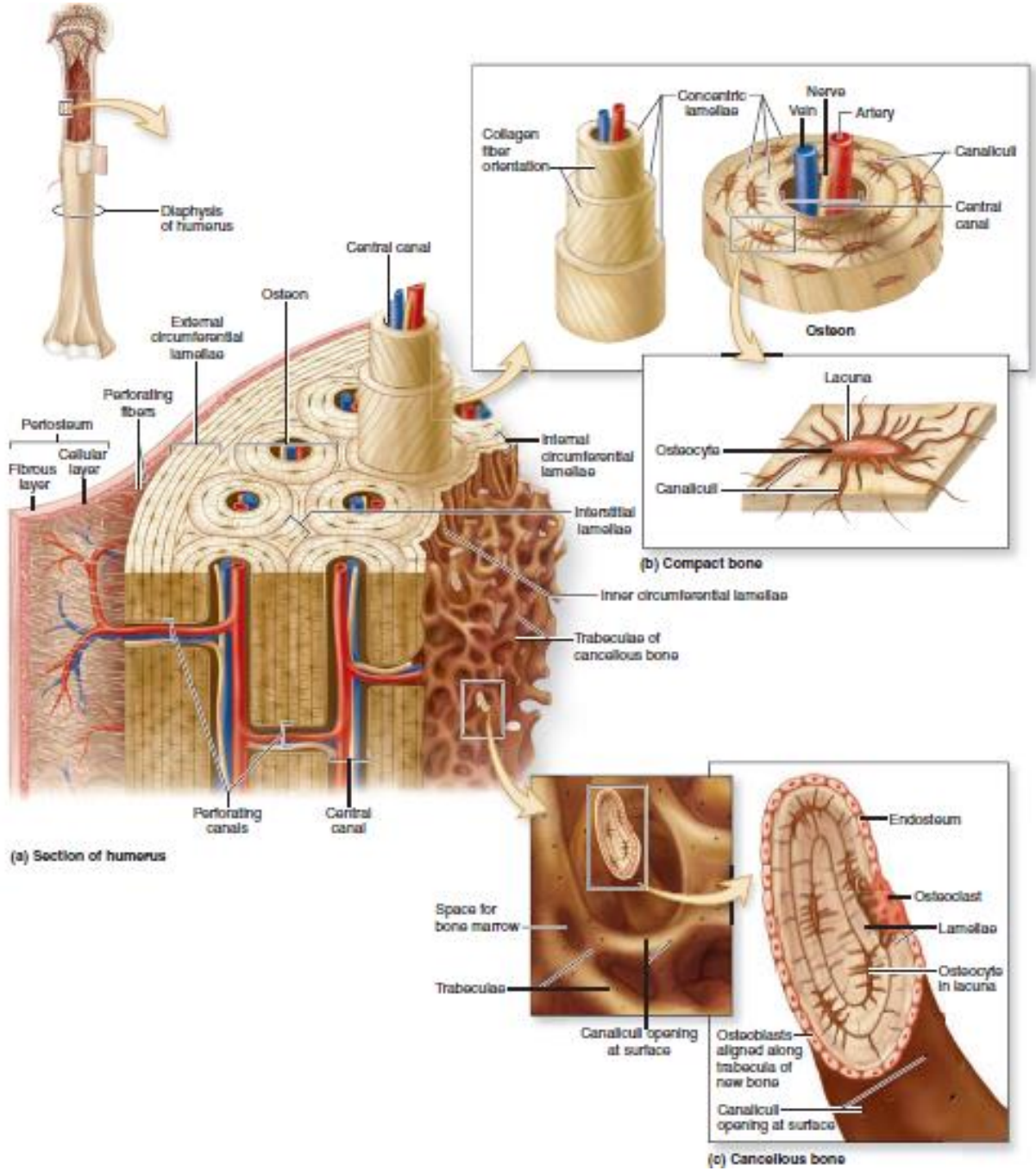
- **Proteoglycan macromolecules** contain a core protein with various numbers of covalently attached side chains of **glycosaminoglycans** (hyaluronan, chondroitin sulfate, and keratan sulfate). They contribute to the compressive strength of bone. They are also responsible for binding growth factors and may inhibit mineralization.

- **Multiadhesive glycoproteins** are responsible for attachment of bone cells and collagen fibers to the mineralized ground substance. Some of the more important **glycoproteins** are **osteonectin,** which serves as a glue between the collagen and hydroxyapatite crystals; **podoplanin (E ll**), which is produced exclusively by osteocytes in response to mechanical stress; **dentin matrix protein (DMP),** which is critical for bone matrix mineralization; and **sialoproteins** such as **osteopontin** (known as **BSP-1),** which mediates attachment of cells to bone matrix, and **BSP-2,** which mediates cell attachment and initiates calcium phosphate formation during the mineralization process.

- **Bone-specific, v itamin K -dependent proteins,** including **osteocalcin,** which captures calcium from the circulation and attracts and stimulates osteoclasts in bone remodeling; **protein S,** which assists in the removal of cells undergoing apoptosis; and **matrix G la-protein (MGP),** which participates in the development of vascular calcifications.

- **Growth factors** and **cytokines,** which are small regulatory proteins such as insulin-like growth factors (IGFs), tumor necrosis factor a (TNF-a), transforming growth factor (3 (TGF-(3), platelet-derived growth factors (PDGFs), **bone morphogenic proteins (BMPs), sclerostin** (BMP antagonist), and **in terleukin s (IL-1, IL-**6**).** The most unique members of this group are BMPs because they induce the differentiation of mesenchymal cells into osteoblasts, the bone-producing cells. Recombinant human **BMP-7,** also known as **osteogenic protein-1 (OP-1),** is now used clinically to induce bone growth after bone surgery involving large bone defects, spinal fusions, or implantation of graft materials.

- Within the bone matrix are spaces called **lacunae** (sing., *lacuna*), each of which contains a bone cell, or **osteocyte.** The osteocyte extends numerous processes into small tunnels called **canaliculi ossei.** Canaliculi course through the mineralized matrix, connecting adjacent lacunae and allowing contact between the cell processes of neighboring osteocytes.
- In this manner, a continuous network of canaliculi and lacunae-containing cells and their processes is formed throughout the entire mass of mineralized tissue. Electron micrographs show that osteocyte processes communicate by gap junctions. Bone tissue depends on the osteocytes to maintain viability.


Diaphysis of humerus
Central canal
Osteon
External circumferential lamellae
Perforating fibers
Periosteum
Fibrous layer
Cellular layer
Internal circumferential lamellae
Interstitial lamellae
Inner circumferential lamellae
Trabeculae of cancellous bone
Perforating canals
Central canal
(a) Section of humerus
Concentric lamellae
Collagen fiber orientation
Nerve
Vein
Artery
Canaliculi
Central canal
Osteon
Lacuna
Osteocyte
Canaliculi
(b) Compact bone
Space for bone marrow
Trabeculae
Canaliculi opening at surface
Endosteum
Osteoclast
Lamellae
Osteocyte in lacuna
Osteoblasts aligned along trabecula of new bone
Canaliculi opening at surface
(c) Cancellous bone

- In addition to osteocytes, four other cell types are associated with bone.
- **Osteoprogenitor cells** are cells derived from mesenchymal stem cells; they give rise to osteoblasts.
- **Osteoblasts** are cells that secrete the extracellular matrix of bone; once the cell is surrounded with its secreted matrix, it is referred to as an **osteocyte.**
- **Bone-lining cells** are cells that remain on the bone surface when there is no active growth. They are derived from those osteoblasts that remain after bone deposition ceases.
- **Osteoclasts** are bone-resorbing cells present on bone surfaces where bone is being removed or remodeled (reorganized) or where bone has been damaged.

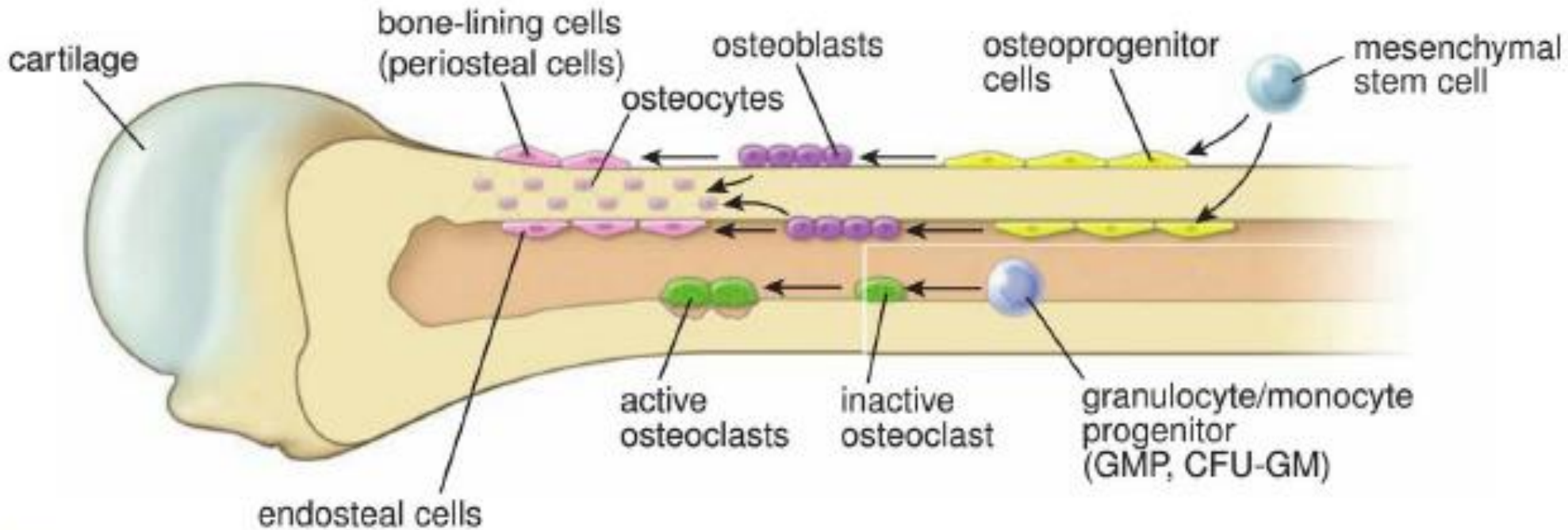


FIGURE 8.8 ▲ Schematic drawing of cells associated with bone. All cells except osteoclasts originate from the mesenchymal stem cells, which differentiate into osteoprogenitor cells, osteoblasts, and finally osteocytes and bone-lining cells. Bone-lining cells on external bone surfaces are part of the periosteum, hence the term periosteal cells. Bone-lining cells on internal bone surfaces are frequently called endosteal cells. Note that osteoprogenitor cells and bone-lining cells have a similar microscopic appearance and are often difficult to distinguish from each other. Osteoclasts originate from hemopoietic progenitor cells, which differentiate into bone-resorbing cells. The specific details of osteoclast differentiation are illustrated in Figure 8.15.

- Osteoprogenitor cells and osteoblasts are developmental precursors of the osteocyte.

- Osteoclasts are phagocytotic cells derived from fusion of hemopoietic progenitor cells in bone marrow that give rise to the neutrophilic granulocyte and monocyte lineages. Each of these cells is described in more detail below.

GENERAL STRUCTURE OF BONES

- **Bones are the organs of the skeletal system; bone tissue is the structural component o f bones.**
- Typically, a **bone** consists of bone tissue and other connective tissues, including hemopoietic tissue, fat tissue, blood vessels, and nerves. If the bone forms a freely movable joint, otherwise referred to as a **synovial joint,** hyaline cartilage is present. The ability of the bone to perform its skeletal function is attributable to the bone tissue, ligaments and, where present, the articular (hyaline) cartilage.

- **Bone tissue is classified as either compact (dense) or spongy (cancellous).**
- If a bone is cut, two distinct structural arrangements of bone tissue can be recognized. A compact, dense layer forms the outside of the bone **(compact bone);** a sponge-like meshwork consisting of **trabeculae** (thin, anastomosing spicules of bone tissue) forms the interior of the bone **(spongy bone).** The spaces within the meshwork are continuous and, in a living bone, are occupied by marrow and blood vessels.

- **Bones are classified according to shape; the location of spongy and compact bone varies with bone shape.**
- Spongy and compact bone tissues are located in specific parts of bones. It is useful, then, to outline briefly the kinds of bones and survey where the two kinds of bone tissue are located. On the basis of shape, bones can be classified into four groups:

a) **Long bones** are longer in one dimension than other bones and consist of a shaft and two ends (e.g., the tibia and the metacarpals).

b) **Short bones** are nearly equal in length and diameter (e.g., the carpal bones of the hand).

c) **Flat bones** are thin and plate-like (e.g., the bones of the calvaria [skull cap] and the sternum). They consist of two layers of relatively thick compact bone with an intervening layer of spongy bone.

d) **Irregular bones** have a shape that does not fit into any one of the three groups just described; the shape may be complex (e.g., a vertebra), or the bone may contain air spaces or sinuses (e.g., the ethmoid bone).

- Long bones have a shaft, called the **diaphysis ,** and two expanded ends, each called an **epiphysis**.
- The articular surface of the epiphysis is covered with hyaline cartilage. The flared portion of the bone between the diaphysis and the epiphysis is called the **metaphysis .** It extends from the diaphysis to the epiphyseal line. A large cavity filled with bone marrow, called the **marrow** or **medullary cavity ,** forms the inner portion of the bone. In the shaft, almost the entire thickness of the bone tissue is compact; at most, only a small amount of spongy bone faces the marrow cavity. At the ends of the bone, the reverse is true. Here, the spongy bone is extensive, and the compact bone consists of little more than a thin outer shell.

- Short bones possess a shell of compact bone and have spongy bone and a marrow space on the inside. Short bones usually form movable joints with their neighbors; like long bones, their articular surfaces are covered with hyaline cartilage.
- Elsewhere, **periosteum ,** a fibrous connective tissue capsule, covers the outer surface of the bone.

OUTER SURFACE OF BONES

- **Bones are covered by periosteum , a sheath of dense fibrous connective tissue containing osteoprogenitor cells.** Bones are covered by a **periosteum** except in areas where they articulate with another bone. In the latter case, the articulating surface is covered by cartilage. The periosteum that covers an actively growing bone consists of an outer fibrous layer that resembles other dense connective tissues and an inner, more cellular layer that contains the osteoprogenitor cells. If active bone formation is not in progress on the bone surface, the fibrous layer is the main component of the periosteum, and the inner layer is not well defined. The relatively few cells that are present, the **periosteal cells,** are, however, capable of undergoing division and becoming osteoblasts under appropriate stimulus.
- In general, the collagen fibers of the periosteum are arranged parallel to the surface of the bone in the form of a capsule. The character of the periosteum is different where ligaments and tendons attach to the bone. Collagen fibers from these structures extend obliquely or at right angles to the long axis of the bone, where they are continuous with the collagen fibers of the extracellular matrix. These fibers are called **perforating** or **Sharpey's fibers.** They extend into the outer circumferential and interstitial lamellae but usually do not enter the osteons.

- **Bones that articulate with neighboring bones possess movable (synovial) joints.**

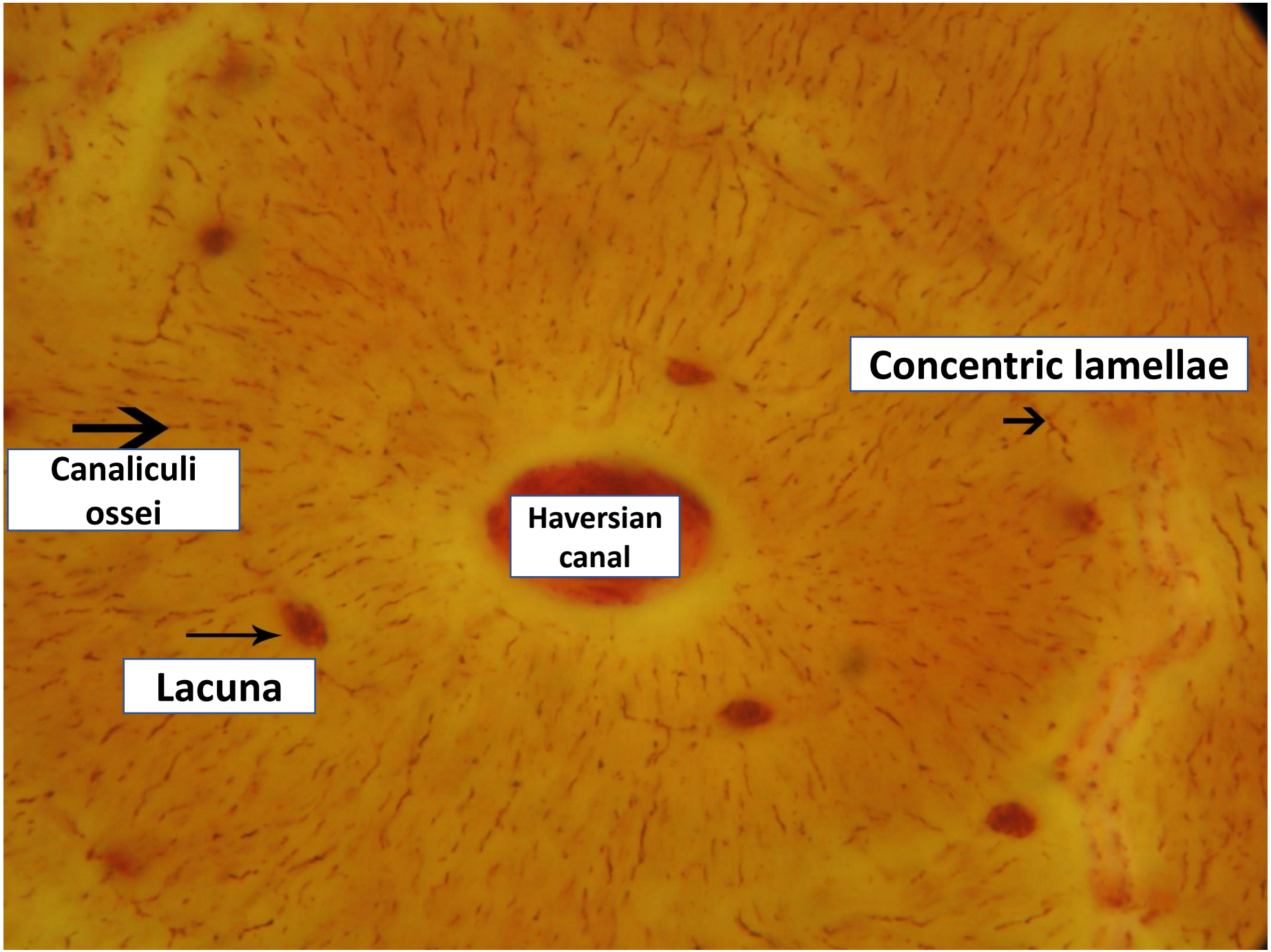
- Where a bone articulates with a neighboring bone, as in **synovial joints ,** the contact areas of the two bones are referred to as **articular surfaces.** The articular surfaces are covered by hyaline cartilage, also called **articular cartilage** because of its location and function; articular cartilage is exposed to the joint cavity. This cartilage is not covered with perichondrium.

BONE CAVITIES

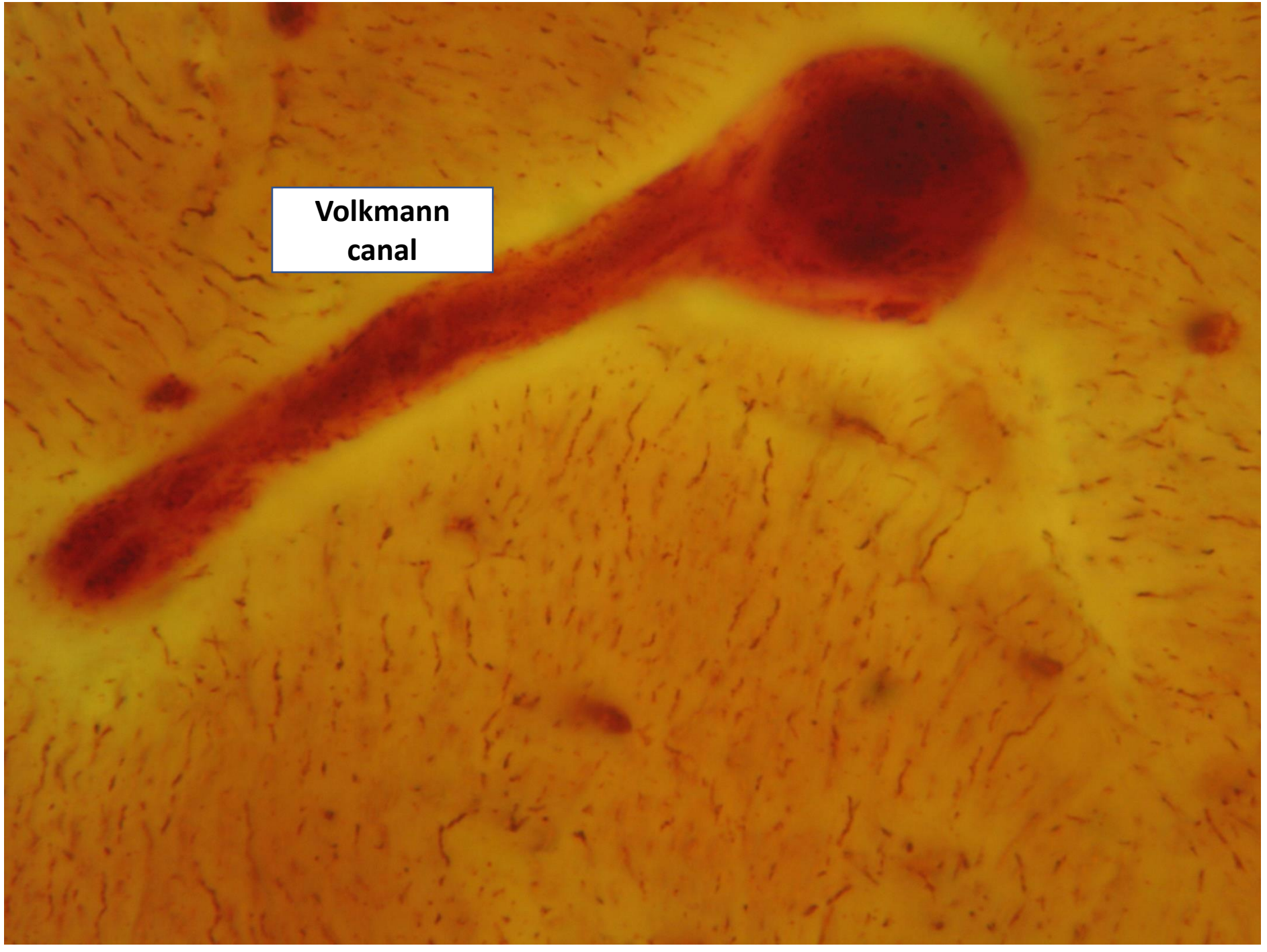
- **Bone cavities are lined by endosteum , a layer of connective tissue cells that contains osteoprogenitor cells.** The **lining tissue** of both the compact bone facing the marrow cavity and the trabeculae of spongy bone within the cavity is referred to as **endosteum .** The endosteum is often only one cell layer thick and consists of osteoprogenitor cells that can differentiate into bone matrix-secreting cells, the osteoblasts, and bone-lining cells. Osteoprogenitor cells and bone-lining cells are difficult to distinguish at the microscopic level. They are both flattened in shape with elongated nuclei and indistinguishable cytoplasmic features. Because of their location within the bone cavities, they are frequently called **endosteal cells.**
- **The marrow cavity and the spaces in spongy bone contain bone m arrow.**
- **Red bone marrow** consists of blood cells in different stages of development and a network of reticular cells and fibers that serve as a supporting framework for the developing blood cells and vessels. As an individual grows, the amount of red marrow does not increase proportionately with bone growth. In later stages of growth and in adults, when the rate of blood cell formation has diminished, the tissue in the marrow cavity consists mostly of fat cells; it is then called **yellow marrow .** In response to appropriate stimuli, such as extreme blood loss, yellow marrow can revert to red marrow. In the adult, red marrow is normally restricted to the spaces of spongy bone in a few locations such as the sternum and the iliac crest. Diagnostic bone marrow samples and marrow for transplantation are obtained from these sites.

TYPES OF BONE TISSUE

- **Mature Bone is composed o f structural units called osteons (Haversian systems).** Mature bone is largely composed of cylindrical units called **o s te o n s** or **H aversian systems**. The osteons consist of **concentric lamellae** (sing., *lam ella)* of bone matrix surrounding a central canal, the **osteonal (Haversian) canal,** which contains the vascular and nerve supply of the osteon. Canaliculi containing the processes of osteocytes are generally arranged in a radial pattern with respect to the canal. The system of canaliculi that opens to the osteonal canal also serves for the passage of substances between the osteocytes and blood vessels. Between the osteons are remnants of previous concentric lamellae called **interstitial lamellae**.
- Because of this organization, mature bone is also called **lamellar bone.** The long axis of an osteon is usually parallel to the long axis of the bone. The collagen fibers in the concentric lamellae in an osteon are laid down parallel to one another in any given lamella but in different directions in adjacent lamellae. This arrangement gives the cut surface of lamellar bone the appearance of plywood and imparts great strength to the osteon. Lamellar bone is also found at sites other than the osteon. **Circumferential lamellae** follow the entire inner and outer circumferences of the shaft of a long bone, appearing much ike the growth rings of a tree.


Concentric lamellae
Canaliculi ossei
Haversian canal
Lacuna

- **Perforating (Volkmann's) canals** are channels in lamellar bone through which blood vessels and nerves travel from the periosteal and endosteal surfaces to reach the osteonal (Haversian) canal; they also connect osteonal canals to one another. They usually run at approximately right angles to the long axis of the osteons and of the bone. Volkmann’s canals are not surrounded by concentric lamellae, a key feature in their histologic identification.


Volkmann
canal

- Mature **spongy bone** is similar in structure to mature compact bone except that the tissue is arranged as **trabeculae** or **spicules;** numerous interconnecting marrow spaces of various sizes are present among the bone tissue. The matrix of the bone is lamellated.
- **Arteries that enter the marrow cavity through the nutrient foramina supply blood to the shaft of long bones. Nutrient foramina** are openings in the bone through which blood vessels pass to reach the marrow. The greatest numbers of nutrient foramina are found in the diaphysis and epiphysis. Metaphyseal arteries supplement the blood supply to the bone. Veins that exit through the nutrient foramina or through the bone tissue of the shaft and continue out through the periosteum drain the blood from bone.
- The **nutrient arteries** that supply the diaphysis and epiphysis arise developmentally as the principal vessels of the periosteal buds. The metaphyseal arteries, in contrast, arise developmentally from periosteal vessels that become incorporated into the metaphysis during the growth process (i.e., through the widening of the bone).

IMMATURE BONE

- Bone tissue initially formed in the skeleton of a developing fetus is called **immature bone.** It differs from mature bone in several respects. Immature bone does not display an organized lamellated appearance. On the basis of its collagen fiber arrangement, such bone is designated **nonlamellar.** Nonlamellar bone is also referred to as **bundle bone** or **woven bone** because of the interlacing arrangement of the collagen fibers. Immature bone contains relatively more cells per unit area than does mature bone.
- The cells in immature bone tend to be randomly arranged, whereas cells in mature bone are usually arranged with their long axes in the same direction as the lamellae. • The matrix of immature bone has more ground substance than does the matrix of mature bone. The matrix in immature bone stains more intensely with hematoxylin, whereas the matrix of mature bone stains more intensely with eosin.

CELLS OF BONE TISSUE

As noted previously, five designated cell types are associated with bone tissue: **osteoprogenitor cells, osteoblasts, osteocytes, bone-lining cells, and osteoclasts**.

With the exception of the osteoclast, each of these cells may be regarded as a differentiated form of the same basic cell type . Each undergoes transformation from a less mature form to a more mature form in relation to functional activity (growth of bone). In contrast, the osteoclast originates from a different cell line and is responsible for bone resorption, an activity associated with bone remodeling.

OSTEOPROGENITOR CELLS

The osteoprogenitor cell is derived from mesenchymal stem cells. Osteogenesis, the process of new bone formation, is essential to normal bone function. It requires a population of renewable **osteoprogenitor cells** (osteoblast precursor cells) that are responsive to molecular stimuli that transform them into bone-forming cells. Osteoprogenitor cells are derived from **mesenchymal Stem cells** in the bone marrow that have the potential to differentiate into many different cell types, including fibroblasts, osteoblasts, adipocytes, chondrocytes, and muscle cells. The key factor that triggers differentiation of osteoprogenitor cells is a transcription factor called **core binding factor alpha-1 (CBFA1)** or **runt-related transcription factor 2 (R U N X 2).**

This protein prompts the expression of genes that are characteristic of the phenotype of the osteoblast. IGF-1 and IGF-2 stimulate osteoprogenitor cell proliferation and differentiation into osteoblasts. bone morphogenic proteins (BMPs) also play a role in the differentiation into osteoblasts.

- **The osteoprogenitor cell is a resting cell that can differentiate into an osteoblast and secrete bone matrix. Osteoprogenitor cells** are found on the external and internal surfaces of bones and may also reside in the microvasculature supplying bone. Morphologically, they resemble the **periosteal cells** that form the innermost layer of the periosteum and the **endosteal cells** that line the marrow cavities, the osteonal (Haversian) canals, and the perforating (Volkmann’s) canals.
- In growing bones, osteoprogenitor cells appear as flattened or squamous cells with lightly staining, elongate, or ovoid nuclei and inconspicuous acidophilic or slightly basophilic cytoplasm. Electron micrographs reveal profiles of rough-surfaced endoplasmic reticulum (rER) and free ribosomes as well as a small Golgi apparatus and other organelles.

OSTEOBLASTS

- **The osteoblast is the differentiated bone-forming cell that secretes bone matrix.** Like its close relatives, the fibroblast and the chondroblast, the **osteoblast** is a versatile secretory cell that retains the ability to divide. It secretes both **type I collagen** (which constitutes 90% of the protein in bone) and **bone matrix proteins,** which constitute the initial unmineralized bone, or **osteoid.** The bone matrix proteins produced by the osteoblast include **calcium - binding proteins** such as osteocalcin and osteonectin, **multi adhesiveglycoproteins** such as bone sialoproteins (BSP-1 [osteopontin] and BSP-2), thrombospondins, various **proteoglycans** and their aggregates, and **alkaline phosphatase (ALP).** Circulating levels of ALP and osteocalcin are used clinically as markers of osteoblast activity.
- The osteoblast is also responsible for the calcification of bone matrix. The calcification process appears to be initiated by the osteoblast through the secretion into the matrix of small, 50- to 250-nm, membrane-limited **matrix vesicles.** The vesicles are rich in ALP and are actively secreted only during the period in which the cell produces the bone matrix. Osteoblasts are recognized in the light microscope by their cuboidal or polygonal shape and their aggregation into a single layer of cells lying in apposition to the forming bone. The newly deposited matrix is not immediately calcified.

- The cytoplasm of the osteoblast is markedly basophilic, and the Golgi apparatus, because of its size, is sometimes observed as a clear area adjacent to the nucleus. Small, periodic acid-Schiff (PAS)-positive granules are observed in the cytoplasm, and a strong ALP reaction associated with the cell membrane can be detected by appropriate histochemical staining.
- In contrast to the secreting osteoblasts found in active matrix deposition, inactive osteoblasts are flat or attenuated cells that cover the bone surface. These cells resemble osteoprogenitor cells. Osteoblasts respond to mechanical stimuli to mediate the changes in bone growth and bone remodeling. As osteoid deposition occurs, the osteoblast is eventually surrounded by osteoid matrix and thereby becomes an osteocyte.

- **Osteoblast processes communicate with the osteoblasts and with osteocytes by gap junctions.**
- At the electron microscope level, osteoblasts exhibit thin cytoplasmic processes that penetrate the adjacent osteoid produced by the cell and are joined to similar processes of adjacent osteocytes by gap junctions. This early establishment of junctions between an osteoblast and adjacent osteocytes (as well as between adjacent osteoblasts) allows neighboring cells within the bone tissue to communicate. The osteoblast cytoplasm is characterized by abundant rER and free ribosomes. These features are consistent with its basophilia observed in the light microscope as well as with its role in the production of collagen and proteoglycans for the extracellular matrix.

- The Golgi apparatus and surrounding regions of the cytoplasm contain numerous vesicles with a flocculent content that is presumed to consist of matrix precursors. These vesicles are the PAS-staining granules seen in light microscopy. The matrix vesicles, also produced by the osteoblast, appear to arise by a different pathway, originating as sphere-like outgrowths that pinch off from the plasma membrane to become free in the matrix. Other cell organelles include numerous rod-shaped mitochondria and occasional dense bodies and lysosomes.

OSTEOCYTES

- **The osteocyte is the mature bone cell enclosed by bone matrix that was previously secreted as an osteoblast.** When completely surrounded by osteoid or bone matrix, the osteoblast is referred to as an **osteocyte**. The process of transformation from osteoblast to osteocyte takes approximately 3 days. Each osteocyte develops on average about 50 cell processes. After bone matrix mineralization, each osteocyte occupies a space, or **lacuna,** that conforms to the shape of the cell. Osteocytes' cytoplasmic processes are enclosed by the **canaliculi** within the matrix. They communicate with processes of neighboring osteocytes and bone-lining cells by means of **gap junctions** formed by a family of bone-expressed connexins.
- Osteocytes also communicate indirectly with distant osteoblasts, endothelial cells of bone marrow vasculature, pericytes of blood vessels, and other cells through the expression of various signaling molecules, such as nitric oxide or glutamate transporters. Osteocyte processes contain **hemichannels** (the unopposed half of gap junction channels) that provide communication between cells and extracellular matrix.

Osteocytes are typically smaller than their precursor cells because of their reduced perinuclear cytoplasm. Often, in routinely prepared microscopic specimens, the cell is highly distorted by shrinkage and other artifacts that result from decalcifying the matrix before sectioning the bone.

In such instances, the nucleus may be the only prominent feature. In well-preserved specimens, osteocytes exhibit less cytoplasmic basophilia than osteoblasts, but little additional cytoplasmic detail can be seen

- **Osteocytes are metabolically active and multifunctional cells that respond to mechanical forces applied to the bone.** In the past, osteocytes were considered passive cells responsible only for maintaining the bone matrix. Recent discoveries show that osteocytes are metabolically active and multifunctional cells. They are involved in the process of **mechanotransduction** in which they respond to mechanical forces applied to the bone.
- Decreased mechanical stimuli (e.g., immobilization, muscle weakness, weightlessness in space) causes bone loss, whereas increased mechanical stimuli promotes bone formation. Due to the slight flexibility of bone, mechanical forces applied to the bone (e.g., to the femur or tibia during walking) cause flow of interstitial fluid out of the canaliculi and lacunae on the compressed side of the bone. Movement of interstitial fluid through the canalicular system generates a **transient electrical potential (streaming potential)** at the moment when the stress is applied.

- An osteocyte responds to reduced mechanical stress by secreting **matrix metallo proteinases (MMPs).** The empty space surrounding osteocytes is the result of enzymatic degradation of bone matrix by MM P. Increased mechanical stress activates molecular mechanisms similar to those found in the matrix-producing osteoblasts. Thus, the osteocytes are responsible for reversible remodeling of their pericanalicular and perilacunar bone matrix. This process is called **osteocytic remodeling.**

Osteocytes appear in different functional states during the osteocytic remodeling of their perilacunar and pericanalicular microenvironment.

Electron microscopy has revealed osteocytes in various functional states related to the **osteocytic remodeling** process. Indeed, there is histologic and microradiologic evidence (i.e., enlarged lacunae and reduced radiodensity) that the osteocyte can remodel the surrounding bone matrix. As mentioned above, osteocytes can modify their microenvironment (the volume of their lacunae or diameter of their canaliculi) in response to environmental stimuli.

Three functional states, each with a characteristic morphology, have been identified based on the appearance of osteocytes in electron micrographs:

Quiescent osteocytes exhibit a paucity of rER and a markedly diminished Golgi apparatus. An osmiophilic lamina representing mature calcified matrix is seen in close apposition to the cell membrane.

Formative osteocytes show evidence of matrix deposition and exhibit certain characteristics similar to those of osteoblasts. Thus, the rER and Golgi apparatus are more abundant, and there is evidence of osteoid in the pericellular space within the lacuna.

Resorptive osteocytes, like formative osteocytes, contain numerous profiles of endoplasmic reticulum and a well-developed Golgi apparatus. Moreover, lysosomes are conspicuous. Degradation of bone by MMPs secreted by the resorptive osteocytes previously was called **osteocytic osteolysis.** The current concept of osteocytic remodeling is that the lytic role of osteocytes is responsible for calcium and phosphate ion homeostasis.

BONE-LINING CELLS

- **Bone-lining cells are derived from osteoblasts and cover bone that is not remodeling.**
- In sites where remodeling is not occurring, the bone surface is covered by a layer of flat cells with attenuated cytoplasm and a paucity of organelles beyond the perinuclear region. These cells are designated simply as **bone-lining cells.** Bone-lining cells on external bone surfaces are called **periosteal cells,** and those lining internal bone surfaces are often called **endosteal cells.** Gap junctions are present where the bone-lining cell processes contact one another.
- Bone-lining cells represent a population of cells that are derived from osteoblasts. They are thought to function in the maintenance and nutritional support of the osteocytes embedded in the underlying bone matrix and regulate the movement of calcium and phosphate into and out of the bone.

OSTEOCLASTS

The osteoclast is responsible for bone resorption.

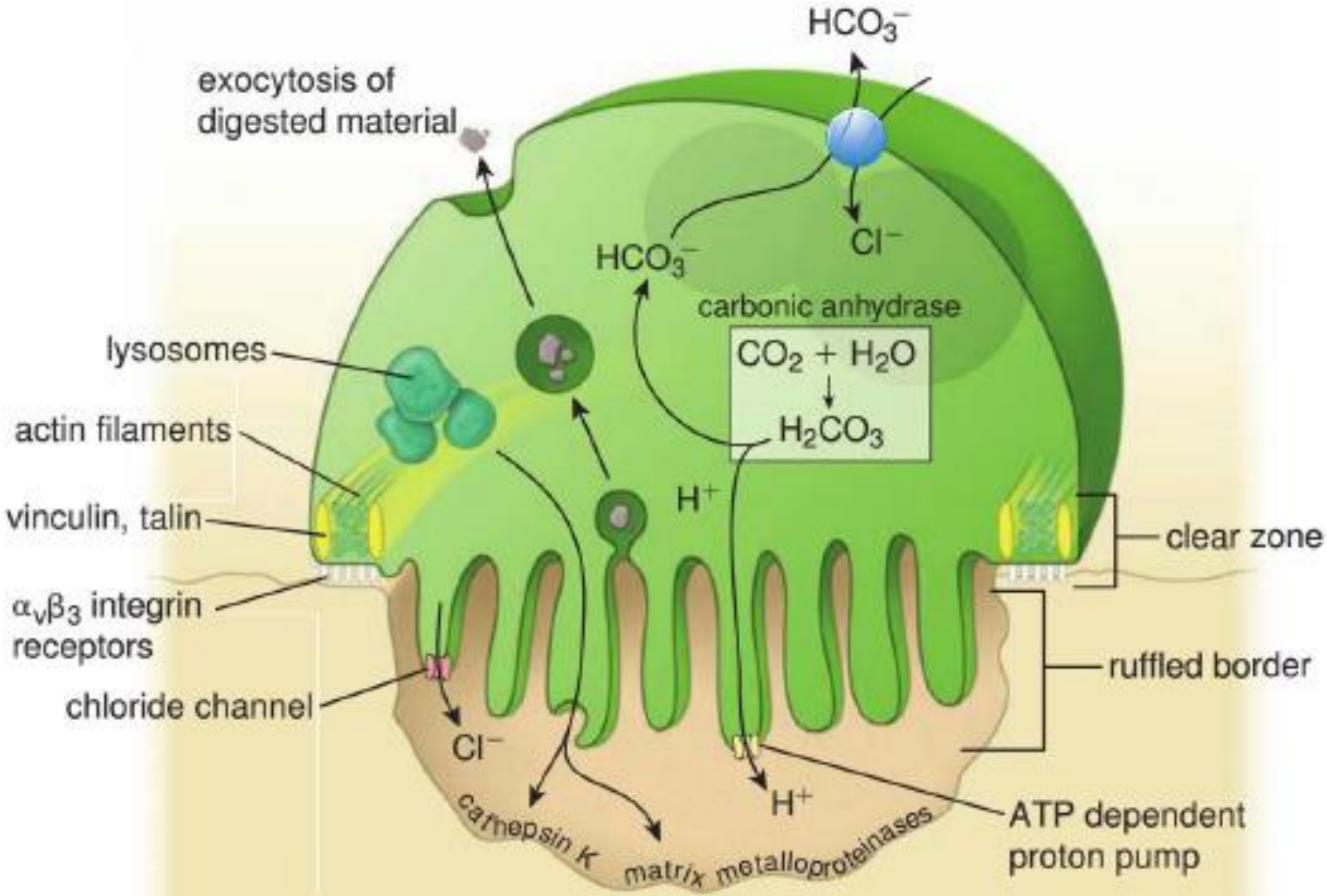
Osteoclasts are large, multinucleated cells found at sites where bone is being removed. They rest directly on the bone tissue where resorption is taking place. As a result of osteoclast activity, a shallow bay called a **resorption bay (Howship's lacuna)** can be observed in the bone directly under the osteoclast. The cell is conspicuous not only because of its large size but also because of its marked acidophilia. It also exhibits a strong histochemical reaction for acid phosphatase because of the numerous lysosomes that it contains.

Osteoclasts are derived from the fusion of mononuclear hemopoietic progenitor cells under the influence of multiple cytokines. Contrary to what was once thought, **osteoclasts** are not related to osteoblasts. They are derived from the fusion of mononuclear hemopoietic cells, namely, **granulocyte / macrophage progenitor cells (GMRCFU-GM)** that give rise to granulocyte and monocyte cell lineages. Osteoclast formation occurs in close association with stromal cells in bone marrow. These cells secrete essential cytokines for differentiation of both osteoclasts and macrophages from GMP progenitor cells, including monocyte colony stimulating factor (M-CSF), TNF, and several interleukins. Initially, cells committed to become osteoclasts (osteoclast precursors) express two important transcription factors, **C-fos** and **NF-k B;** later, a receptor molecule called **receptor activator of nuclear factor-K B (RANK)** is expressed on their surface.

- The RANK receptor interacts with **R A N K ligand molecule (R A N K L)** produced and expressed on the stromal cell surface. The **RANK -R A N K L signaling mechanism** is essential for osteoclast differentiation and maturation.
- Alternatively, during **inflammation ,** activated T lymphocytes can produce both membrane-bound and soluble RANKL molecules. Therefore, inflammatory processes can stimulate osteoclast-mediated bone resorption. This pathway can be blocked by **osteoprotegerin (O P G),** which serves as a “decoy” receptor for RANKL. Lack of available ligand affects the RANK-RANKL signaling pathway and acts as a potent inhibitor of osteoclast formation.

- **Newly formed osteoclasts undergo an activation process to become bone-resorbing cells.**
- The newly formed **osteoclast** must be activated to become a bone-resorbing cell. During this process, it becomes highly polarized. When actively resorbing bone, osteoclasts exhibit three specialized regions:
- The **ruffled border** is the part of the cell in direct contact with bone. It contains numerous deep plasma membrane infoldings forming microvillous-type structures responsible for increasing surface area for the exocytosis of hydrolytic enzymes and secretion of protons by ATP-dependent proton pumps, as well as endocytosis of degradation products and bone debris. Internal to the ruffled border and in close proximity are numerous mitochondria and lysosomes. The nuclei are typically located in the part of the cell more removed from the bone surface. In this same region are profiles of rER, multiple stacks of Golgi apparatus, and many vesicles.

- The **clear zone** (sealing zone) is a ring-like perimeter of cytoplasm adjacent to the ruffled border that demarcates the bone area being resorbed. Essentially, the clear zone is a compartment at the site of the ruffled border where resorption and degradation of the matrix occurs. It contains abundant actin filaments but essentially lacks other organelles. The actin filaments are arranged in a ring-like structure surrounded on both sides by actin-binding proteins such as vinculin and talin. The plasma membrane at the site of the clear zone contains cell and **extra cellular matrix adhesion molecules** that are responsible for providing a tight seal between the plasma membrane and mineralized matrix of the bone. Several classes of **integrin extra cellular receptors** (i.e., a vp3 vitronectin receptor, a 2pi type I collagen receptor, or a vpi vitronectin/fibrinogen receptor) help maintain the seal.
- The **basolateral region** functions in the exocytosis of digested material. Transport vesicles containing degraded bone material endocytosed at the ruffled border fuse here with the cell membrane to release their contents. TRAP has been found within these vesicles, suggesting its role in the fragmentation of endocytosed material.


HCO_3^-
exocytosis of digested material
Cl^-
HCO_3^-
carbonic anhydrase
$CO_2 + H_2O$
H_2CO_3
lysosomes
actin filaments
vinculin, talin
H^+
clear zone
$\alpha_v\beta_3$ integrin receptors
ruffled border
chloride channel
Cl^-
H^+
cathepsin K
matrix metalloproteinases
ATP dependent proton pump

- **Osteoclast function is regulated by many factors.** Digested materials from the resorbed bone are transported in endocytic vesicles across the osteoclast. The content of the endocytic vesicles that originate at the ruffled border is released at the basement membrane, which is usually in contact with blood vessels. Therefore, numerous coated pits and coated vesicles are present at the ruffled border. Osteoclasts are observed at sites where bone remodeling is in progress. Thus, at sites where osteons are being altered or where bone is undergoing change during the growth process, osteoclasts are relatively numerous.
- **Parathyroid hormone (PTH)** secreted by the principal (chief) cells of the parathyroid glands is the most important regulator of calcium and phosphate levels in the extracellular fluid. Because osteoclasts do not have PTH receptors, PTH exerts only an indirect effect on osteoclasts. In contrast, osteocytes, osteoblasts, and T lymphocytes all have **PTH receptors** that activate adenyl cyclase, increasing intracellular levels of cAMP. Brief intermittent exposure to PTH increases bone mass through the cyclic adenosine monophosphate (cAMP)/IGF-l pathway in osteocytes and osteoblasts.

- However, prolonged continuous exposure to PTH increases the production of RANKL byT lymphocytes and osteoblasts, leading to osteoclastic hyperactivity and eventually osteoporosis. Estrogen suppresses RANKL production by T lymphocytes.
- **Calcitonin,** secreted by the parafollicular cells of the thyroid gland, has the singular effect of reducing osteoclastic activity.

BONEFORMATION

- The distinction between endochondral and intramembranous formation rests on whether a cartilage model serves as the precursor of the bone **(endochondral ossification)** or whether the bone is formed by a simpler method, without the intervention of a cartilage precursor **(intramembranous ossification).** The bones of the extremities and those parts of the axial skeleton that bear weight (e.g., vertebrae) develop by endochondral ossification. The flat bones of the skull and face, the mandible, and the clavicle develop by intramembranous ossification.

INTRAMEMBRANOUS OSSIFICATION

- The first evidence of intramembranous ossification is seen mammalian gestation within embryonic connective tissue, the mesenchyme. Some of the spindle-shaped, pale-staining **mesenchymal cells** migrate and aggregate in specific areas (e.g., the region of flat bone development in the head), forming **ossification centers.** This condensation of cells within the mesenchymal tissue initiates the process of intramembranous ossification. Mesenchymal cells in these ossification centers elongate and differentiate into **osteoprogenitor cells.**
- The osteoprogenitor cell cytoplasm changes from eosinophilic to basophilic, and a clear Golgi area becomes evident. These cytologic changes result in the differentiated **osteoblast,** which then secretes the collagens (mainly type I collagen molecules), bone sialoproteins, osteocalcin, and other components of the bone matrix (osteoid). The osteoblasts accumulate at the periphery of the ossification center and continue to secrete osteoid at the center of the nodule. As the process continues, the osteoid undergoes mineralization and the entrapped osteoblasts become **osteocytes**. Within the bony matrix, osteocytes increasingly separate from one another as more matrix is produced, but they remain attached by thin cytoplasmic processes. With time, the matrix becomes mineralized, and the interconnecting cytoplasmic processes of osteocytes are contained within canaliculi.

ENDOCHONDRAL OSSIFICATION

- **Endochondral ossification** also begins with the proliferation and aggregation of mesenchymal cells at the site of the future bone. Under the influence of different fibroblastic growth factors (FGFs) and bone morphogenic proteins (BMPs) the mesenchymal cells initially Express type II collagen and differentiate into chondroblasts that, in turn, produce cartilage matrix.
- **Initially, a hyaline cartilage model with the general shape of the bone is formed.** Once established, the **cartilage model** (a miniature version of the future definitive bone) grows by interstitial and appositional growth. The increase in the length of the cartilage model is attributed to interstitial growth. The increase in its width is largely the result of the addition of cartilage matrix produced by new chondrocytes that differentiate from the chondrogenic layer of the perichondrium surrounding the cartilage mass.

- At this stage, the perichondrial cells in the midregion of the cartilage model no longer give rise to chondrocytes. Instead, **bone-forming cells** or **o steoblasts** are produced. Thus, the connective tissue surrounding this portion of the cartilage is no longer fu n ctionally a perichondrium ; rather, because of its altered role, it is now called periosteum .
- Moreover, because the cells within this layer are differentiating into osteoblasts, an **osteogenic layer** can now be identified within the periosteum. Because of these changes, a layer of bone is formed around the cartilage model. This bone can be classified as either periosteal bone, because of its location, or intramembranous bone, because of its method of development. In the case of a long bone, a distinctive cuff of periosteal bone, the **bony collar,** is established around the cartilage model in the diaphyseal portion of the developing bone.

- As the chondrocytes enlarge, their surrounding cartilage matrix is resorbed, forming thin irregular cartilage plates between the **hypertrophic cells.** The hypertrophic cells begin to synthesize alkaline phosphatase; concomitantly, the surrounding **cartilage matrix** undergoes **calcification.**
- With the death of the chondrocytes, much of the matrix breaks down, and neighboring lacunae become confluent, producing an increasingly large cavity. While these events are occurring, one or several blood vessels grow through the thin diaphyseal bony collar to vascularize the cavity.
- **Mesenchymal stem** cells residing in the developing periosteum migrate along the penetrating blood vessels and differentiate into osteoprogenitor cells in the bone marrow cavity. **Hemopoietic stem cells (HSCs)** also gain access to the cavity via the new vasculature, leaving the circulation to give rise to the marrow, including all the blood cell lineages. As the calcified cartilage breaks down and is partially removed, some remains as irregular spicules. When the osteoprogenitor cells come in apposition to the remaining calcified cartilage spicules, they become osteoblasts and begin to lay down bone matrix (osteoid) on the spicule framework. Thus, the bone formed in this manner may be described as endochondral bone. This first site where bone begins to form in the diaphysis of a long bone is called the **primary ossification center**). The combination of bone, which is initially only a thin layer, and the underlying calcified cartilage is described as a **mixed spicule.**

BIOLOGIC MINERALIZATION AND MATRIX VESICLES

Mineralization occurs in the **extracellular matrix** of bone, cartilage, and in the dentin, cementum, and enamel of teeth. The matrices of all of these structures except enamel contain collagen fibrils and ground substance. Mineralization is initiated at the same time within the collagen fibrils and in the ground substance surrounding them. In enamel, mineralization occurs within the extracellular matrix secreted by the enamel organ.

Despite the extracellular location of biologic mineralization and the fact that physicochemical factors are basic to the process, biologic mineralization is a cell-regulated event.

- In places where the mineralization of bone, cartilage, dentin, and cementum is initiated, the local concentration of Ca2+ and PO4 ions in the matrix must exceed the normal threshold level. Several events are responsible for this mineralization:
- • The binding of extracellular Ca2+ by **osteocalcin** and other sialoproteins creates a high local concentration of this ion.
- • The high Ca2+ concentration stimulates the osteoblasts to secrete **alkaline phosphatase (ALP),** which increases the local concentration of PO4 ions. The high PO4 concentration stimulates further increases in Ca2+ concentration where mineralization will be initiated.
- • At this stage of high extracellular Ca2+ and PO4 concentration, the osteoblasts release small (50- to 200-nm) **matrix vesicles** into the bony matrix by exocytosis. The matrix vesicles contain ALP and pyrophosphatase that cleave PO4 ions from other molecules of the matrix.

- The matrix vesicles that accumulate Ca2+ and cleave PO4 ions cause the local isoelectric point to increase, which results in **crystallization of CaP**O4 in the surroundig matrix vesicles.

- • The CaPO4 crystals initiate matrix mineralization by the formation and deposition of **hydroxyapatite crystals** [Ca10(PO4)6(OH)2] in the matrix surrounding the osteoblasts.

PHYSIOLOGIC ASPECTS OF BONE

The maintenance of normal blood calcium levels is critical to health and life. Calcium may be delivered from the bone matrix to the blood if the circulating blood levels of calcium fall below a critical point. Conversely, excess blood calcium may be removed from the blood and stored in bone.

These processes are regulated by **parathyroid hormone (PTH),** secreted by the principal (chief) cells of the parathyroid glands, and **calcitonin,** secreted by the parafollicular cells of the thyroid gland.

PTH acts on the bone to *raise low blood calcium levels* to normal.

Calcitonin acts on the bone to *low er elevated calcium levels* to normal.

PTH stimulates both osteocytes and osteoclasts (indirectly via RANK-RANKL signaling pathways because osteoclasts do not have PTH receptors) to resorb bone, thereby releasing calcium into the blood.

Calcitonin inhibits bone resorption, specifically inhibiting the effects of PTH on osteoclasts.

- Several recent discoveries of novel hormones produced by osteoblasts and osteocytes include skeleton in the group of endocrine organs responsible for mineral and nutrient homeostasis. These hormones include the following:
- **Fibroblast growth factor 23 (FGF-23),** which is produced by osteocytes, regulates serum phosphate levels by altering the levels of active vitamin D and the activity of specific phosphate transporters in the kidney. FGF-23 is an important factor in aiding PTH in the disposal of excess phosphate released from hydroxyapatites during bone resorption.
- **Osteocalcin,** which is produced by osteoblasts, is linked to a new pathway regulating energy and glucose metabolism. It targets adipocytes and insulin-producing cells in the pancreas.

BIOLOGY OF BONE REPAIR

- Repair of bone fracture can occur in two processes: direct or indirect bone healing.
- **Direct (primary) bone healing** occurs when the fractured bone is surgically stabilized with compression plates, thereby restricting movement completely between fractured fragments of bone. In this process, bone undergoes internal remodeling similar to that of mature bone. The cutting cones formed by the osteoclasts cross the fracture line and generate longitudinal resorption canals that are later filled by bone-producing osteoblasts residing in the closing cones. This process results in the simultaneous generation of a bony union and the restoration of Haversian systems.
- **Indirect (secondary) bone healing** involves responses from periosteum and surrounding soft tissues as well as endochondral and intramembranous bone formation. This type of bone repair occurs in fractures that are treated with nonrigid or semirigid bone fixation (i.e., treatment with casts, fracture braces, external fixation, intramedullary nailing, or application of metal plates over the fracture gap).

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