BONE TISSUE

Bone tissue is a specialized form of connective tissue and is the main element of the skeletal tissues. It is composed of cells and an extracellular matrix in which fibers are embedded. Bone tissue is unlike other connective tissues in that the extracellular matrix becomes calcified.

FUNCTIONS OF BONE TISSUE

- The skeleton is built of bone tissue. Bone provides the internal support of the body and provides sites of attachment of tendons and muscles, essential for locomotion.
- Bone provides protection for the vital organs of the body: the skull protects the brain; the ribs protect the heart and lungs.
- The hematopoietic bone marrow is protected by the surrounding bony tissue.
- The main store of calcium and phosphate is in bone. Bone has several metabolic functions especially in calcium homeostasis.

Bone is a hard, but brittle, tissue and is relatively light per unit volume. Bone is a dynamic tissue, which throughout life bone tissue is continually being formed and resorbed. This **remodelling and reorganization** of bone tissue is the result of many factors including:

  - mechanical stimuli
  - metabolic causes (lack of dietary calcium, illness, aging)
  - endocrine changes
  - effects of drugs.

MACROSCOPIC STRUCTURE OF BONE

There are two main categories of bone :

- **Spongy bone (trabecular bone, cancellous bone)**
- **Compact bone (cortical bone)**

Spongy bone

Spongy bone is composed of a lattice or network of branching bone spicules or trabeculae. The spaces between the bone spicules contain bone marrow.

Compact bone

Compact bone appears as a mass of bony tissue lacking spaces visible to the unaided eye.

Anatomical classification of bones

Bones are characterized anatomically as:

- **long bones** (e.g. humerus, femur)
- **flat bones** (membrane bones)
- **irregular bones** (such as the vertebrae)

All these bone types, regardless of their anatomical form, are composed of both spongy and compact bone.

Macroscopic structure of long bones

The main shaft of long bones is called the **diaphysis**. At the extremities of the long bone are the **epiphyses** (in articulating joints). The region involved in bone elongation between the diaphysis and epiphysis in growing bones is called the **metaphysis**. The shaft (or diaphysis) is composed of compact (cortical or diaphyseal) bone. The epiphyses are mainly composed of trabeculae of spongy bone. The articulating surface of the epiphyses of synovial joints is covered with articular cartilage.

Bones are covered with a connective tissue called the **periosteum** (absent from the articular cartilage surfaces). A thinner layer of connective tissue, known as the endosteum, surrounds the bone marrow spaces and trabeculae of spongy bone. The periosteum and endosteum are a source of new bone-forming cells (**osteoprogenitor cells**) and are described as possessing **osteogenic potential**. The periosteum and endosteum are also involved in bone repair after injury. Blood vessels of the periosteum and endosteum are involved in nutrition of the bone.

Macroscopic structure of flat bones

The flat bones or "membrane" bones of the skull are composed in a sandwich-like fashion of an outer layer of compact bone (**outer table**), a middle layer of spongy bone (**diploe**), and an inner layer of compact bone (**inner table**). Periosteum covers the flat bone on the outer side (near the scalp) and on the inner side the periosteum is thicker and continuous with the duramater (outer

meningeal layer of the brain).

PREPARATION OF HISTOLOGICAL SECTIONS OF BONE

Because bone tissue is hard and calcified, special histological techniques are used to prepare sections.

- **Decalcification**. The most common techniques involve calcium removal from the tissue (decalcification) after fixation and prior to wax embedding. Acids, such as formic acid or nitric acid, can be used as decalcifying agents. After decalcification the tissue is soft and can be embedded and processed as in standard histology. It is also possible to use chelating agents, such as EDTA, which specifically bind calcium. These chelating agents are less damaging to the tissue than acids, but the decalcification process may be quite long (several weeks or more).
- **Ground sections**. It is possible to grind the bone until the sample is sufficiently thin for histological observation. The cells and organic tissue are destroyed in such preparations, though the canaliculi and cell lacunae are well seen. (Similar techniques are used by geologists to prepare thin sections of rock samples).
- **Sections of non-decalcified bone**. It is possible to embed bone tissue in a hard resin and section it with special knives (tungsten-carbide). Small samples for electron microscopy can be cut with diamond knives.

MICROSCOPIC STRUCTURE OF BONE

This is best seen in compact bone, for example, in transverse sections of the diaphysis of a long bone. The cells constitute only a very small percentage of the bone tissue, whereas the bulk of the tissue is occupied by the intercellular, calcified, bone matrix.

The bone matrix has two main components :

- **Organic matrix**
- **Inorganic salts.**

Organic matrix

The organic matrix is composed of **type I collagen fibers** (about 95%) embedded in an **amorphous ground substance** consisting of:

- **sulfated glycosaminoglycans**

  (chondroitin-4-sulfate, chondroitin-6-sulfate, keratan sulfate)

- various **bone proteins** (bone sialoprotein, osteocalcin).

The relative amounts of sulfated GAG's are far less than in hyaline cartilage, and bone matrix appears acidophilic after regular staining (H&E).

Inorganic salts

The main calcium deposits in the bone matrix are in the form of crystals of **hydroxyapatite** $Ca_{10}(PO_4)_6.(OH)_2$

BONE CELLS

4 different cell types are found in developing bone:

- **Osteoprogenitor cells**
- **Osteoblasts**
- **Osteocytes**
- **Osteoclasts**

Osteoprogenitor cells

Bone, like other connective tissue in the embryo, is derived from mesenchyme cells. After birth, flattened, poorly-differentiated, mesenchyme-like cells, are found in the periosteum and endosteum. These cells can divide (mitosis) and differentiate into bone cells (osteogenic potential) and as a result are known as osteoprogenitor cells.

Osteoblasts

The first cells to develop from the osteoprogenitor cells are the osteoblasts. Osteoblasts are involved in the formation of bone and are found on the boundaries of developing and growing bone. The cells are typically oval, with a large eccentric nucleus, and the cytoplasm is fairly basophilic. These cells are very active in synthesizing and secreting the components of the bone matrix and have well-developed rough endoplasmic reticulum (RER), Golgi bodies and granules. Osteoblasts are rich in the enzyme **alkaline phosphatase**, which

plays a major role in the formation of the mineral deposits in the matrix. The collagen fibers are synthesized and secreted by the osteoblasts.

The matrix closest to the osteoblasts is not yet calcified and is known as **osteoid** or **prebone**. This osteoid is rich in collagen fibers. Small membrane-bound **matrix vesicles** (not visible by light microscopy) are budded off processes of the osteoblast cell membrane and secreted to the matrix. These play an important role in the calcification process of the matrix.

Osteocytes

Osteocytes are mature bone cells that develop from osteoblasts and are located in lacunae within the bony matrix. Osteocytes have cytoplasmic processes located in **canaliculi**, which penetrate the bony matrix. Cytoplasmic processes from one osteocyte make contact with the processes from neighboring osteocytes and can communicate via gap junctions. Because the bony matrix is calcified there is no possibility of diffusion except via the network of canaliculi.

Osteoclasts

Osteoclasts are the largest of the bone cells (20-100μm diameter) and are multinuclear (with up to 50 nuclei). Osteoclasts are involvedin **bone resorption** and can be found on the eroding surfaces of bone, often in cavities known as **Howship's lacunae**. The osteocytic cell membrane closest to the bone undergoing resorption has multiple invaginations and is known as the "**ruffled border**". Thcells are metabolically very active, possess large numbers of mitochondria (resulting in the acidophilia of regular staining) and have well-developed Golgi bodies. Osteocytes synand secrete the enzyme **acid phosphatase**, which is involved in the erosion of the bony matrix. (More specifically the enzyme is known as Tartrate-resistant Acid Phosphatase or TRAP and histochemical localization of TRAP enzymatic activity is a useful marker for identifying osteoclasts in sections).

Osteoclasts originate from monocytes and are included in the **mononuclear phagocyte system**.

OSTEOGENESIS

Woven bone (Immature bone, Primary bone)

Osteogenesis is the name given to the development of bone tissue. The first bone to develop is a form of spongy bone known as woven bone (immature bone or primary bone). This is a primitive form of bone tissue that can be identified by the lack of order of the lacunae (of osteocytes) and the thick, irregular "woven" network of collagen fibers in the matrix. Woven bone is found temporarily in the developing embryo, before undergoing rearrangement (remodeling) resulting in the development of lamellar bone.

Woven bone is not usually found in people aged over 14 except for some specific locations including the vicinity of sutures of flat bones of the skull, in tooth sockets, and some tendon insertions. Woven bone also develops temporarily in cases of bone fracture and repair.

Lamellar bone (Mature bone, Secondary bone)

Most bone tissue is lamellar bone in which the tissue is well organized and regular. The lacunae (of osteocytes) are regularly arranged as are the collagen fibers of the matrix. The term **lamella** ("leaf") refers to the layer of matrix between two rows of lacunae. The lamellar arrangements are best illustrated in the cortical (compact) bone of the diaphysis of long bones.

Mature compact bone is composed of three **lamellar arrangements** :

- **Osteons (Haversian Systems)**
- **Circumferential Systems**
- **Interstitial Systems**

Osteons (Haversian Systems)

Osteons (or Haversian Systems) are cylindrical structures of compact bone, which in transverse section are seen to be formed of 4-20 regular concentric lamella surrounding a central vascular channel (**Haversian canal**). The diameter of each osteon typically ranges from 20-110μ m. The collagen fibers in each lamella are regularly arranged and display anisotropy (birefringence) when examined by polarizing microscopy. The direction of the collagen fibers alternates from lamella to lamella, so that at any one time the anisotropy is visible only in every alternate lamella. At the periphery of each osteon, and separating it from adjacent osteons or interstitial systems, is a **cement line**. The cement lines do not calcify, have relatively little collagen, but are rich in glycoproteins and stain differently from the matrix of lamella. The antibiotic, tetracycline, if injected, is incorporated into the matrix of developing lamella and can be seen by fluorescence microscopy. If a second injection of

tetracycline is injected, it is possible to measure the distance between the two fluorescent lines and to determine the rate of osteon development (about 4-5 weeks).

Each osteon comprises a single trophic unit. Each Haversian canal contains a blood vessel involved in the common nutrition of the osteon, consequently osteons represent the main morphofunctional unit of compact bone. The blood vessels of the Haversian canals are supplied with blood from vessels from the periosteum. These blood vessels penetrate the osteons in a transverse direction and are known as **Volkmann's canals**. Volkmann's canals can be identified as they do not have concentric lamella surrounding them.

Circumferential Systems

Immediately below the periosteum, at the periphery of compact bone of the diaphysis, the lamellae surround the bone in a continuous manner. These are known as the **outer circumferential lamellae**. A similar system of continuous lamellae adjacent to the endosteum is also found and is known as the **inner circumferential lamellae**. Bundles of collagen fibers, known as **Sharpey's fibers** or **perforating fibers**, anchor the periosteum to the outer circumferential lamellae, especially in sites of tendon insertions.

Interstitial Systems

Remodeling of bone is a continuous process involving resorption of osteons and the rebuilding of new osteons. Interstitial systems of compact bone represent the remnants of osteons after remodeling. They are present between regular osteons and can be identified as irregular lamellar structures that lack a central Haversian canal.

Remodelling

The resorption of osteons involves osteoclasts from the Haversian canals eroding parts of lamella leading to the formation of **resorption cavities**. These may connect with resorption cavities from adjacent osteons. When sufficient resorption has occurred, osteoblasts appear in the resorption cavity and start building a new generation of osteons. When the new osteon is completed, the remnants of the previous osteon result in an interstitial system. This process of remodeling continues throughout life.

Trabecular bone

The spicules or trabeculae of spongy bone are also formed of lamellae, however, these are not arranged into systems as in compact bone. The trabeculae of spongy bone are not penetrated by blood vessels, but receive their nutrition via diffusion from the endosteum lining the bone marrow spaces.

Osteogenesis

There are two different types of bone formation (osteogenesis):

- **Intramembranous ossification**
- **Endochondral ossification**

In both cases the first bone tissue to be formed is primary (woven or immature) bone, which is temporary only, prior to its replacement by secondary (lamellar or mature) bone.

Intramembranous ossification involves the direct formation of bone within primitive connective tissue, whereas with endochondral ossification there is a cartilage model prior to the development of the bone.

Intramembranous ossification

Intramembranous ossification occurs during the embryonic development of many flat bones of the skull ("membrane bones") and jaw. During the initial stages of the process there is a proliferation and aggregation of **mesenchyme cells**, and simultaneously in the area one finds the development of many small blood vessels. The long processes of the mesenchyme cells are in contact with those of neighboring mesenchyme cells. The mesenchyme cells begin to synthesize and secrete fine collagen fibrils and an amorphous gel-like substance into the intercellular spaces. This is followed by the differentiation of the mesenchyme cells into **osteoblasts** (identified by their basophilia and eccentric nuclei). The osteoblasts synthesize and secrete the components of the **osteoid** (prebone) which, at a later stage, becomes calcified resulting in the development of bone spicules or trabeculae.

The process of intramembranous ossification is well seen in histological preparations of the embryonic calvaria. The newly formed bone matrix of developing trabeculae is stained acidophilic (pink) after regular staining. A layer of osteoblasts is present on the surface of the developing trabeculae, whereas osteocytes occupy lacunae in the bone matrix. Even at this early stage osteoclasts are present on the surface of the trabeculae and are active in bone resorption. Primitive blood vessels are seen in the connective tissue located

between the trabeculae. At a later stage the connective tissue surrounding the developing flat bone forms the **periosteum**.

Endochondral ossification

Endochondral ossification is best illustrated in the developing long bones.

- The first stages involve the development of a **hyaline cartilage model** with surrounding **perichondrium**. A layer of woven bone (the **periosteal collar**) develops around the central shaft of the cartilage as a result of **intramembranous ossification**.

- **Primary (diaphyseal) center of ossification.**

  The chondrocytes in the developing central sha(primary center of ossification) hypertrophy (enlarge with swollen cytoplasm) and their lacunae also become enlarged. The intercellular matrix becomes calcified. As a result, there is no diffusion via tmatrix and the chondrocytes degenerate and die, leaving a network of calcified cartilage. At the same time, blood vessels and mesenchyme-like cells from the periosteum penetrate this region of the diaphysis. Osteoblasts differentiate from the mecells and begin forming primary bone tissue on the calcified cartilage framework.

- A **bone marrow cavity** forms in the developing diaphysis as a result of osteoclastic activity eroding the primary spongy bone trabeculae. The bone cavity enlarges accompanied by further vascularization. The further elongation of long bones occurs in the growth plates of the metaphysis.

- Examination of the **growth plates** reveals an orderly columnar arrangement of chondrocytes involved in the process of endochondral ossification.

  Several zones can be identified according to the arrangement and appearance of the chondrocytes:

- **resting zone** (small flattened lacunae)
- **zone of proliferation** (site of mitoses, and larger elliptical lacunae)
- **zone of hypertrophy** (greatly enlarged and rounded chrondrocytes in enlarged lacunae)
- **zone of calcification** of the matrix and degeneration of the chondrocytes

- **zone of ossification**. Osteoblasts are involved in forming bone trabeculae on the remains of the calcified cartilage.
- **primary spongiosa** (primary spongy bone) where the newly-formed trabeculae are continuously being eroded by osteoclastic activity and remodelled.

During bone elongation there is a continuous addition of new cartilage cells and subsequent endochondral ossification, accompanied by the enlargement of the diaphyseal bone marrow cavity and erosion of the primary spongy bone.

- **Secondary (epiphyseal) center of ossification**

At a later stage of development blood vessels penetrate the epiphyses accompanied by hypertrophy of the more central cartilage cells and calcification of the matrix and degeneration of the chondrocytes. Osteoblasts start building trabecular bone on the skeleton of the calcified cartilage. The trabecula are radially arranged.

- **Closure of the epiphyses.** At ages 14-17, the bone cavities of the diaphysis and epiphyses unite, with the loss of the growth plates. This closure of the epiphyses prevents the further elongation of the long bones.

Long bones have two sources of bone trabeculae: the trabeculae formed by endochondral ossification at the growth plates and the trabeculae of the diaphysis formed by intramembranous ossification. During developmental stages the trabeculae formed by endochondral ossification can be recognized by the more basophilic staining of their calcified cartilage (lacking in trabeculae formed from the diaphyseal collar).

Long bones grow in width by the addition of bone tissue by osteoblastic activity in the region of the periosteum, whereas in parallel there is erosion of bone tissue by osteoclastic activity from the inner regions of the bone. As a consequence the bone marrow cavity is enlarged.

Bone is continuously being remodelled throughout life. The bone mass is constantly changing and with aging there is a net loss of bone and the quality of bone becomes impaired. Osteoporosis is common in the elderly.

Bone fracture and repair

Although bone is hard, it is also brittle and liable to fracture. The fracture is accompanied by hemorrhage. Cells of the periosteum and endosteum respond to the injury. There is a rapid proliferation of fibroblasts, which are involved in the formation of cartilage and fibrocartilage (**fibrocartilaginous callus**) that fills the injured gap. On the basis of the fibrocartilaginous callus, osteoclasts begin forming bone matrix, resulting in a **bony callus** of primary (woven, immature bone). Subsequently the primary bone is remodelled into a secondary (lamellar) bone.

Synovial joints

Bones are connected to each other by joints. The most common joint type is the **diarthrosis** of articulating joints, which has a fibrous connective tissue capsule (**ligament**), continuous with the periosteum of the two bones and which permits a degree of freedom of movement between the two bones. The inner part of the capsule consists of the **synovial membrane**, which may extend as a fold (**synovial fold**). The synovial membrane is well vascularised with both blood and lymph vessels. The main cell type present in the synovial membrane, are fibroblast-like cells, and involved in the formation of the **synovial fluid**. This fluid is rich in **hyaluronic acid** and fills the joint cavity. Macrophage-like cells are also found in the synovial membrane and are responsible for keeping the synovial fluid clean and free of cell fragments. The synovial fluid plays an important role in the lubrication of the joint and in providing nutrition for the articular cartilage of the epiphyses. **Fat deposits** or pads, found between the synovial membrane and the ligament, function as mechanical shock absorbers.

Aging changes to joints, in particular pathological changes of the articular cartilage (**osteoarthritis**), are very common in the elderly.

Physiology of bone

Most of the calcium stored in the body is in bone tissue and can be released to the blood according to physiological demands or alternatively can be used to produce new bone. Calcium levels in the extracellular fluid of the body are very closely regulated. Three hormones, in particular, are involved in **calcium homeostasis**:

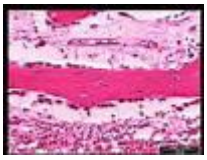
- **Parathyroid hormone**
- **Calcitonin**
- **Vitamin D3**

The main organ systems involved in calcium homeostasis are the bony skeleton, the kidney and the intestine.

Parathyroid hormone is involved in increasing blood calcium levels by stimulating osteoclastic activity and bone resorption. **Calcitonin** has an opposite effect and is involved in reducing blood calcium levels. Calcitonin encourages bone tissue formation and can be used in clinical treatment of osteoporosis.
The active metabolites of **Vitamin D3** are involved in particular in stimulating dietary calcium absorption through the small intestine. Lack of vitamin D_3 can result in improper calcification of bone tissue and the development of **rickets**.



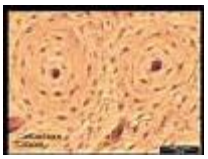

Hyaline Cartilage - Trachea Elastic Cartilage - External ear Elastic Cartilage Intramembranous Ossification




Intramembranous Ossification Osteoclasts Endochondral Ossification Endochondral Ossification

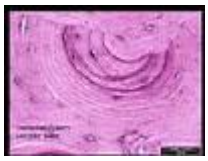



Endochondral Ossification Endochondral Ossification Growth Plate Synovial Joint

Compact bone Compact bone Compact bone Lamellar bone



Lamellar bone Lamellar bone Interstitial System Interstitial System




Flat bone Intervertebral disk Intervertebral disk Compact Bone

Trabecular bone lamellae

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