Histology

Cartilage and bone

Bone

Bone is one of the three *Specialised Connective Tissues*. Rigid for weight-bearing. The matrix is largely composed of calcium salts (60% by weight). Collagen fibres are present. The cells are called *osteocytes*, and lie in lacunae which interconnect via narrow tunnels (*canaliculi*). Processes of the osteocytes extend throughout the canaliculi, which are continuous with the longitudinal, central [Haversian] canals and the radial, perforating [Volkmann's] canals, which carry blood vessels and nerves. The concentric layers or *lamellae* of matrix around a central canal form *osteons* [Haversian systems].

The inside and outside free surfaces of bone are covered by a layer of dense connective tissue, (*endosteum* and *periosteum*, respectively), which contains undifferentiated, pluripotent mesenchymal cells and [Sharpey's] collagen fibres.

Bone is not fixed, but is continuously being *remodelled* by organised processes of breakdown and formation of new osteons. The cells responsible for the resorption of bone are *osteoclasts*, which are giant cells with 200-300 nuclei, 15-20 of which may be visible in a single section. Osteoclasts are invariably found lying on the surface of the bone. Their cytoplasm is acidophilic (red with H&E) and highly vacuolated ('foamy') and they contain hydrolytic enzymes.

Bone may be classified as *spongy* or *dense*. Spongy (also known as ‘*cancellous’*) bone consists of a trabecular (lattice-like) structure, with many spaces between the strands. The strands of bone are covered by a thin layer of CT — *endosteum*. Dense (or ‘compact’) bone has no spaces within it. Compact bone is almost entirely composed of osteons; spongy bone may contain osteons; if it does not, it is known as ‘woven bone’.
Two types of bone can be distinguished macroscopically:

- Trabecular bone (also called cancellous or spongy bone) consists of delicate bars and sheets of bone, trabeculae, which branch and intersect to form a sponge-like network. The ends of long bones (or epiphyses) consist mainly of trabecular bone.

- Compact bone does not have any spaces or hollows in the bone matrix that are visible to the eye. Compact bone forms the thick-walled tube of the shaft (or diaphysis) of long bones, which surrounds the marrow cavity (or medullary cavity). A thin layer of compact bone also covers the epiphyses of long bones.

Bone is, again like cartilage, surrounded by a layer of dense connective tissue, the periosteum. A thin layer of cell-rich connective tissue, the endosteum, lines the surface of the bone facing the marrow cavity. Both the periosteum and the endosteum possess osteogenic potency. Following injury, cells in these layers may differentiate into osteoblasts (bone forming cells) which become involved in the repair of damage to the bone.

**Compact Bone**

- **Osteocytes - mature bone cells**
- **Lacunae - small cavities in bone that contain osteocytes**
- **Canaliculi - hairlike canals that connect lacunae to each other and the central canal**
- **Haversian system, or osteon - the structural unit of compact bone**
  - **Lamella - weight-bearing, column-like matrix tubes composed mainly of collagen**
  - **Haversian, or central canal - central channel containing blood vessels and nerves**
  - **Volkmann's canals - channels lying at right angles to the central canal, connecting blood and nerve supply of the periosteum to that of the Haversian canal**
PROPERTIES OF BONE

Bone Cells: Osteocytes, osteoblasts, osteoclasts, osteoprogenitor cells

Organic Matrix:

Ground Substance: complex polysaccharides and glycoproteins

Collagen: Type I

Mineral: calcium phosphate hydroxyapatite

Physical Properties:

Strength- resistance to compression, shear and tensile strength
(protects vital organs and provides for motion due to muscle contraction)

- Mobilizable reservoir of calcium
- Adapts to growth and weight changes by remodeling
- Self repair
- Site of hematopoiesis

Composition and Structure of Bone

- Stiffness - ratio of stress to strain in a loaded material (stress divided by the relative amount of change in structure's shape)

- Compressive strength - ability to resist pressing or squeezing force

- Building Blocks of Bone
  - Minerals (calcium carbonate and calcium phosphate ~ 60-70% of bone weight)
    - source of stiffness and compressive strength
  - Collagen (protein) ~ 10%
    - source of flexibility and tensile strength
- Aging causes decrease in collagen and, as a result, increase in fragility
  - Water ~ 25-30%
- Important contributor to bone strength
- **Cortical Bone** (compact mineralized tissue with low porosity) vs. **Trabecular Bone** (less compact with high porosity)

### Histological Organisation of Bone

![Histological Organisation of Bone](image)

**Compact Bone**
- Compact bone consists almost entirely of extracellular substance, the matrix.
- Osteoblasts deposit the matrix in the form of thin sheets which are called lamellae.
- Lamellae are microscopical structures. Collagen fibers within each lamella run parallel to each other.
- Collagen fibers which belong to adjacent lamellae run at oblique angles to each other.
- Fiber density seems lower at the border between adjacent lamellae, which gives rise to the lamellar appearance of the tissue.
• Bone which is composed by lamellae when viewed under the microscope is also called lamellar bone.

• In the process of the deposition of the matrix, osteoblasts become encased in small hollows within the matrix, the lacunae.

• Unlike chondrocytes, osteocytes have several thin processes, which extend from the lacunae into small channels within the bone matrix, the canaliculi. Canaliculi arising from one lacuna may anastomose with those of other lacunae and, eventually, with larger, vessel-containing canals within the bone.

• Canaliculi provide the means for the osteocytes to communicate with each other and to exchange substances by diffusion.

• In mature compact bone most of the individual lamellae form concentric rings around larger longitudinal canals (approx. 50 urn in diameter) within the bone tissue. These canals are called Haversian canals.

• Haversian canals typically run parallel to the surface and along the long axis of the bone.

• The canals and the surrounding lamellae (8-15) are called a Haversian system or an osteon. A Haversian canal generally contains one or two capillaries and nerve fibres.

• Irregular areas of interstitial lamellae, which apparently do not belong to any Haversian system, are found in between the Haversian systems.

• Immediately beneath the periosteum and endosteum a few lamella are found which run parallel to the inner and outer surfaces of the bone. They are the circumferential lamellae and endosteal lamellae.

• A second system of canals, called Volkmann's canals, penetrates the bone more or less perpendicular to its surface.

• These canals establish connections of the Haversian canals with the inner and outer surfaces of the bone.

• Vessels in Volkmann's canals communicate with vessels in the Haversian canals on the one hand and vessels in the endosteum on the other.
A few communications also exist with vessels in the periosteum.

Trabecular Bone

- The matrix of trabecular bone is also deposited in the form of lamellae. In mature bones, trabecular bone will also be lamellar bone.
- However, lamellae in trabecular bone do not form Haversian systems.
- Lamellae of trabecular bone are deposited on preexisting trabeculae depending on the local demands on bone rigidity.
- Osteocytes, lacunae and canaliculi in trabecular bone resemble those in compact bone.

Bone Matrix and Bone Cells

Bone matrix consists of:

- **collagen fibres** (about 90% of the organic substance) and ground substance.
  - Collagen type I is the dominant collagen form in bone.
- The hardness of the matrix is due to its content of inorganic salts (**hydroxyapatite; about 75% of the dry weight of bone**), which become deposited between collagen fibres. Calcification begins a few days after the deposition of organic bone substance (or osteoid) by the osteoblasts. Osteoblasts are capable of producing high local concentration of calcium phosphate in the extracellular space, which precipitates on the collagen molecules. About 75% of the hydroxyapatite is deposited in the first few days of the process, but complete calcification may take several months.

Bone Cells

*Osteoblasts* - bone-forming cells, Derived from *Mesenchymal precursor cells / stem cells in bone marrow / osteoprogenitor cells of periosteum*

Functions:
1. Make and mineralize bone extracellular matrix.
2. Produce matrix proteins:
Type 1 collagen (90% of the protein in bone)
osteocalcin
osteopontin
osteonectin
proteoglycans
alkaline phosphatase

3. Deposit osteoid on pre-existing mineralized or calcified surfaces only (= the mineralization front) Requires vitamin C.

4. Become trapped in lacunae within the Matrix of bone as osteocytes (appositional growth).

5. Produce factors that stimulate osteoclasts.

Osteocytes - mature bone cells Derived from: osteoblasts, mesenchymal origin
Characteristics:
- represent inactive osteoblasts trapped in lacunae
- cytoplasmic processes in canaliculi
- communicate via gap junctions
- surrounded by extracellular fluid in lacunae and canaliculi (periosteocytic space, site of calcium resorption)
- small golgi and RER
- nondividing (NO interstitial bone growth)
Functions:
- osteocytic osteolysis role in calcium regulation via plasma [Ca++]
- assist in nutrition of bone
- mechanotransduction (regulate bone response to the mechanical environment via release of factors that recruit preosteoblasts)

Osteoclasts - large cells that resorb or break down bone matrix
Derived from: Hematopoetic stem cells in bone marrow
Characteristics:
- large, motile, multinucleated, acidophilic cells
- some features of macrophages
- located on bone surfaces in Howship's lacuna
- ruffled border of the cell membrane
- cytosolic organelle-free region, or 'clear zone' (actin ring), seal
- adhere to the bone surface via integrins (specialized cell surface low-affinity receptors)
- many mitochondria, golgi, vesicles (lysosomes), RER
- nondividing

Function:
- Resorption of bone matrix: initially involves mineral dissolution, followed by degradation of the organic components (collagen)

*Osteoid* - unmineralized bone matrix composed of proteoglycans, glycoproteins, and collagen

Formation of Bone
Bones are formed by two mechanisms:

**Intramembranous Ossification**
(bones of the skull, part of the mandible and clavicle) or endochondral ossification.

- Intramembranous ossification occurs within a membranous, condensed plate of mesenchymal cells.
- At the initial site of ossification (ossification centre) mesenchymal cells (osteoprogenitor cells) differentiate into osteoblasts.
- The osteoblasts begin to deposit the organic bone matrix, the osteoid.
- The matrix separates osteoblasts, which, from now on, are located in lacunae within the matrix.
The collagen fibres of the osteoid form a woven network without a preferred orientation, and lamellae are not present at this stage.

Because of the lack of a preferred orientation of the collagen fibres in the matrix, this type of bone is also called woven bone.

**Endochondral Ossification**

- Begins in the second month of development
- Uses hyaline cartilage "bones" as models for bone construction
- Requires breakdown of hyaline cartilage prior to ossification

**Stages of Endochondral Ossification**

- Formation of bone collar
- Cavitation of the hyaline cartilage
- Invasion of internal cavities by the periosteal bud, and spongy bone formation
- Formation of the medullary cavity; appearance of secondary ossification centers in the epiphyses
- Ossification of the epiphyses, with hyaline cartilage remaining only in the epiphyseal plates

**Reorganisation and Restoration of Bone**

Changes in the size and shape of bones during the period of growth imply some bone reorganisation.

- Osteoblast and osteoclast constantly deposit and remove bone to adjust its properties to growth-related demands on size and/or changes of tensile and compressive forces.
- Although the reorganisation of bone may not result in macroscopically visible changes of bone structure, it continues throughout life to mend damage to bone (e.g. microfractures) and to counteract the wear and tear occurring in bone.
- Osteoclasts and osteoblasts remain the key players in this process. Osteoclasts "drill" more or less circular tunnels within existing bone matrix.
• Osteoblasts deposit new lamellae of bone matrix on the walls of these tunnels resulting in the formation of a new Haversian system within the matrix of compact bone.
• Parts of older Haversian systems, which may remain between the new ones, represent the interstitial lamellae in mature bone. Capillaries and nerves sprout into new Haversian canals.
• Restorative activity continues in aged humans (about 2% of the Haversian systems seen in an 84 year old individual contained lamellae that had been formed within 2 weeks prior to death!).
• However, the Haversian systems tend to be smaller in older individuals and the canals are larger because of slower bone deposition. If these age-related changes in the appearance of the Haversian systems are pronounced they are termed osteopenia or senile osteoporosis.
• The reduced strength of bone affected by osteoporosis will increase the likelihood of fractures

Summary of Bone Formation
a) Growth in length — occurs by the addition of new cartilage at the neck of the bone (epiphysis). At the extremity of the epiphysis the chondrocytes are small (Quiescent Zone), but towards the shaft (diaphysis) they are mitotic, forming columns of small cells (Proliferative Zone), which then increase in size (Maturation Zone).
b) Ossification . Cells in the connective tissue sheath around the cartilage (perichondrium) differentiate into osteoblasts and form bone on the surface of the cartilage . This process continues, adding to the growth of the bone.

At the same time, the chondrocytes in the middle of the shaft hypertrophy, the lacunae enlarge and the amount of matrix is correspondingly reduced and calcified (becoming basophilic) . (In favourable circumstances it may be possible to see a ‘tide-mark’ of
calcification where the matrix becomes basophilic — blue with H&E). The chondrocytes then die and the matrix is dissolved leaving only the thicker plates (trabeculae) like stalactites hanging down into the marrow cavity of the bone.

Blood vessels and cells grow into the spaces which are enlarged to form the primary marrow cavity. Some cells become osteoblasts and form bone on the remaining matrix — the whole constituting the primary ossification centre. This process extends outwards from the centre. The periosteal collar thickens to support the eroded cartilage.

Finally, bone is resorbed in the centre so that the thickness of the wall remains approximately constant while the overall diameter increases. The primary marrow cavity becomes filled with small precursor cells of blood and is therefore densely basophilic because their nuclei are so close together.

Thus, ossification forms four more zones (from end to middle): Calcification, Retrogression (death of chondroblasts and dissolution of matrix), Ossification, Resorption.

A secondary ossification centre develops at the epiphysis and expands, leaving cartilage only on the articular surface and as a thin epiphyseal plate or disc. It is from the diaphyseal edge of this plate that further cartilage formation and growth occurs in childhood.

Growth in length of the bone ceases when the proliferation of the chondrocytes is not sufficient to keep pace with the rate of ossification and the epiphyseal disc becomes completely ossified.
Cancellous Bone

Trabeculae

Spaces containing bone marrow and blood vessels

Lamellae

Canaliculus

Osteocytes

Osteoclasts

Osteoblasts