# BONE

Bone is the main component of the skeleton in the adult human. Like cartilage, bone is a specialized form of dense connective tissue. Bone gives the skeleton the necessary rigidity to function as attachment and lever for muscles and supports the body against gravity. It is hard and rigid because of mineralization of the extracellular matrix. Bone also serves as a reservoir for calcium.

Bone has a rich vascular supply (unlike cartilage).

Bone tissue is classified morphologically into two types:

• **Spongy bone** (also known as cancellous or trabecular bone)

# • Compact bone

Most bones are composed of both compact and spongy 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 spongy 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 the damage to the bone.



#### Tibia

Bone is a specialized connective tissue that provides support. Most bones contain both compact (or cortical) and spongy (or cancellous).

The distal end of the tibia as seen by scanning electron microscopy (SEM). Spongy bone is contained by a thin outer shell of compact bone.

- Compact Bone (blue) composed of osteons.
- Spongy Bone (yellow) composed of a spongelike network of trabeculae.



## **Compact and Spongy Bone**

Bone must be decalcified (by exposure to strong acids) so it can be cut into thin sections. The remaining material is mostly collagen.

This slide contains two samples:

- Long Bone partial cross-section from the end of a long bone found in the extremities.
- Flat Bone cross-section of a bone from the skull cap (calvarium).

Most bones contain both compact and spongy bone.

Two structural arrangements of bone tissue are seen:

- Compact Bone a dense layer that forms the outer shell or cortex of bones.
- Long Bone forms the outer circular surface.
- Flat Bone forms the outer and inner flat surfaces.
- Spongy (Cancellous or Trabecular) bone anastomosing network of spicules found in the interior of bones.
- Long Bone often found near the ends of long bones.
- Flat Bone fills the narrow interior of flat bones.

A three-dimensional view of compact and spongy bone is seen in <u>EM 215 Tibia</u> by scanning electron microscopy.



• Long Bone - partial cross-section from the end of a long bone found in the extremities.



Spongy (Cancellous or Trabecular) bone - anastomosing network of spicules found in the interior of bones. Long Bone - often found near the ends of long bones.



• Flat Bone - cross-section of a bone from the skull cap (calvarium).





• Flat Bone - forms the outer and inner flat surfaces.



Spongy (Cancellous or Trabecular) bone - anastomosing network of spicules found in the interior of bones.
Flat Bone - fills the narrow interior of flat bones.





## **Spongy Bone**

The spine is made up of 24 bones, called vertebrae, that surround the spinal cord. These irregularly shaped bones have a thin cortical shell which surrounds spongy (cancellous or trabecular) bone (#1 and #2).

Spongy bone is formed by an anastomosing network of trabeculae that form interconnecting spaces containing bone marrow.

Articular hyaline cartilage (#1 and #2) can also be seen.



Vertebrae

and

spinal



Articular hyaline cartilage (#1 and #2) can also be seen.



Osteon (or Haversian System)

The large amount of calcium in bones prevents the cutting of tissue sections. The bone must be demineralized (with acids or chelating agents) leaving behind only the organic components.

Osteons (or Haversian system) are the structural unit of compact bone. They are cylindrical structures of concentric layers of bone (lamellae) aligned with the long axis of a bone.

Examine osteons in the cross-section and longitudinal sections of a decalcified long bone.





Haversian Canals - circular openings at the center of osteons.



Haversian Vessels - remnants of blood vessels and nerve fibers are seen in some canals.



Osteoblasts - found on the surface of some canals.



• Haversian Lamellae - concentric layers of mineralized matrix that surround a Haversian canal.



• Cement Line - thin, basophilic boundary of an osteon.



• Osteocytes - remnants of these cells are found inside lacunae located in the matrix of the bone.



Longitudinal Section



• Haversian Canals - longitudinal sections of the channel at the center of osteons.

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Haversian Vessels - remnants of blood vessels and nerve fibers are seen in some canals.



Osteoblasts - line the inside of some canals.





#### **Compact Bone**

An alternative technique for examining bone is to saw bone into thin wafers and use abrasive surfaces to produce thin "ground" sections. Organic material is lost leaving only the mineral matrix of bone. The open spaces filled with air appear darker than the mineralized matrix.



• Circumferential Lamellae - parallel layers of mineralized matrix on the surface of bone.

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• Outer Circumferential Lamellae - form the outer surface and receive nutrients from blood vessels in the periosteum.





• Osteons (#1, #2 and #3) - composed of concentric layers of mineralized matrix that surround a central canal.



- (Also see <u>EM 217 Osteon</u>.)
- Haversian Canals circular opening at their center.
- Lacunae small, lenticular spaces in the surrounding matrix.
- Volkmann's Canals openings that run perpendicular to Haversian canals connecting them with the surface (#1 and #2) and each other (#3 and #4). They carry blood vessels and nerve fibers into the Haversian system.

Many structures are easier to identify in sections of ground bone stained with dark dyes to reveal small, open spaces. See <u>MH 202 Ground Bone</u> (Schmorl's stain) and <u>MH 233 Ground Bone</u> (India ink).



## Osteon

Bone is a connective tissue with a mineralized extracellular matrix. The osteon (or Haversian system) is the basic structural unit of bone.

An osteon in cross-section as seen by scanning electron microscopy (SEM).



- Osteon (purple) round, concentric layers of lamellae that surround a central canal.
- Haversian Canal (dark purple) central canal at the center of the osteon.
- Haversian Lamellae (purple) concentric layers of calcified matrix.
- Lacunae (black) oblong spaces found between lamellae that contain a single osteocyte.
- Cementing Line (yellow) the boundary that surrounds an osteon.
- Interstitial Lamellae (blue) remnants of osteons that remain from bone remodeling.



### **Compact Bone (Schmorl's Stain)**

An alternative technique for examining bone is to saw bone into thin wafers and use abrasive surfaces to produce thin "ground" sections. Many structures are easier to identify after staining with a dark dye (Schmorl's stain for this specimen). The higher contrast allows open spaces to be seen in greater detail.



• Osteons (#1, #2 and #3) - cylindrical structures that are the structural unit of compact bone. They are arranged parallel to the lines of stress in a bone.



- Haversian Canals circular opening at their center.
- Haversian Lamellae concentric layers of mineralized matrix that forms most of the osteon.
- Lacunae darkly stained, open spaces in the mineralized matrix that contain an osteocyte.
- Canaliculi (#1 and #2) darkly stained, small channels that connect the lacunae with each other. Osteocytes share nutrients by extending cellular processes through canaliculi.



- Interstitial Lamellae (#1, #2 and #3) found between osteons and are remnants left from remodeling of bone. The darker appearance of their matrix reflects higher mineralization than in osteons.
- Canaliculi lacunae in interstitial lamellae connect to those in osteons.

The structure of an osteon can also be seen in <u>EM 217 Osteon</u> by scanning electron microscopy. An osteocyte inside a lacunae is shown in <u>EM 218 Osteocyte</u>.



#### Osteocyte

Osteocytes are trapped in lacunae of osteons.

An osteocyte as seen by scanning electron microscopy (SEM).

- Lacunae oblong spaces found between lamellae that contain a single osteocyte.
- Osteocyte (purple) reside in a lacunae and extend long cytoplasmic processes to other osteocytes.
- Canaliculi small canals between lacunae that contain cytoplasmic processes between adjacent osteocytes.
- Osteoid (tan) unmineralized collagen surrounding an osteocyte.

• Bone (blue) - calcified bone matrix. (A bone fragment has dropped into the lacunae to the right of the osteocyte.)



## **Compact Bone(India Ink)**

An alternative technique for examining bone is to saw bone into thin wafers and use abrasive surfaces to produce thin "ground" sections. Many structures are easier to identify after staining with a dark dye (India ink for this specimen).

The higher contrast allows open spaces to be seen in greater detail. The more intense staining of osteons compared to interstitial lamellae reflects their lower mineralization (*i.e.*, higher permeability to the dye).



• Osteons (#1, #2 and #3) - cylindrical structures that are the structural unit of compact bone. They are arranged parallel to the lines of stress in a bone.

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- Haversian Canals circular opening at their center.
- Haversian Lamellae concentric layers of mineralized matrix that forms most of the osteon.
- Lacunae darkly stained, lenticular spaces in the mineralized matrix that contain an osteocyte.
- Canaliculi darkly stained, small channels that connect lacunae with each other. Osteocytes share nutrients by extending cellular processes through canaliculi..



• Interstitial Lamellae (#1, #2 and #3) - lightly stained remnants from remodeling of bone.



Remnants of Osteons - parts of previous osteons, such as Haversian canals



- or sections of the Haversian lamellae, are often seen within these areas.
  - Canaliculi relatively few are stained compared to those in osteons

## Histological Organisation of Bone

### **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 fibres within each lamella run parallel to each other. Collagen fibres which belong to adjacent lamellae run at oblique angles to each other. Fibre 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, lacunae. Unlike chondrocytes, osteocytes have several thin processes, which extend from the lacunae into small channels within the bone, the canaliculi. Canaliculi arising from one lacuna may anastomose with those of other lacunae and, eventually, with 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  $\mu$ m in diameter) within the bone tissue. These canal 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 some 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 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.

? Note the distinction between macroscopic (visible to the eye) and microscopic (only visible under the microscope) appearance when the bone is named. Lamellar bone forms both trabecular bone and compact bone, which are the two macroscopically recognizable bone forms.

Suitable Slides

sections of compact bone (usually part of the diaphysis of a long bone) - ground (unstained), Schmorl stained or H&E  $\,$ 

Compact Bone, human - Schmorl stain Lamellae which run parallel to the surface of the bone are visible both on the outer, convex surface of the bone (circumferential lamellae) and on the inner, concave surface of the bone facing the marrow cavity (endosteal lamellae). The surface formed by the endosteal lamellae is often more irregular than the surface formed by the circumferential lamellae. The space between these two sets of lamellae is filled by Haversian systems and interstitial lamellae. Only few of the Haversian systems are "textbook" circular. Osteocyte lacunae are visible between the lamellae. Canaliculi become visible at high magnification (not illustrated). You should be able to see, draw and identify Haversian systems, interstitial and circumferential lamellae and/or endosteal lamellae.



Compact Bone. human ground (unstained) The osteocyte lacunae are the main feature of the ground section. They are visible as elongated black spots in the bone matrix. Canaliculi, radiate from the lacunae into the surrounding bone matrix. Some lamellae are visible in the ground section. There is actually no distinct border between most lamellae, but our brain can use the elongated osteocyte their orientation to "reconstruct" the lamellae. lacunae and This is one of the cases where it may pay off to close down the iris diaphragm. The canaliculi should stand out more clearly if you do so. Remember to open the diaphragm afterwards!

Draw a small section of the a Haversian system at high magnification and identify in your drawing lacunae, canaliculli and (if visible) lamellae.



Suitable Slides sections of part of a vertebra or an epiphysis of a long bone - H&E, van Gieson Sections prepared to show articular cartilage will often also contain trabecular bone in the epiphysis below the articular cartilage.

Articular Cartilage, bovine - H&E Thin sheets and bars of bone, trabeculae, are visible at low magnification. Although they may appear as individual pieces in sections, they form an interconnected meshwork in the living bone. The spaces between the trabeculae, the marrow cavity of the diaphysis, is filled by either red bone marrow or yellow bone marrow. At high magnification, elongated osteocyte lacunae, which in well preserved tissue still contain osteocytes, are visible in the matrix. If the H&E stain also turned out well, it should be visible that the matrix of the trabecular bone is formed by lamellae. Canaliculi are present but hard to identify in most H&E stained sections. Haversian systems are not present in the trabeculae. In trabecular bone obtained from young individuals, in which the bone is still growing, small areas of calcified cartilage are occasionally seen in the bone trabeculae. They are remnants of the cartilage scaffold on which bone matrix was deposited during the formation of the trabeculae. With the reorganisation of bone such areas will eventually be lost. Draw trabecular bone at high and low magnification. Make sure that it is visible that the trabecular bone is also lamellar bone. Include enough detail of the marrow as you think you will need to identify this type of preparation.



# **Bone Matrix and Bone Cells**

## **Bone Matrix**

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**

Osteoprogenitor cells (or stem cells of bone)

are located in the periosteum and endosteum. They are very difficult to distinguish from the surrounding connective tissue cells. They differentiate into

Osteoblasts (or bone forming cells).

Osteoblasts may form a low columnar "epitheloid layer" at sites of bone deposition. They contain plenty of rough endoplasmatic reticulum (collagen synthesis) and a large Golgi apparatus. As they become trapped in the forming bone they differentiate into

Osteocytes.

Osteocytes contain less endoplasmatic reticulum and are somewhat smaller than osteoblasts.



## Osteoclasts

are very large (up to 100  $\mu$ m), multi-nucleated (about 5-10 visible in a histological section, but up to 50 in the actual cell) bone-resorbing cells. They arise by the fusion of monocytes (macrophage precursors in the blood) or macrophages. Osteoclasts attach themselves to the bone matrix and form a tight seal at the rim of the attachment site. The cell membrane opposite the matrix has deep invaginations forming a ruffled border. Osteoclasts empty the contents of lysosomes into the extracellular space between the ruffled border and the bone matrix. The released enzymes break down the collagen fibres of the matrix. Osteoclasts are

stimulated by parathyroid hormone (produced by the parathyroid gland) and inhibited by calcitonin (produced by specialised cells of the thyroid gland). Osteoclasts are often seen within the indentations of the bone matrix that are formed by their activity (resorption bays or Howship's lacunae).

## **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

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. The osteoid calcifies leading to the formation of primitive trabecular bone.

Further deposition and calcification of osteoid at sites where compact bone is needed leads to the formation of primitive compact bone.

? Note the distinction between macroscopic and microscopic apperance when the bone is named. We again have the two macroscopically different forms of bone - trabecular bone and compact bone - but their early developmental ("primitive") forms consist of woven bone.

Through subsequent reorganisation the primitive compact and trabecular bone is converted into mature compact and trabecular bone. During reorganisation and growth, woven bone will, in time, be replaced by lamellar bone.

Intramembranous ossification does not require the existence of a cartilage bone model.

## Suitable Slides

sections of the developing mandible (or some other bones of the skull) or clavicle -H&E, van Gieson Sections prepared to show endochondral ossification (see below) may be an alternative if no specifically prepared slides of intramembranous ossification are available. The periosteal collar, i.e. the manchette of bone forming around the diaphysis of the cartilage model of the bone, is formed by a mechanism similar to intramembranous ossification and results in the deposition of woven bone.

Mandible, intramembranous ossification. H&E The developing bone will in sections usually be associated with a number of other tissues which develop in close association with it. In case of the mandible, there can be developing glands. teeth. the tongue, skin and salivary The first job - best done at low magnification - is to find the developing bone. It should look like a coarse meshwork (trabecular bone) of pink tissue surrounding patches of much lighter or unstained tissue. Lamellae are not visible (woven bone) and the lacunae are larger than lacunae in mature bone. Ossification centres appear as areas of a gradual transition from connective tissue to bone. Light, pinkish bone matrix is found between the osteoblasts.

Depending on the state of development of the bone, it is occasionally possible to find bone trabeculae which are lined by a layer of osteoblasts. These osteblasts are depositing the first lamellae on the already existing trabeculae. The trabeculae will therefore have a core of woven bone, which is surrounded by lamellar bone. Compare the shapes, sizes and frequencies of lacunae in lammellar and woven bone if both types of bone are present. Draw a part of trabecular bone consisting of woven bone and an ossification centre. Label the features in your drawings.



**Endochondral Ossification** 

Most bones are formed by the transformation of cartilage "bone models", a process called endochondral ossification. A thin sheet of bone, the periosteal collar, is deposited around the shaft of the cartilage model. The periosteal collar consists of woven bone. Concurrently a periosteal bud invades the cartilage model and allows osteoprogenitor cells to enter the cartilage. At these sites, the cartilage is in a state of hypertrophy (very large lacunae and chondrocytes) and partial calcification, which eventually leads to the death of the chondrocytes.

Invading osteoprogenitor cells mature into osteoblasts, which use the framework of calcified cartilage to deposit new bone. The bone deposited onto the cartilage scaffold is lamellar bone. The initial site of bone deposition is called a primary ossification centre. Secondary ossification centres occur in the future epiphyses of the bone.

Close to the zone of ossification, the cartilage can usually be divided into a number of distinct zones :

- 1. Reserve cartilage, furthest away from the zone of ossification, looks like immature hyaline cartilage.
- 2. A zone of chondrocyte proliferation contains longitudinal columns of mitotically active chondrocytes, which grow in size in
- 3. the zone of cartilage maturation and hypertrophy.
- 4. A zone of cartilage calcification forms the border between cartilage and the zone of bone deposition.

Primary and secondary ossification centres do not merge before adulthood. Between the diaphysis and the epiphyses a thin sheet of cartilage, the epiphyseal plate, is maintained

until adulthood. By continuing cartilage production, the epiphyseal plate provides the basis for growth in the length of the bone. Cartilage production gradually ceases in the epiphyseal plate as maturity is approached. The epiphyseal plate is finally removed by the continued production of bone from the diaphyseal side.

Bone formation and bone resorption go hand in hand during the growth of bone. This first deposited trabecular bone is removed (<u>By which cells?</u>) as the zone of ossification moves in the direction of the future epiphyses. This process creates the marrow cavity of the bones. Simultaneously, bone is removed from the endosteal surface of the compact bone forming the diaphysis and deposited on its periosteal surface which allows the bone to grow in diameter.

Suitable Slides

sections of bones during the early stages of their development - H&E, van Gieson

Foetal Vertebral Column, human, H&E Hold the section against a light background. The cartilage models will stain very light, and the outlines of the skeletal structures they will form can often be identified. If an ossification centre is present, it will appear as a darker area within the cartilage model. The zonation of the cartilage should be visible in all ossification centres. How much bone is present depends on how far ossification has proceeded. The newly formed bone trabeculae will often consist of lamellar bone with a more or less extensive core of darkly staining calcified cartilage. The lamellar organisation of the bone may not be visible. How the ossification centre excactly will look also depends on the plane of the section in relation to the ossification centre. **Draw the ossification centre/zone, include the adjacent cartilage and identify the zones within it in your drawing.** 



# Suitable Slides sections of the epiphyseal disc of growing bones - H&E



Growing Bone H&E The cartilage model has almost entirely been transformed into bone. The only remaining cartilage is found in the epiphyseal disk. Zones of cartilage proliferation, hypertrophy and calcification are visible at high magnification, but only on one side of the epiphyseal disk - towards the diaphysis, which increases in length as the cartilage generated by the epiphyseal disc is transformed into bone.

Osteoclasts may be found on the newly formed trabeculae or associated with parts of the cartilage scaffold. Draw the region of the epiphyseal disks. Identify in your drawing the epiphyseal disk and the bone of the epiphysis and diaphysis. Indicate the direction of cartilage proliferation in the epiphyseal disk and the direction of bone growth.

## **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



compact bone remodelling

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.