Bone tissue

- Special connective tissue.
- Cartilages + Bones = Skeleton.
  - ✓ Osseous = refers to bone.

- **Functions of the bone:**

1. Support: It forms the framework of our body; most of the skeleton in our body is formed by bones.

2. Protection:
   -The cranial bones provide protection for the brain.
   -Thoracic cage provides protection for the lungs and the heart.
   -Vertebral canal provides protection for the spinal cord.
   -The pelvic cavity provides protection for the urinary bladder and the reproductive organs.

3. Movement: bones don't move, but muscles which are inserted into the bones transform their action into bodily movement.

4. Mineral homeostasis: bones act as reservoir for minerals that are important to our body such as calcium and phosphate. For example, a certain levels of calcium inside the blood should be maintained, so when the body needs calcium, calcium will be mobilized from the bone to increase calcium blood level.
   - On the other hand, if you increase your intake of calcium, more deposition of calcium inside the bone occurs.
   - 99% of the calcium in our body is reserved in the bone. It is just like a storage site for calcium.
   - ✓ If we **need** calcium, **mobilization** of calcium from bones will take place.
   - ✓ If we **have extra** calcium, **deposition** of calcium inside bones will take place.

-Bone is a special type of connective tissue, it is composed of:

A. Cells	**B. ECM**
Osteoblasts/ osteocytes	**- Fibers**: Mainly collagen type 1. This is why the bone is strong. - **Ground substance**: little amount of proteoglycans and glycoproteins. - Two thirds of the ECM of bone is **inorganic material (minerals)**. This is why the bone is hard, because the ECM is highly mineralized.

- ✓ These minerals are deposited inside the ECM of the bone and form crystals called **Hydroxyapatite Crystals**. Main minerals are calcium and phosphate, other minor minerals are magnesium and potassium.

It is hard to break the bone, compare it with steel, it is hard to push or pull on bone and make it break

5. Hematopoiesis: the process of production of the formed elements of the blood (formation of red, white blood cells and platelets).
   -Occurs inside the red bone marrow.
   - At birth, our bones contain in their cavities red bone marrow. With aging this red bone marrow is gradually replaced by the yellow bone marrow. The red bone marrow remains in flat bones for example such as the sternum for the production of the blood cells.
6. Storage of adipose tissue: yellow bone marrow.
   -Yellow bone marrow inside the cavities is adipose tissue.
   -Storage of lipids inside the bone as a yellow bone marrow.

- **Types of bones**:
  - Anatomically: according to their shape:
  1. Long bone: composed of shaft or body (Diaphysis), proximal and distal ends (Epiphysis). For example phalanges are long bones because they have proximal end, distal end and a shaft.
  2. Short bones: carpal and tarsal bones
  3. Flat bones: bones of the skull
  4. Irregular: vertebrae
  5. Sesamoid: located within tendons such as patella which is located within the quadriceps tendon.

- Macroscopically: if we have a longitudinal section through the long bone, there are 2 different types of bones:

1. Dense bone/Compact bone/Cortical bone:
   - It is compacted, has no spaces and looks dense.
   - Cortical refers to cortex which means shell.

2. Spongy bone/Cancellous bone/ trabecular bone
   -It has many spaces/holes that are filled with bone marrow, so they are called marrow cavities.
   - It looks like a sponge but it is hard.
   -It is composed of irregularly shaped bony structures or bony spicules, called trabeculae (called also trabecular bone)

✓ **Remember** always and always: the spongy bone is covered by a layer of compact bone.

✓ **What is the difference in appearance between the compact and spongy bone?** The compact one is compacted and the spongy has many spaces or pores, so the spongy bone is more lightly weighted.

✓ **Why our bones have cancellous/spongy bone?**
Cancellous bone is not as strong as the compact bone and it is lightly weighted. Imagine if our bones are composed of only compact bones, then we would be so heavy and movement would be so difficult. The spaces inside the cancellous bones are sites for bone marrow as well

➢ **The structure of the long bone:**

- Notice inside the diaphysis of the long bone: there is a tube-like canal called **medullary canal or cavity** (at birth it contains red bone marrow). Within the spaces of the cancellous bone, there are cavities called **medullary/marrow cavities** that are filled with red bone marrow.
- With aging this red bone marrow will be replaced by a yellow bone marrow except in the epiphysis, some red bone marrow sites remain for the production of blood.
- Epiphyses (proximal and distal ends) are composed of spongy bone covered by a layer of compact bone (cortical bone), whereas diaphysis is composed of compact bone. However, a thin layer of spongy bone lines the medullary canal. Blue area: hyaline cartilage or articular cartilage. The articular cartilage has no surrounding perichondrium and it gets its nutrition from the synovial fluid inside the joint cavity.

- **Notice:**

  -**Diaphysis**: constricted portion of the body of the long bone.
  -**Epiphysis**: proximal and distal round ends.
  -**Epiphyseal line**: The line between the epiphysis and diaphysis, it is completely composed of bone, and it is the place where the growth plate was. It was composed of hyaline cartilage.
  -**Metaphysis**: is the zone of transformation between the diaphysis (constricted portion) and epiphysis (rounded in shape).

✓ **What is the difference between epiphyseal line and metaphysis?**

-The epiphyseal line is part of metaphysis; the most proximal part or superior edge of the metaphysis if we are talking about the proximal end.

-Once the diaphysis (constricted portion) starts to flare out, the bone inside becomes spongy and the medullary canal ends; this area is called metaphysis.

Again: The diaphysis is mostly compact bone while proximal and distal ends are composed mostly of spongy bone. The epiphysis is mainly composed of cancellous bone and it is covered by a layer of cortical bone, same concept with diaphysis which is mainly composed of cortical bone and the medullary canal is lined by a thin layer of spongy bone. (Refer to slides 7 and 9 in the powerpoint)

Growth plate is hyaline cartilage and is responsible for the growth of bone in length, which means whenever we still have cartilage, we can grow in height but once it is completely replaced with bone (growth plate is closed), the growth stops and it is called epiphyseal line. For example, a 30-year-old man has in his long bone epiphyseal **line** but not epiphyseal **plate**.

Periosteum: A double-layered membrane that covers the outer surface of bone (same as perichondrium), it is irregular dense connective tissue surrounding the bone .

- The outer layer is fibrous (contains collagen type I)
- The inner layer is cellular, contains osteogenic (osteoprogenitor) cells which are able to differentiate into osteoblasts (similar to the structure of perichondrium)

The microscopic structure of the compact bone: (Refer to slide 21 in the powerpoint)

✓ No spaces (except for the presence of tiny canals)

✓ External cover (Periosteum which is composed of outer fibrous layer and inner cellular layer)

✓Consists of structural units called **osteons or haversian systems**, each unit is a tall column of bone. It looks like tree trunk

✓ The long axis of the osteon is parallel to the long axis of the long bone.

Now the Osteon is:

- Composed of concentric (متحدة المركز) rings of bone tissue surrounding a central canal called **Haversian canal (central canal)** which contains blood vessels, nerves and lymphatics. These concentric rings are called lamellae. The bone is highly vascular and highly innervated, that's why bone fractures are very painful
- Remember bone tissue is composed of cells and ECM
  Osteocytes are located between these lamellae. So lamellae are actually the ECM of the bone.

▪ ECM is composed of:

1. 33% organic material, mainly collagen type I (responsible for the bone's strength), glycoproteins and proteoglycans.

2. 67% inorganic material (minerals): mainly Calcium and phosphate, and they are responsible for the hardness of bone.

And that means if you exclude the minerals from the bone you would end up with a soft structure, and if you exclude collagen the bone would be brittle

- Each ring is called lamella (صفيحة) (the plural is lamellae), each lamella is composed of bone ECM. The osteocytes lie in between these lamellae.

Why we can demarcate each lamella from the adjacent one?

- Simply because of the different orientation of collagen type I between lamellae.

Refer to slide number 21: notice collagen fibers in a single lamella run helically and parallel to each other. In the adjacent lamella the fibers run also parallel to each other but at a perpendicular angle to the first lamella, so that's why we can outline each lamella

This arrangement is very important for bone strength. How?

- if you arrange Lego pieces in rows above and parallel to each other to build a wall, this wall can be easily broken if a force is applied, but if you arrange the pieces in rows perpendicular to each other, then you get a stronger wall.

Types of lamellae:

1) Concentric lamellae: Circles within circles around haversian canals (osteons)

2) Interstitial lamellae: The lamellae lie in between the osteons. Interstitial lamellae represent the old osteon system (this is an indication of the continuous process of bone resorption and bone deposition, the word "old" here means partially resorbed)

3) Outer circumferential (محيطية) lamellae: Located exactly under the periosteum and surround the whole circumference of the bone.

4) Inner circumferential lamellae: surround the medullary canal

Volkmann's canal: A transverse canal that contains blood vessels and nerve supply communicating with Haversian canals of osteons and the blood vessels of periosteum and endosteum

Osteocyte:

A cell that has many branches (processes) that pass through the bone tissue in small tiny canals created within the hard ECM, those canals are called canaliculi (plural , and the singular is canaliculus).

Why do they have processes?? To allow communication between Osteocytes and blood vessels of central canals, they are used for exchange of nutrients and waste products. The processes connect by gap junctions

Why canaliculi ?

- Bone is a hard tissue so wastes and nutrients can't diffuse and can't reach osteocytes unless there are such canals.

Structure of Spongy bone:

- ✓ Composed of trabeculae (plural, singular is trabecula: Piece of bone). The spaces between them are filled with bone marrow.
- ✓ It has lamellae that run parallel to each other rather than forming concentric rings around a central canal, so there is no central canal in the middle (no osteons)

Does it contain osteocytes? YES

Do these osteocytes have canaliculi? YES

Is spongy bone composed of osteons? NO

Where can you find the spongy bone?

In the epiphysis, and in flat bones (spongy bone sandwiched between 2 layers of cortical bone)

Remember: Spongy bone is never ever exposed; it is always covered by a layer of compact bone

- ➢ Diploë (pronounced dip-lo-we) is anatomical definition for the area of spongy bone between the two parts of cortical bone.

Endosteum

- ➢ is an inner-lining membrane
- ➢ it lines all cavities of the bone

Sharpey's fibers: Collagen I fibers anchoring the periosteum to the bone tissue. The sharpey's fibers emerge from the outer layer of Periosteum then merge with collagen type I of the bone matrix.

✔ Clinical importance: When the surgeon reflects (removes) the periosteum, he/she must cut the sharpey's fibers.

What is the histological difference between Periosteum and Endosteum ?

-They differ in location and number of layers:

Endosteum ➞ lining, single layer of osteogenic cells.

Periosteum ➞ covering, double layer (outer fibrous and inner cellular containing osteogenic cells)

Spongy bone Vs. Compact bone

Compact bone is stronger but spongy bone is more lightly weighted

4 types of cells present in bone tissue:
1- Osteoprogenitor cells (osteogenic)
2- Osteoblasts
3-Osteocytes
4- Osteoclasts

Osteoprogenitor cells:

- Origin: From mesenchyme
- Unspecialized stem cells: able to differentiate into bone-forming cells (osteoblasts).
- They can undergo mitosis
- Found in 2 places (cellular layer of Periosteum, the Endosteum)

Osteo**b**lasts:

- **B**uilding cells, they first secrete organic ECM (collagen I (mainly), proteoglycans, glycoproteins) and later they deposit minerals around and in between collagen fibers so the ECM is mineralized.
- Active cells in ECM synthesis.
- After the deposition of ECM, osteoblasts are encased within lacunae (small spaces) but they communicate with each other by canaliculi, now they are called osteocytes.

- Origin: Osteoprogenitor cells, so you find them in periosteum and endosteum
- Arranged next to each other so they look like simple cuboidal epithelium.
- They synthesize ECM toward the bone surface (old bone)
- The matrix synthesized is called osteoid (like bone-still unmineralized)
- Osteoblasts secrete osteocalcin, osteocalcin binds Ca^{2+} with high affinity, thus raising the local concentration of these ions. Osteoblasts also release very small matrix vesicles which contain alkaline phosphatase and other enzymes. These enzymes hydrolyze PO_4^- ions from various macromolecules, creating a high concentration of these ions locally. (Refer to slide 52)
- The high ion concentrations cause deposition of minerals (crystallization) in and around the matrix vesicles. The crystals grow and mineralize further with formation of small growing masses of calcium hydroxyapatite, which surround the collagen fibers and all other macromolecules. Eventually the masses of hydroxyapatite merge as a more solid bony matrix as calcification of the matrix is completed.
- Again: ⬆ [minerals]➞ leads to their deposition as **crystals➞** these crystals unite to form the mineralized matrix of bone .

<u>Osteocyte:</u>

The inactive form of osteoblast. Thus, it is expected to have the same histological appearance of the inactive cell (fewer ER, condensed Golgi apparatus)

- Smaller than osteoblasts
- Situated inside lacunae, one cell in each lacuna.
- Cells have processes (filopodial) passing through canaliculi in the thin surrounding matrix.
- Adjacent cells make contact through gap junctions in the processes.

The osteocytes can communicate with each other through their processes which have gap junctions. (**<u>Note that:</u>** gap junctions are not exclusive to the epithelial cells, it is also located between the osteocytes)

Osteocytes are important to maintain the ECM

Osteoclasts:

- We talked about them when we considered the mononuclear phagocyte system (different macrophage- like cells in different locations (in bone they are called osteoclasts)
- Originate from monocytes (fusion of monocytes) (from hematopoietic stem cells in bone marrow)
- Multi nucleated: their nuclei number can reach up to fifty nuclei within one cell
- In histological sections, we are not able to see fifty nuclei, we usually see from (5-10) nuclei in each osteoclast
- The main function of them is resorption of bone [be aware that when we say resorption of bone it doesn't necessarily mean a disease, throughout our life, the bone is exposed to a continuous state of remodeling (resorption and deposition).
- They secrete different lysosomal enzymes into ECM (hydrolytic enzymes such as collagenase), and they pump protons (H+), creating an acidic microenviroment (low ph) in the ECM, resulting in digestion and demineralization of the ECM.
- It's also located at the surfaces of the bone (periosteum and endosteum), and they are anchored by actin filaments to the bone surface.

- The zone of the osteoclast that is bounded to the bone is rich in actin filaments in order to adhere to the bone [this border of the osteoclast is called Ruffled border, it looks like microvilli, it is thrown into folds in order to increase the surface area].
- When resorption of the matrix takes place, the calcium ions and phosphate travel through the osteoclasts to the blood capillaries (by transcytosis), to increase the calcium/ phosphate level inside the blood
  for example, the osteoclasts are activated when the concentration of the calcium in the blood is very low
- Regulation of osteoclasts and osteoblasts: It is achieved by hormones; certain hormones for example increase/decrease the osteoclastic activity and other hormones increase/decrease the osteoblastic activity.
- For example, normally, Estrogen (a hormone presents mainly during the reproductive life of females) inhibits the osteoclastic activity, but after the menopause low levels of Estrogen is present, so more bone resorption occurs, that is why osteoporosis is more common in females.

- It is very important for a female to increase dietary calcium/ calcium supplement especially after the menopause

Osteoporosis (هشاشه العظام)

- The density of the bone is less, because the activity of the osteoclast is high which causes spaces (pores). In osteoporosis, the bone deposition by osteoblasts is also less (the activity of osteoclast should be parallel to the activity of the osteoblast). The bone becomes weak and the possibility of fractures is high. In osteoporosis, the bone breaks with relatively minor injury that normally would not cause a bone to fracture. It is common in females after menopause. Because during the reproductive period estrogen (female sex hormone) inhibits the action of osteoclasts

How to prepare a histological section of Bone for microscopic examination?

- We have 2 methods (because ordinary microtomes can't cut the bone "it is a hard tissue"):

  1-**Decalcified bone section**: Removing minerals from bone tissue after putting it in a decalcifying agent, only soft structures (only cells and organic matrix) are left so I can do embedding, cutting, staining and so on ...
- So the bone after decalcification will resemble **tendon** (because the bone now contains collagen type I and osteocytes).
- This method leads to distortion of osteons so you can't see the highly-organized structure of osteons, simply because of minerals removal.

  2- **Ground bone section** (ground is an adjective that means crushed) (مطحون), it is produced by fine grinding of bone specimen into small pieces then examining under microscope without staining.

- In this method the morphology of bone is preserved, because bone dust will accumulate inside the spaces of bone (like empty lacunae, canaliculi, Haversian canal, etc ..) .

- Notice that the lacunae are empty because osteocytes were eliminated during grinding, no fixatives were used

- ✓ Bone tissue is also classified into primary bone and secondary bone.

- ✓ In the beginning, the first bone that is deposited by the osteoblasts is called primary bone, this bone will be replaced by a more mature type of bone called secondary bone (cortical and spongy bone are both types of secondary bone).
- ✓ Primary bone can also be called immature type of bone, or woven bone.
- ✓ Secondary bone, also known as mature bone, or lamellar bone.

Why primary bone is called woven bone?

The extracellular matrix of the bone is composed of collagen type one fibers which are parallel to each other in the single ″ lamella″ with little amount of ground substance, but in the woven bone, collagen fibers are *haphazardly* arranged, or *irregularly shaped or "woven"* compared to the lamellar bone.

This type of bone is temporary, and will be replaced by the more mature secondary type of bone with organized *osteons* of cortical bone, or *trabeculae* of spongy bone.

Woven bone has a lower mineral content, so in x-rays we can differentiate between woven bone and secondary bone, as it is **less mineralized**, it appears **less white**, or **less radio-opaque** than the secondary bone. (Woven bone is still a mineralized tissue, so it appears white in color but LESS white than secondary type of bone because it is less mineralized) so secondary bone appears *more whitish*.

Woven bone has a higher number of osteocytes with larger lacunae (remember that osteocytes are found within lacunae)

Primary Bone	Secondary Bone
Higher number of osteocytes	Lower number of osteocytes
Larger osteocytes	Smaller osteocytes
Larger lacunae	Smaller lacunae
In decalcified sections, collagen fibers (eosinophilic) are woven in appearance.	The collagen fibers run parallel to each other in decalcified sections.

- Primary bone is formed during:

1- Growth period

The bone is formed mostly within a cartilaginous model.
The first type deposited is primary bone, then osteoclasts remove it and new secondary bone will be formed.

2-During the process of repair

When there is a fracture, the first bone to be deposited will be woven bone then it will be replaced by a more mature type of bone, it takes a long time for traces of fracture to be completely gone, as we have fibrocartilage, primary bone, and then secondary bone deposition.

A fracture is a break in the continuity of bone. Fractures can occur for any number of reasons, such as falls, sporting injuries, and increased mechanical stress applied to the bone. As we age, our bones become weaker and more brittle, thus the majority of fractures occur in the elderly, as a result of low bone density or the condition known as osteoporosis.