Tissue Engineering
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Lecture - 32
Bone Tissue Engineering - Part 1

Good morning everyone, I am Hemalatha Kanniyappan, doing my research under the guidance of Dr. Vignesh Muthuvijayan. Today I will be explaining about Bone Tissue Engineering.

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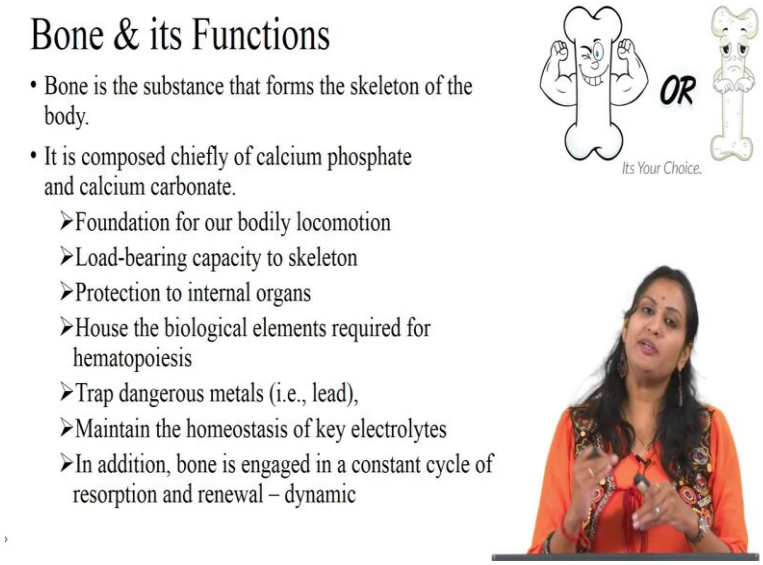

I start with a brief introduction about bone and its functions. What is a bone? Bone is a substance that forms the skeleton of the body. To be more clear, it is a natural composite material that is composed of organic collagen phase and inorganic hydroxyapatite phase. The hardness of the bone is mainly due to the presence of hydroxyapatite, which is mainly composed of calcium phosphate and calcium carbonate. Whereas, the toughness and the viscoelastic behavior is mainly due to the presence of collagen.

The major function of the bone, as we all know, it makes the foundation for our bodily locomotion, and it acts as the load-bearing capacity to our skeleton. It protects the internal organs. It also houses the biological elements that are required for hematopoiesis, which is the process that is responsible for the formation of blood cellular components. It traps the dangerous metals; for example, lead. And also, it maintains the

homeostasis of key electrolytes, like it maintains the equilibrium between the cellular components.

In addition to above all the function, bone is engaged in a constant cycle of resorption and renewal, which makes the bone, dynamic in nature.

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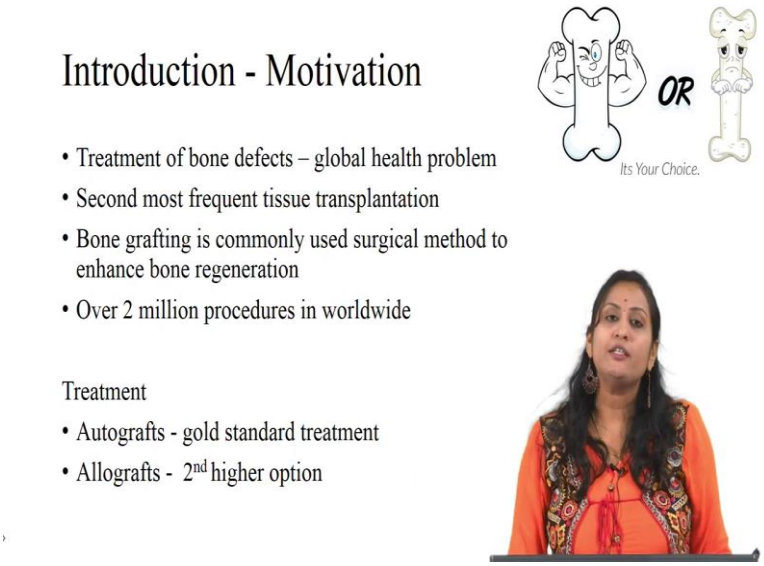

The defect in bone and the treatment of bone defects due to severe trauma or several pathological disorders, like a bone tumor or infection, possesses a major threat to the surgeons worldwide, and it is a serious global health problem. Amongst the transplants made, bone is considered to be the second most transplanted tissue after blood. Bone loss due to damage is a serious challenge to orthopedic surgeons, especially when bone loss is massive. Bone grafting is a commonly used surgical method to enhance bone regeneration. Over 2 million bone grafting procedures are performed worldwide every year.

The treatment of bone grafting includes autografting. In this, we graft tissue from the same individual. It is considered to be the gold standard treatment because of its ideal biocompatibility in terms of structural as well as immunological point of view. The second higher option is allografts, where we graft tissue from a different individual of the same species. Though autografts or allografts are considered to be the standard treatment for bone grafting, an autograft requires the harvest of the site. It not only increases the pain but also increases the time of surgery, bleeding, and donor site morbidity. It also

leads to nerve injuries at multiple harvest sites due to surgeries. Whereas in the case where we need a large amount of tissue to be transplanted, it cannot be done using autograft procedure because the availability of the tissue is limited.

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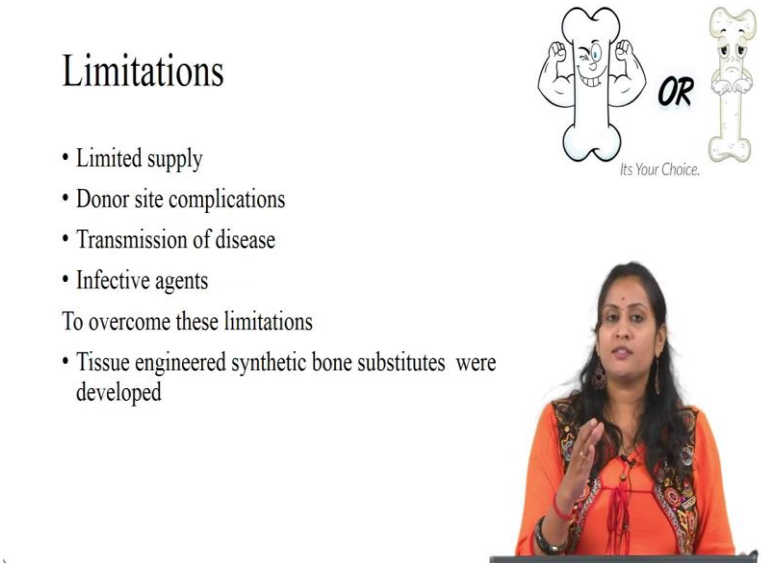

Whereas, in the allograft, it is associated with pathogen transfer and also immune rejection. So, we need a better treating option to enhance bone regeneration or to repair the bone defects, where it overcomes all other above said limitations.

Tissue-engineered synthetic bone substrates were developed, which will ideally eliminate the above-said limitation and aims to develop an ideal bone graft that enhances bone regeneration. Before getting into tissue engineering aspects of our bone tissue engineering application, its status, issues, we need to understand the anatomy of bone.

So, in this session, I will be explaining about the anatomy of the bone and the modeling and remodeling of the bone. Then the later session will be discussing about tissue engineering aspects.

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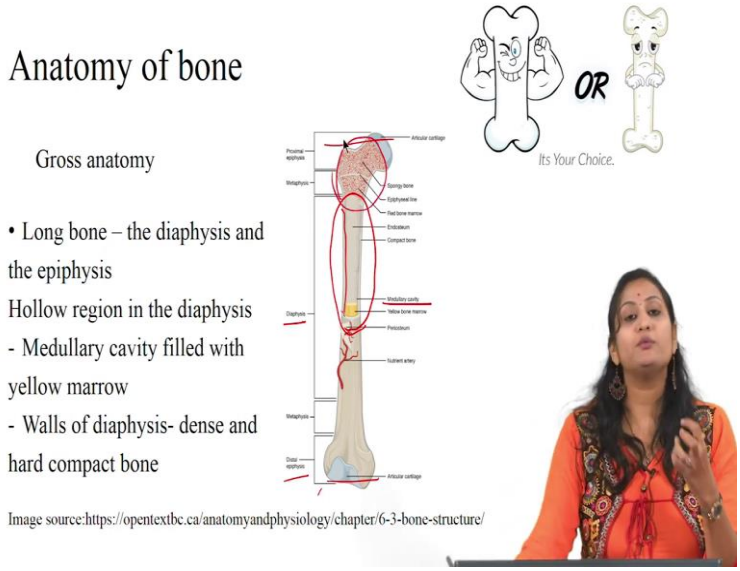

The anatomy of the bone, the first gross anatomy. Gross anatomy means the study of the anatomy of the bone at the visible level. The structure of the long bone explores the best visualization of all parts of the bone. The long bone is divided into two parts; diaphysis and the epiphysis. The diaphysis, the tubular region that runs between the proximal and the distal ends of the bone. The hollow region in the diaphysis is called a medullary cavity, which is filled with yellow bone marrow. The walls of the diaphysis are made up of hard and dense compact bone.

The wider sections at each side of the bone are the epiphysis, and it is filled with red marrow. The cavity is filled with spongy spaces, and red marrow fills these spaces. An epiphysis connects to the diaphysis at the metaphysis. The metaphysis is the narrow area that has epiphysial growth plate and then cartilage layer, hyaline cartilage layer. This epiphysial growth plate becomes an epiphysial line when bone growth stops at early adulthood; approximately at the age of 18 to 21 years, and the hyaline cartilage layer is replaced with osseous tissue. The inner medullary cavity is made up of intramembranous delicate lining called endosteum.

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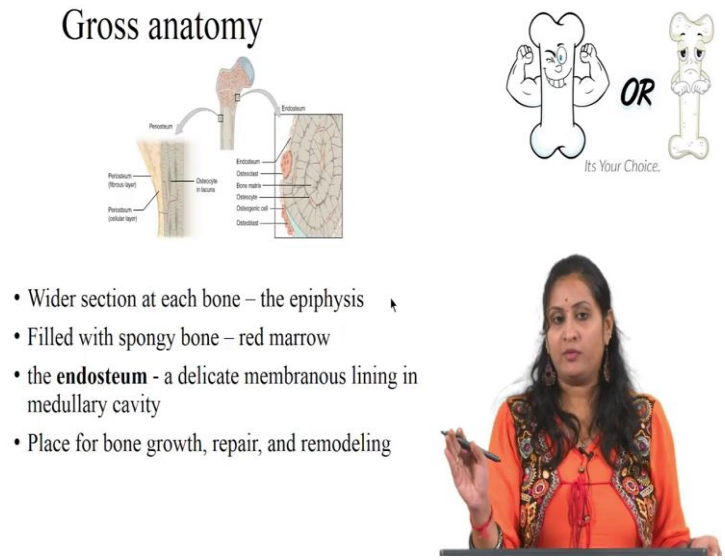

Endosteum is the place for bone growth, repair and remodeling occur. I will explain bone growth repair and remodeling in detail and what are the cellular components involved, in the latter part of the session.

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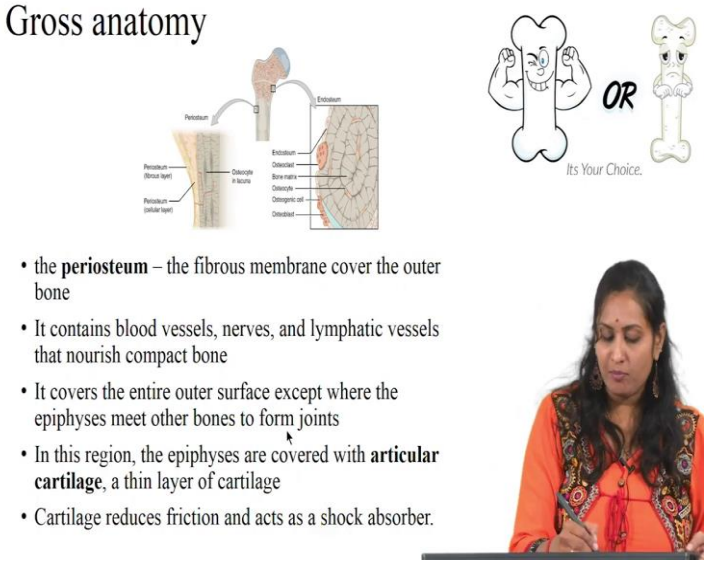

The periosteum is the fibrous membrane covers the outer surface of the bone. It has blood vessels, nerves, lymphatic vessels that nourish the compact bone. This periosteum covers the entire outer surface of the bone, except at the epiphysis region, where it has articular cartilage. And, this acts as the shock absorber and reduces the friction.

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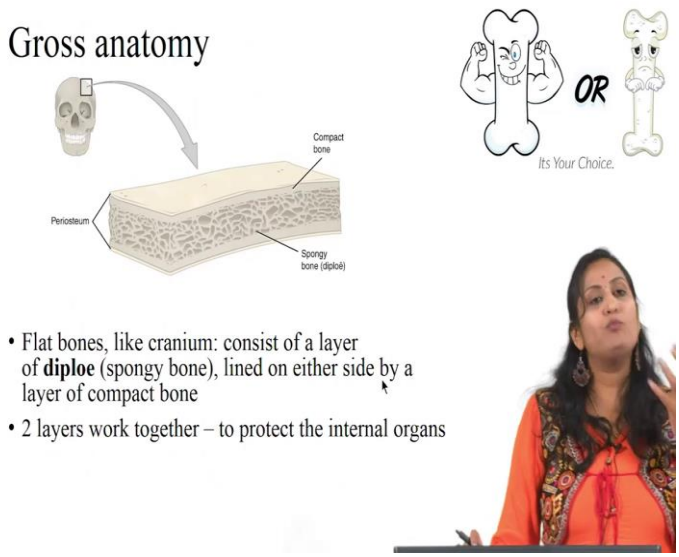

Whereas flat bones like cranium, it is made up of a layer of a spongy bone and lined on either side of the layer of the compact bones. So, the layer of spongy bone and the two layers of the compact bone works together to protect the internal organs. If there is any fracture in the outer cranial bone, still the brain is protected by the inner contact layer of the compact bones.

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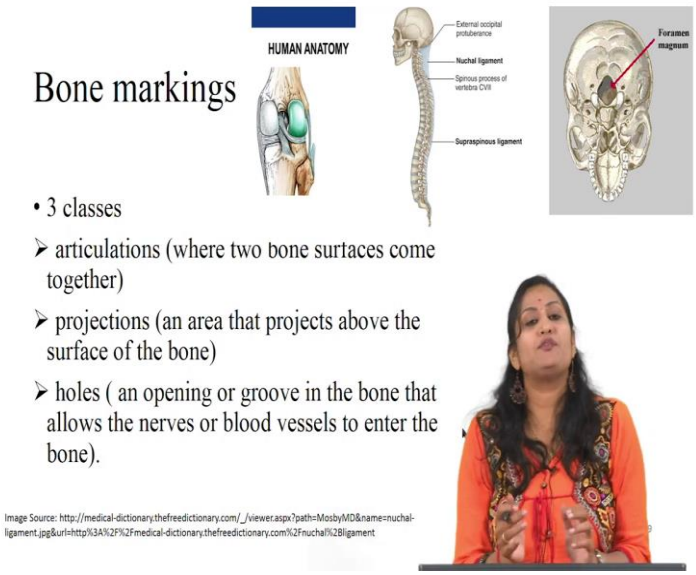

The surface features of the bone markings. There are three general classes of bone markings: articulations, projections, and holes. The first class of bone marking

articulation, as the name implies, where two bones surfaces come together. These surfaces tend to conform to one another, such as one being grounded, the other being curbed in order to facilitate the function of articulation. Articulation means joint, for example, knee joint.

The second bone marking general class is called as projections. It is an area that projects above the surface of the bone. Tendons and ligaments are attached to the periosteum through this marking. The spinous process of the vertebra is an example of the projection kind of bone marking. The third general class of bone marking is holes where it is an opening or groove in the bone that allow the nerves or blood vessels to enter the bone. For example, foramen, where the blood vessels pass through the bone.

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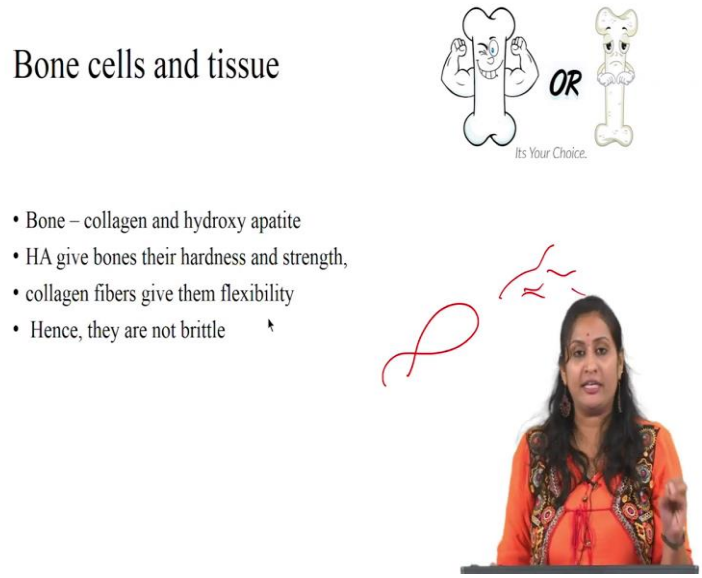

Next, we need to discuss about bone cells and tissue, which plays a major role in tissue engineering aspects; we need to know about what are the cells present in the bone. As I explained in the introduction session, bone is made up of collagen and hydroxyapatite. Hydroxyapatite gives bone their strength and hardness, and whereas, collagen gives them flexibility. If hydroxyapatite is not there, the bone will become more elastic. If collagen is not there, the bone will become brittle. Bone is composed of a smaller volume of cells. Although the bone composed of a smaller volume of cells, it plays a very important role in the nth function.

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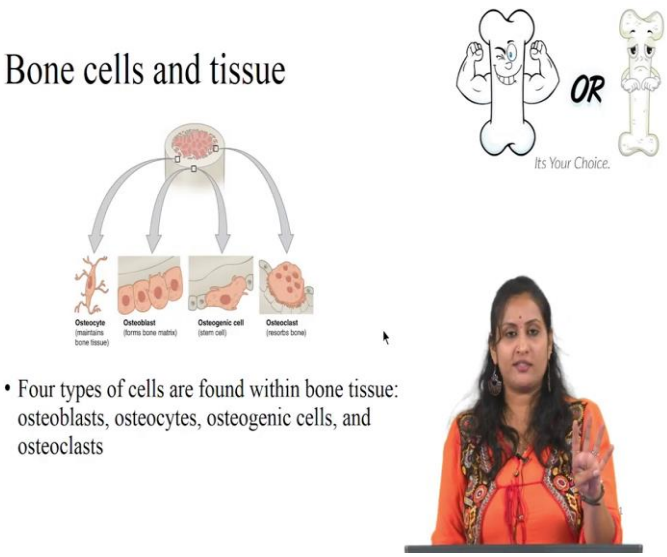

There are four types of cells; osteocyte, osteoblast, osteogenic cell, and osteoclast cells. Osteoblasts cells are the bones cells responsible for the formation of bone. It is present in the growing structures, and it is called osteocyte, which is the primary cell for the mature bone. And, the places where the osteocytes located are called as lacunae. Osteocytes maintain the mineral concentration within the matrix. Osteoblast and osteocytes communicate with each other and exchange their nutrients through the long cytoplasmic processes via canaliculus, which is present inside the bone matrix. Both osteocyte and osteoblast lack mitosis, then there arises the question if both the cells lack mitosis, how are they replenished when the old one dies?

The answer lies in the third category of the cells, which is osteogenic cells. It is a highly undifferentiated cell, and it is present in the deeper regions of periosteum and marrow cavities. It is the only bone cell that can divide; it divides and differentiates into osteoblast cells. So, while explaining the function of bone, the last function where I said, bone is dynamic in nature, which means the new bone is constantly formed, and the old bone or the damaged bone or the repaired bone should be resorbed continuously, which is done by the cells called osteoclast cells. Osteoclast cells are responsible for bone resorption.

So, there should be a constant balance between osteoblast cells, which are responsible for the formation of new bone, and osteoclast cells, which are responsible for bone resorption, in order to maintain the structural integrity of the bone.

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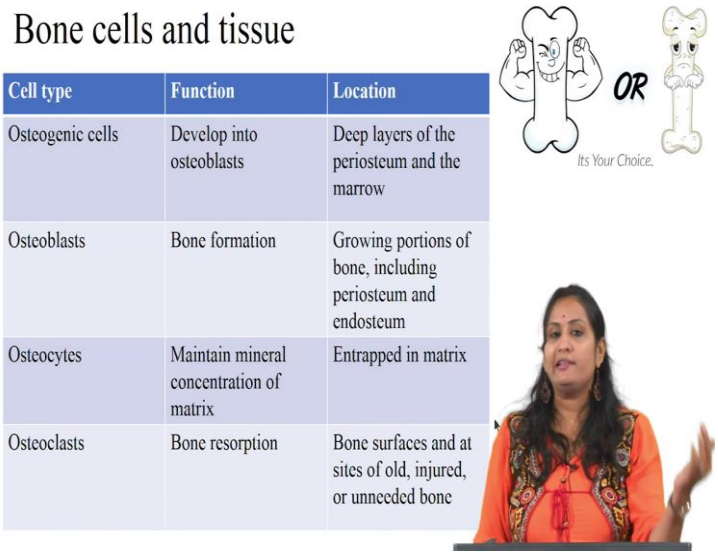

This reviews the cell type, its function and location in the bone. The first cell type is osteogenic cells; as I said, it is the only cell that can divide, and it develops into osteoblast. It is present in the deep layers of periosteum and the marrow. And, the next is osteoblasts cells, which are responsible for the formation of bone and present in the growing portions of bone including periosteum and endosteum. Osteocytes, which are the primary cell for a matured bone, and the location where it is located is known as lacunae and it maintains the mineral concentration of matrix. Osteoclasts cells are responsible for bone resorption, and it is present at the bone surfaces and at sites of old injured or unneeded bone. These osteoclasts cells are formed from monocytes or macrophages, which are two white blood cells; they are not originated from osteogenic cells.

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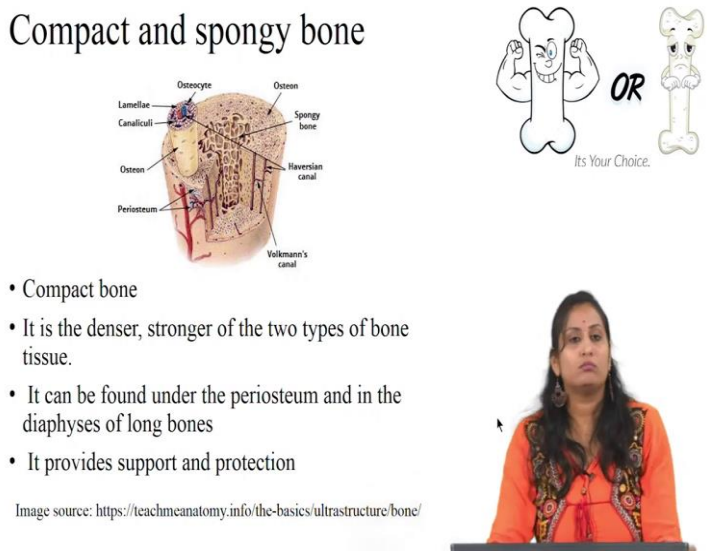

The details of compact and spongy bone are well explored by its histology. The compact bone is the stronger bone of the two, and it gives support and protection. It is denser, stronger than the spongy bone, and it is found under the periosteum and diaphysis of the long bone. The microstructural unit of compact bone is called as osteon. Each osteon is made up of concentric rings of a calcified matrix; this is also called a Haversian system. This osteon is made up of concentrated rings of the calcified matrix, which are called as lamellae.

Running towards the center to the osteon, it is the central canal where it has blood vessels, lymphatic vessels, and nerves. This is also called as Haversian canal, and this blood vessel, nerves, and branches are at the right angles through the Volkmann's canal or perforating canal, which connects to the periosteum or endosteum.

The osteocytes present in the lacunae are found at the adjacent of the lamellae of the osteons. As I explained earlier, the nutrients exchanged is done by canaliculi. So, one canaliculus are connected to another canaliculus, eventually to the central canal, where it has the blood vessels and lymphatic vessels. The osteocytes are nourished with the presence of these lymphatic vessels, and nutrients are transported through the central canal.

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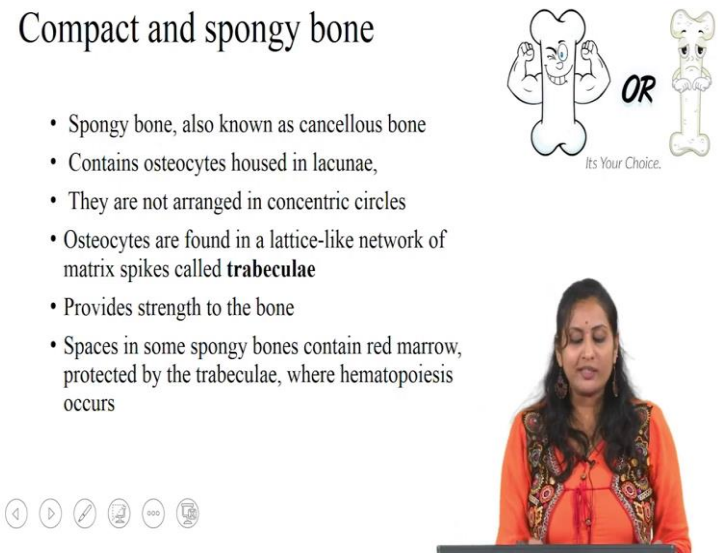

Spongy bone is also called as cancellous bone. Unlike the compact bone, spongy bone is not made up of concentric rings, whereas it is made up of a lattice-like network of matrix spikes called trabeculae. This provides strength to the bone. The spaces in some spongy bones that contain red marrow, which is protected by the trabeculae is where hematopoiesis occurs. Hematopoiesis is nothing but the process of formation of blood cellular components.

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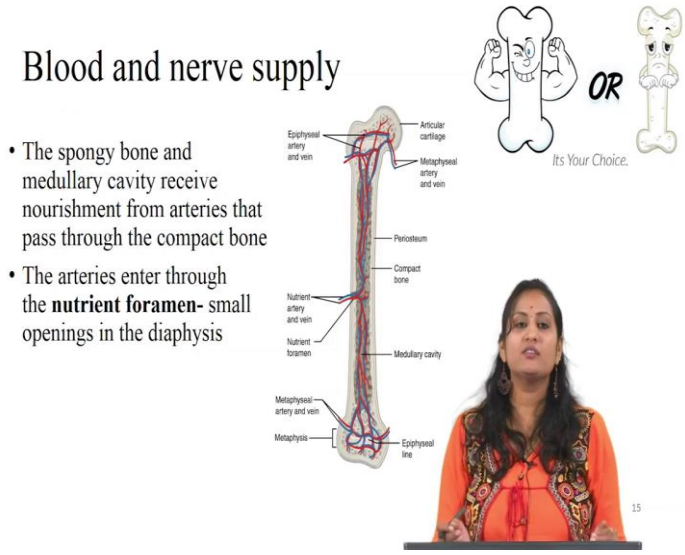

Blood supply and nerve supply to the bone. The spongy bone and medullary cavity receive nourishment from arteries that pass through the compact bone. The osteocytes the arteries enter through the nutrient foramen, which is the small opening present in the diaphysis. The osteocytes present in the spongy bone get nourished by the blood vessels and the arteries that enter through the periosteum and into the marrow cavities. Once it passes through the marrow cavities, it is collected by the veins, and it passes out from the bone.

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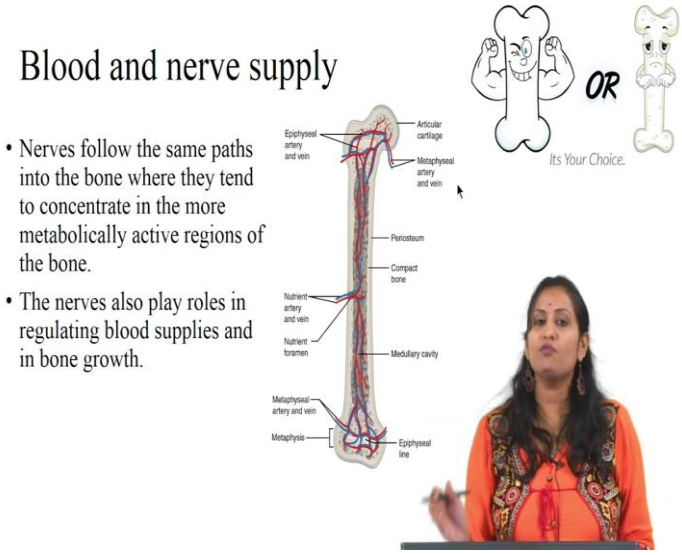

Similarly, nerves also follow the same paths into the bone, where they tend to concentrate on the more metabolically active regions of the bone. Nerves also sense pain; the nerves also plays a very important role in regulating blood supplies and bone growth.

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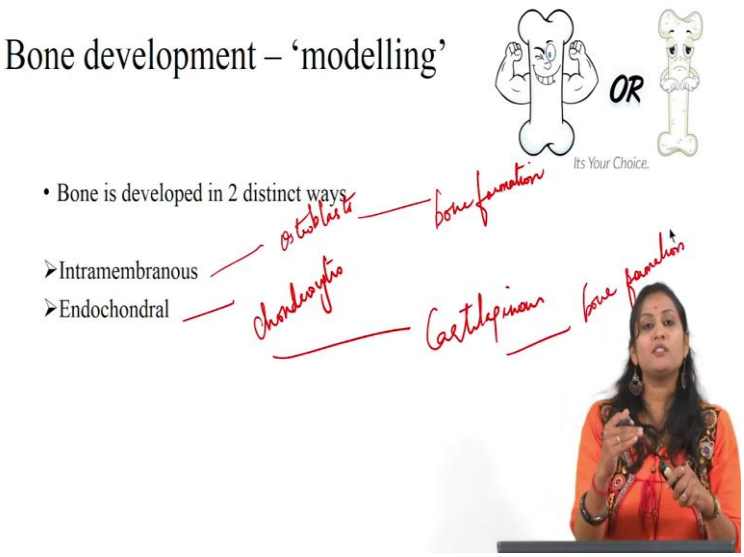

With this, the detailed explanation about bone anatomy is done. Now, we move on to the formation of bone, which is modeling and remodeling. Initially, bone is formed by modeling; it is developed in two distinct ways; the intramembranous pathway and the endochondral pathway. In either of the cases, there is a mesenchymal cellular condensation that occurs, and it serves as the template for the formation of bone.

In the intramembranous bone, where mesenchymal stem cells are directly developed into osteoblast cells, and subsequent bone formation occurs. For example, bones in mandible, clavicle, or few cranial bones are formed by this process.

Whereas, in the endochondral pathway, where most of the bones like long bones in our body formed through this pathway. Here, the mesenchymal progenitor cells are developed into chondrocytes. First, they are developed into chondrocytes. Then they form a cartilaginous layer, calcified matrix, then the subsequent bone formation occurs. So, these are the two pathways where the formation of bone occurs.

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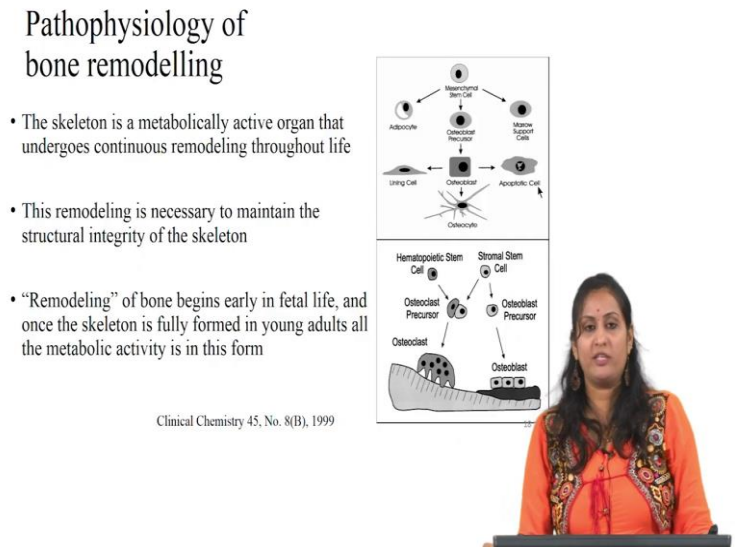

Then the remodeling of bone takes place; we know that the skeleton is a metabolically active organ that undergoes continuous remodeling throughout life. This remodeling is necessary in order to maintain structural integrity as well as to maintain the mineral concentration within the matrix. Remodeling of bone begins at the early fetal stage and also once the skeleton is fully formed in young adults; afterwards, all the metabolic activity will take place in this form.

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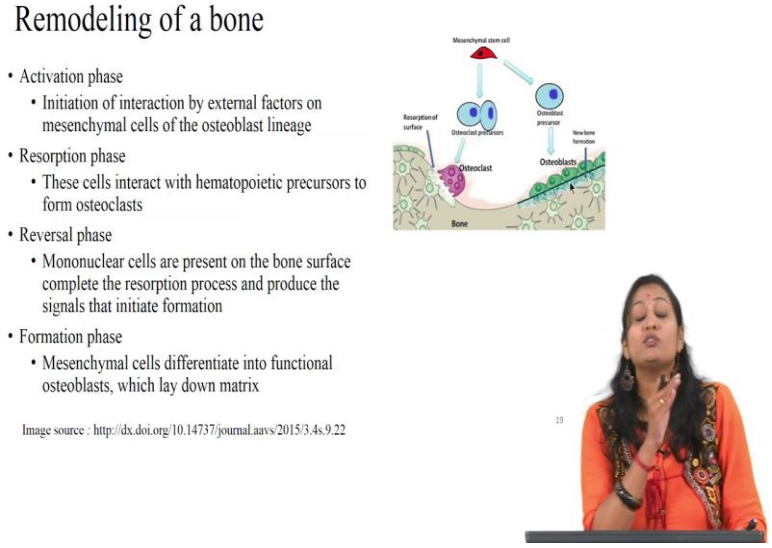

This remodeling consists of a series of highly regulated steps that involve the interaction of two cell lineages.

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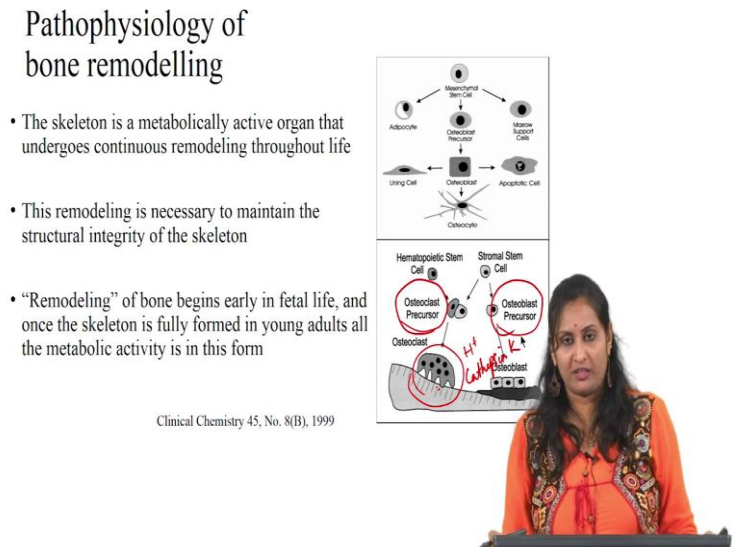

They are mesenchymal osteoblastic cells and hematopoietic osteoclastic cells. The interaction between mesenchymal osteoblastic cells and hematopoietic osteoclastic precursors cells leads to the start of the remodeling phase. And, there are four phases in the remodelling of a bone: activation phase, resorption phase, reversal phase, and formation phase. The first phase is the activation phase, where the interaction between the two precursors cell lines happens, which results in the differentiation, fusion, and formation of the large osteoclasts cells.

Osteoclast cells are present at the surface of the bone matrix; these cells will tend to secrete hydrogen ions as well as the lysosomal enzymes, especially cathepsin K. These two will degrade all bone cellular components, including collagen at low pH. This is the resorption phase. The resorption phase, where the cells interact with the hematopoietic precursors to form osteoclasts, and the reversal phase, the complete resorption takes place. The complete resorption of bone by osteoclasts takes place in the reversal phase and initiates the formation phase.

In this formation phase, mesenchymal osteoblastic cells will start to produce osteoblasts, which fills the cavity, which are resorbed by the osteoclasts; thereby, it laid down the bone matrix. So, this is how the remodeling of bone occurs. The first is the interaction

between two precursors cell lines, which are hematopoietic osteoclasts cell and mesenchymal osteoblasts. And then they form the osteoclasts cells. This osteoclast cell involves in the bone resorption, and it resorbs the bone matrix completely, whereas, there is a formation of osteoblast cells, that fills the cavity of the bone resorbed matrix. So, this is how bone modeling and remodeling, the formation of bone occurs.

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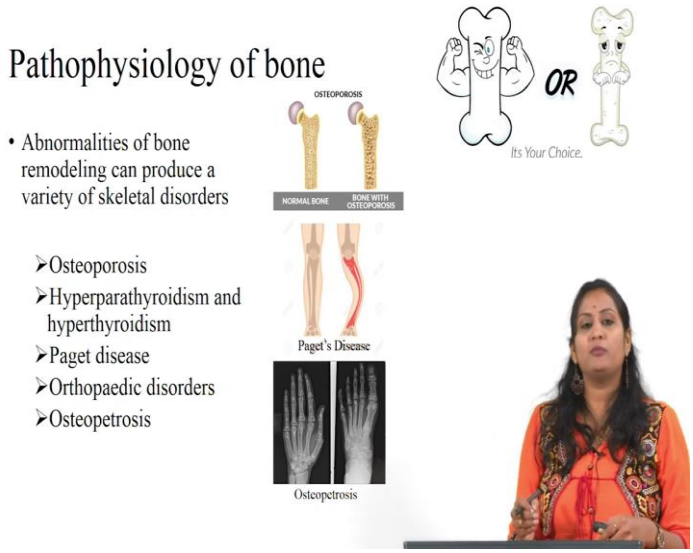

If there are any abnormalities present in this bone remodeling will lead to various skeletal disorders. For example, osteoporosis, hyperparathyroidism, and hyperthyroidism, Paget disease, orthopedic disorders, osteopetrosis. These are the skeleton disorders that are due to abnormalities happen in the bone remodeling cycle. Osteoporosis is defined as the loss of bone mass and strength which leads to the increase in propensity to fracture. It is type 1 and type 2; type 1 is called as postmenopausal osteoporosis and type 2 is called as senile osteoporosis.

Recently, literature has proved that deficiency of estrogen, which is an important systemic hormone for bone turnover; if there is a deficiency of estrogen, it leads to osteoporosis. The osteoporosis can be mainly due to three reasons; one the peak bone mass is not formed completely, or there is an imbalance between osteoclast function and osteoblast function, or the over activeness of osteoclast. Like, osteoclasts are activated too much, thereby it resorbs bone more than the bone formation, that is osteoporosis.

In Paget's disease, the complete mechanism or the clear mechanism is not yet understood. But, they say that because of some viral infection, these osteoclast cells are activated abnormally, and thereby bone resorption is more, where it changes the structure of the bone, where it shows evident in that picture; that is Paget disease.

In osteopetrosis, osteoblasts function is lost; bone formation is not that great when compared to bone resorption. So, there should be a balance between the formation of bone or the resorption of bone in order to maintain the proper shape of the bone; otherwise, it will lead to a variety of skeletal disorders.

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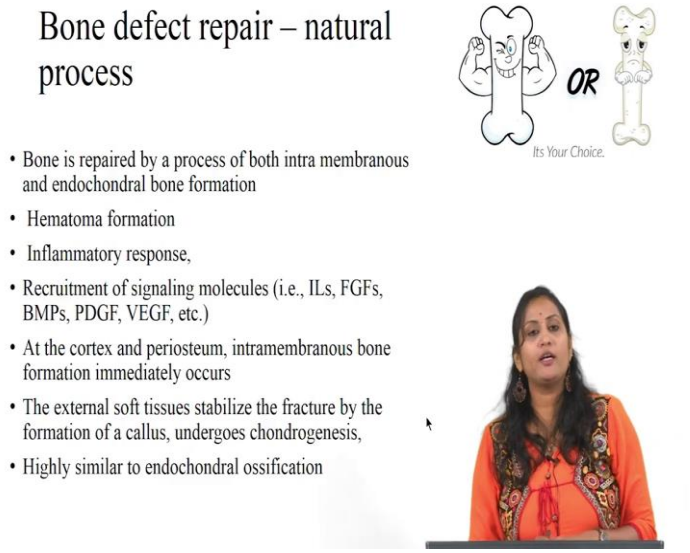

Now I will be explaining about the normal process of bone healing. Once there is any fracture, bone can itself heal by its own process. It can be repaired by the process of both intramembranous and endochondral bone formation. First, it starts with the hematoma formation accompanied by the inflammatory response; then, it starts recruiting the signaling molecules. For example, interleukin, fibroblast growth factors, bone morphogenetic proteins, which are responsible for the formation of bone.

Once after recruiting signaling molecules at the place of cortex and periosteum, intramembranous bone formation immediately occurs. Then it stabilizes the fracture by the formation of callus by chondrogenesis. Chondrogenesis is the activation of chondrocytes, which is highly similar to endochondral ossification.

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Then once the tissue reaches its maturity, the chondrocyte proliferation decreases, and they calcify the matrix, then growing blood vessels, which carries chondroclasts and osteoblastic progenitors. These chondroclasts will resorb the calcified cartilage, whereas the osteoblastic progenitors will help in the formation of new bone, and the remodeling of new bone will start; thus, by it heals the repair or fracture.

As we know that bone is highly vascularized tissue, though it can heal by itself, beyond a critical point, clinical intervention is required; where the future treatment option is bone tissue engineering. Bone tissue engineering is considered to be a future treatment option. In the next session, we will be discussing about is status or key components involved in bone tissue engineering.

Thank you.