Osteology -

At the end of this module, you should be able to :

1. **Describe the process of bone development;**
2. **Appreciate the role of specialised cells in this process;**
3. **Understand the processes of bone growth, remodelling, and healing.**

The majority the human skeleton is made up of a bony framework which facilitates locomotion, and also acts as a protective cage for internal organs. It is strong and light-weight, but is also a constantly changing tissue, undergoing a remodelling process throughout life. Structurally, the skeleton consists of bone tissue, cartilage, bone marrow, and periosteum, but there are many other terms used in the description of bone structure and function.

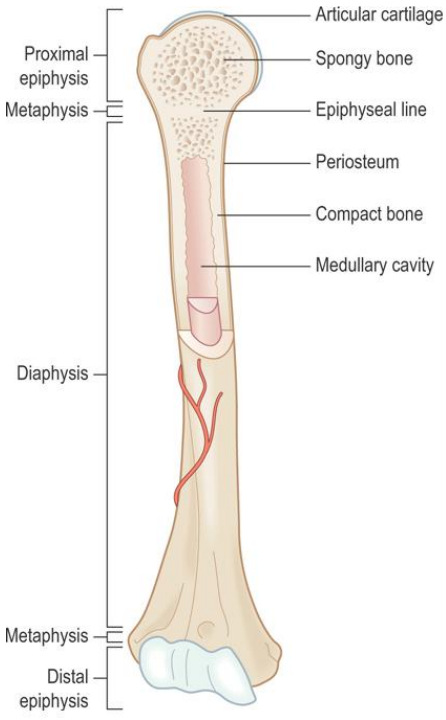
Diaphysis – the shaft, or main portion of a bone

Epiphysis – the distal or proximal end of a bone

Metaphysis – the area where diaphysis and epiphysis merge. In growing bone this is usually represented by the epiphyseal plate

Medulla – the central cavity which may contain bone marrow

Periosteum – the membrane surrounding the non-articular surface of the bone. The outer part of the periosteum is fibrous and contains blood vessels and nerves, whilst the inner, osteogenic layer also contains blood vessels as well as osteoclasts and osteoblasts.



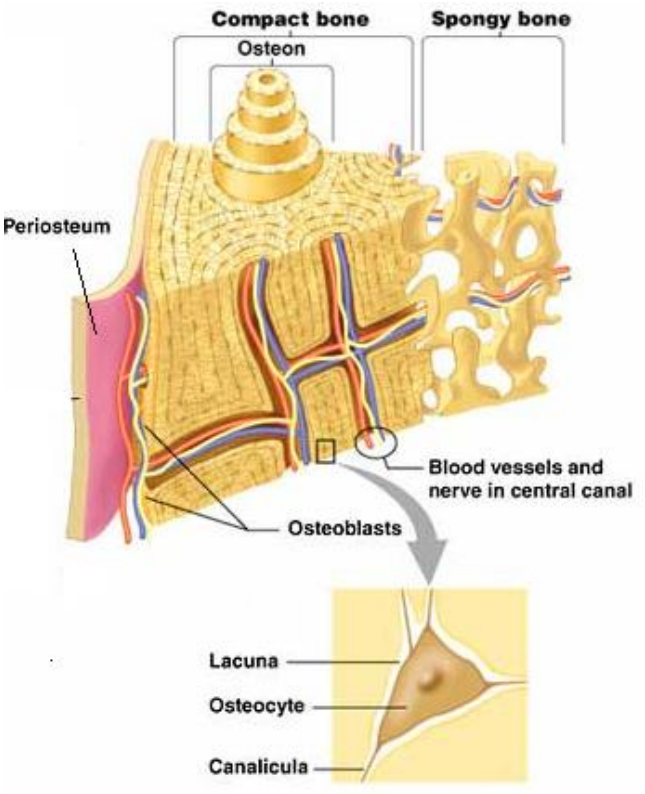
Bone consists of a matrix, which contains different types of cells. The matrix contains around 25% each of water and collagen fibres, and 50% mineral salts. The four types of cells in the matrix are osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts. Osteoprogenitor cells are unspecialised cells with the ability to develop into osteoblasts, the cells which are able to form bone. Osteocytes are mature bone cells that are derived from osteoblasts which have become surrounded by deposited bone, and can no-longer secrete bone material. Their role is maintain regular cellular activity rather than to produce new bone. Osteoclasts are involved in bone resorption and breakdown, and are therefore important in maintaining healthy bone tissue.

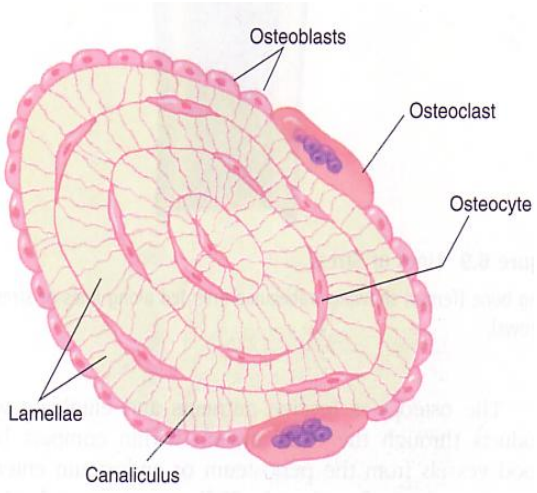
Bone matrix contains high levels of minerals, mainly in the form of hydroxyapatite crystals, with some calcium carbonate. Also present are smaller amounts of magnesium, fluoride, and sulphate. These minerals are deposited on the collagen fibres, which then harden in a process of calcification (mineralisation). The presence of collagen fibres is of great importance in this process, as they add strength – without collagen bone would be fairly brittle and easily damaged. The collagen provides tensile strength, allowing the bone to resist stretching and torsion. In addition, if no collagen is produced in the bony matrix, then no mineral salts will accumulate, and there is no calcification.

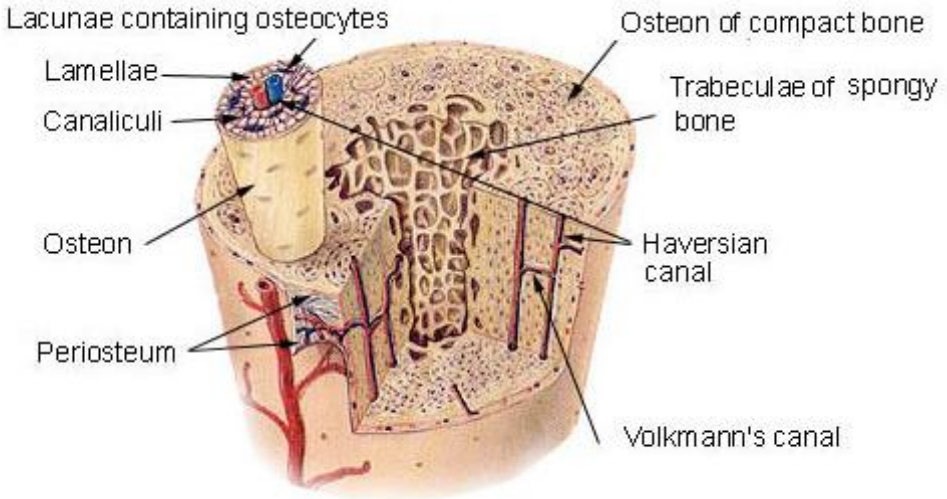
The overall architecture of bone is divided into cancellous bone (also referred to as trabecular bone or spongy bone) and cortical bone (also referred to as compact bone). Cortical bone forms a compact shell around the more delicate cancellous bone, which is formed by an inter-connecting latticework of trabeculae. In general, the peripheral skeleton is composed primarily of cortical bone, while the axial skeleton is composed of both cancellous and cortical bone. Because the surface area of cancellous bone far exceeds that of cortical bone, and is more metabolically active, cancellous bone is more severely affected if bone remodelling becomes uncoupled.

Compact (cortical) Bone

This type of bone is very dense, with few spaces within its structure, and forms the bulk of the diaphysis of the long bones, as well as the external layer of all bones in the body. Its role is to provide support and protection, and forms approximately 80% of skeletal mass. Adult compact bone typically has an arrangement of concentric rings. Blood vessels and nerves from the periosteum penetrate the compact bone through perforating canals (Volkmann's canals), and connect with the medullary cavity, the periosteum, and the central canals (Haversian canals). These central canals run longitudinally through the bone, surrounded by concentric lamellae, which are rings of calcified bone matrix. Between the lamellae are very small spaces (lacuna), where there are osteocytes. There are many canals radiating from the lacunae, called canaliculi, which contain extracellular fluid, as well as the fine processes which extend from the osteocytes. This provides a communication system throughout the compact bone, providing a route for nutrients and minerals, as well as waste disposal. Osteocytes can also communicate with each other via the canaliculi. Each central canal with its associated lamellae, lacunae, osteocytes, and canaliculi is referred to as an osteon. Between the osteons are fragments of older osteons which have been reabsorbed during growth and redevelopment.

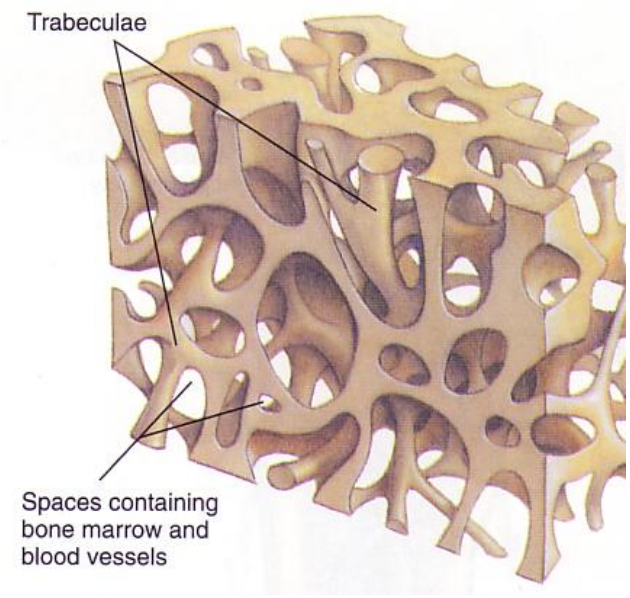

Compact bone
Spongy bone
Osteon
Periosteum
Blood vessels and nerve in central canal
Osteoblasts
Lacuna
Osteocyte
Canalicula


Osteoblasts
Osteoclast
Osteocyte
Lamellae
Canaliculus


Lacunae containing osteocytes
Osteon of compact bone
Lamellae
Canaliculi
Trabeculae of spongy bone
Osteon
Haversian canal
Periosteum
Volkmann's canal

Spongy (trabecular, cancellous) Bone

Spongy bone represents around 20% of the skeletal mass. It is less dense, more elastic and has a higher turnover rate than cortical bone. It is found in the epiphyseal and metaphyseal regions of long bones and throughout the interior of short bones. Spongy bone constitutes most of the bone tissue of the axial skeleton - bones of the skull, ribs and spine. It consists of lamellae arranged in an irregular lattice of thin plates of bone known as trabeculae. There are relatively large spaces between the trabeculae which are filled with blood vessels and red bone marrow, producing erythrocytes. Within the trabeculae are osteocytes that lie within lacunae, with radiating canaliculi. These osteocytes receive their nutrients directly from the surrounding blood vessels, therefore the arrangement of osteons seen in compact bone is not required in spongy bone.



Bone requires a good blood supply as it is highly metabolically active. The highest number of blood vessels are found in the spongy bone. Blood vessels pass into bones from the periosteum. Taking a typical long bone as an example, the artery to the diaphysis is referred to as the nutrient artery via an opening called the nutrient foramen. Before reaching the medullary cavity it sends off branches to supply the Haversian canals as well as branches to

supply the epiphysis. Once it passes to the medullary cavity it splits into a proximal and distal branch, supplying bone marrow, the inner portion of compact bone, metaphysis, and diaphysis. Periosteal arteries, accompanied by nerves, enter the diaphysis in many areas through perforating canals, and supply the outer area of compact bone, and the diaphysis. Veins accompany the arteries, as do a number of nerves, although the nerve supply is not extensive, and primarily the supply is sensory.

Bone formation

This process is known as ossification, which initially begins around the sixth to seventh week of fetal life. There are two patterns to this process : intramembranous, and endochondral.

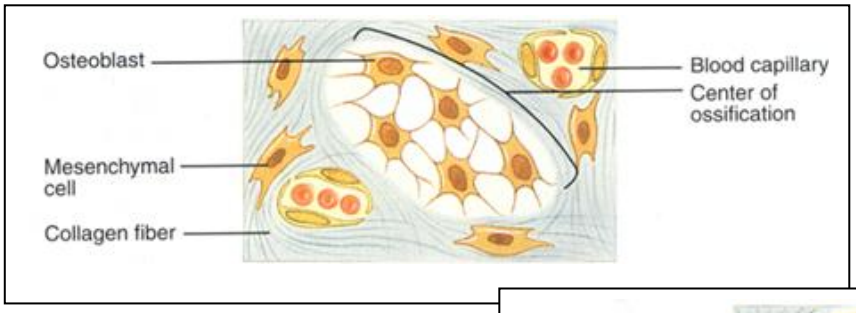
> **Intramembranous ossification** – this is the formation of bone directly on or within loose connective tissues. These bones form directly from the embryonic mesenchyme without first being cartilaginous.
>
> **Endochondral ossification** – the formation of bone within hyaline cartilage. Here, mesenchyme is transformed into chondroblasts which then produce cartilage, and then is eventually replaced by bone.

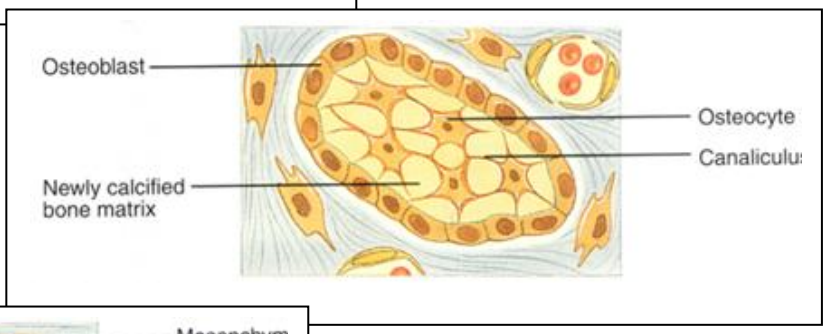
The end product of both of these processes is bone, whose structure is the same no matter by which method it was produced. The first stage in both of these processes is the migration of embryonic mesenchymal cells to areas where bone formation will occur. The mesenchyme cells increase in size and number, and become osteoprogenitor cells. Where capillaries are small in number, these may develop into chondroblasts, but in areas where capillary numbers are high, they develop directly into osteoblasts.

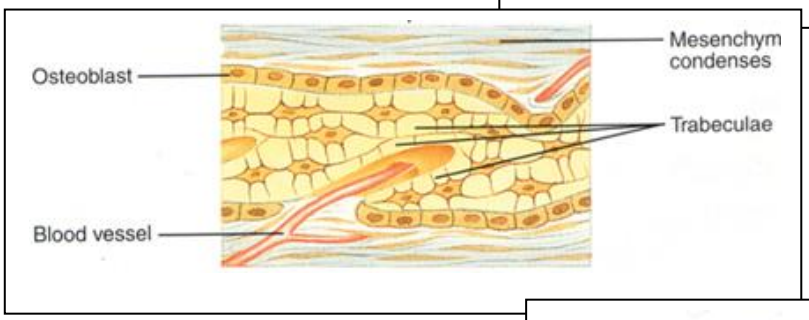
Intramembranous ossification

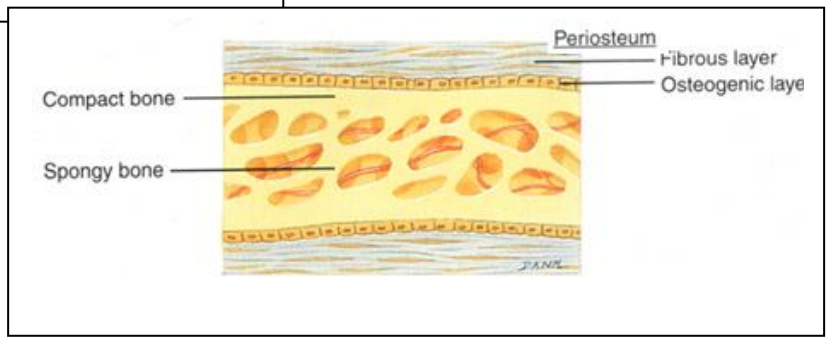
With intramembranous ossification, mesenchymal cells in fibrous connective tissue begin to cluster together and differentiate into osteoprogenitor cells, and then osteoblasts, forming a centre of ossification. Osteoblasts begin to secrete bone matrix (osteoid) until they around completely surrounded by it.

At this point matrix secretion stops, and the cells become mature osteocytes, sitting in their lacunae with communicating canaliculi. Calcium hydroxyapatite crystals are then deposited on collagen fibrils within the matrix, which then hardens. As the matrix develops it forms trabeculae which fuse with each other, creating the classical appearance of spongy bone. The spaces fill with vascularised mesenchyme which then differentiates into red bone marrow. On the outer surface of the bone, the vascularised mesenchyme condenses, and develops into the periosteum. Eventually, most of the spongy bone is replaced by compact bone, which is then remodelled during the growth and development process.







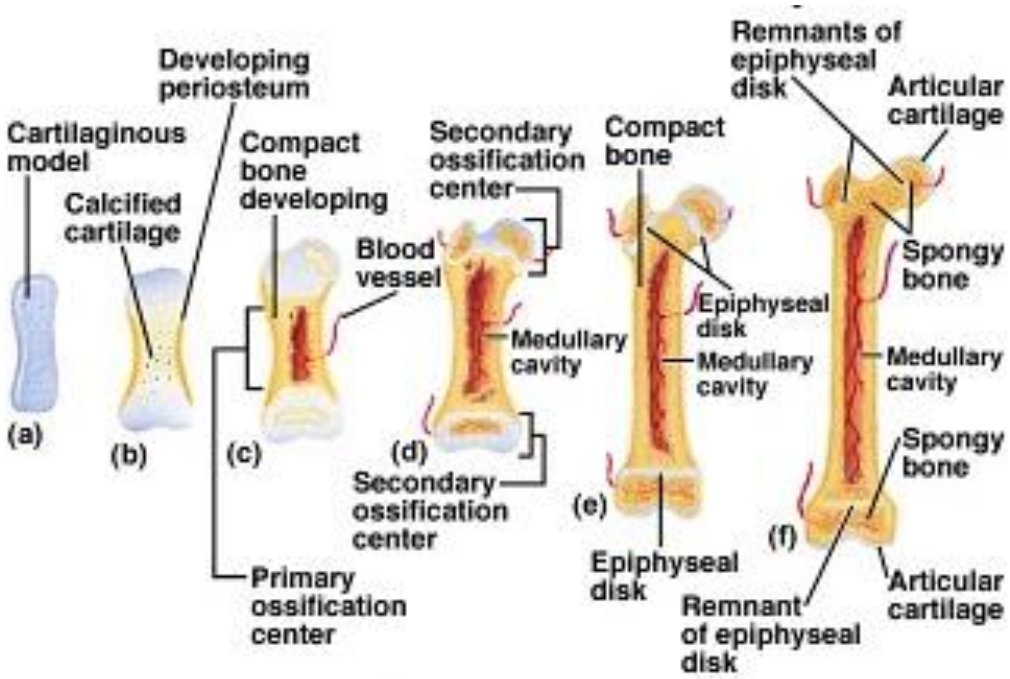


Endochondral ossification

In endochondral ossification, mesenchymal cells in fibrous connective tissue begin to cluster together and differentiate into chondrocytes which produce cartilage matrix. Additionally, the perichondrium (membrane) develops around these cells. The cartilage grows in length as the chondrocytes continue to divide, producing further matrix, resulting in interstitial growth (growth from within). Increase in thickness is due to the increased production of matrix by the chondrocytes. As the area if cartilage continues to grow, cells in its midregion begin to get larger, and eventually lyse (burst), releasing their contents into that matrix. This creates a change in pH, and triggers the calcification process. Once this occurs, other chondrocytes begin to die because nutrients cannot pass through the ossified areas. The spaces where these cells were are left empty, and the partitions between the lacunae break down, forming cavities. Whilst this process is occurring, elsewhere a nutrient artery has penetrated the perichondrium, stimulating osteoprogenitor cells in that area to differentiate into osteoblasts. These cells begin to lay down a layer of compact bone under the perichondrium, known as the periosteal bone collar. Eventually, this area becomes known as the periosteum.

Periosteal capillaries begin to grow into the area of calcified cartilage, forming a periosteal bud, and taking within them osteoblasts, osteoclasts, and red bone marrow cells. The presence of this bud stimulates the growth of a primary ossification centre, where bone tissues replaces most of the cartilage. Osteoblasts then begin to produce more bone matrix over the remains of calcified cartilage, and begin the formation of the spongy bone. As the ossification centre enlarges towards the ends of the bone, osteoclasts break down the newly formed spongy trabeculae, leaving a cavity (medullary cavity), which then fills with red bone marrow. The diaphysis, which was originally cartilage, is replaced by compact bone filled with red bone marrow. Blood vessels enter the epiphyses, where secondary ossification centres develop, usually occurring around the time of birth. In these secondary centres bone formation is similar to that in the primary centres, apart from

one difference. Spongy bone remains in the interior of the epiphyses, with no medullary cavity. Also, cartilage remains between the diaphysis and epiphysis in the form of the epiphyseal plate, which will remain until growth and ossification is complete.



Blood supply

Certain regions of bone containing red bone marrow have a very good blood supply that passes from the periosteum into the interior of the bone. The periosteal arteries are accompanied by nerves and they enter the diaphysis through the perforating Volkmann's canals. They supply the periosteum and the compact bone. Near the centre of the diaphysis there is a large nutrient artery that passes obliquely through the compact bone through a hole - the nutrient foramen. When the nutrient artery reaches the medullary cavity it divides into proximal and distal branches, which supply both the inner layers of compact bone and spongy bone of the diaphysis and the red marrow as far as the epiphyseal growth plates. The number of nutrient foramina varies from bone to bone, the tibia has only one nutrient artery while the femur has

many, the number being related to the size of the bone and its relative amount of red bone marrow. The ends of the bone are supplied by the metaphyseal and epiphyseal arteries. These arteries arise from the arteries that supply the joint. Both the metaphyseal and epiphyseal arteries also enter the bone and supply the red bone marrow in their respective regions. There are usually one or two nutrient veins that accompany the artery in the diaphysis, and there are many epiphyseal and metaphyseal veins which also exit with the respective arteries.

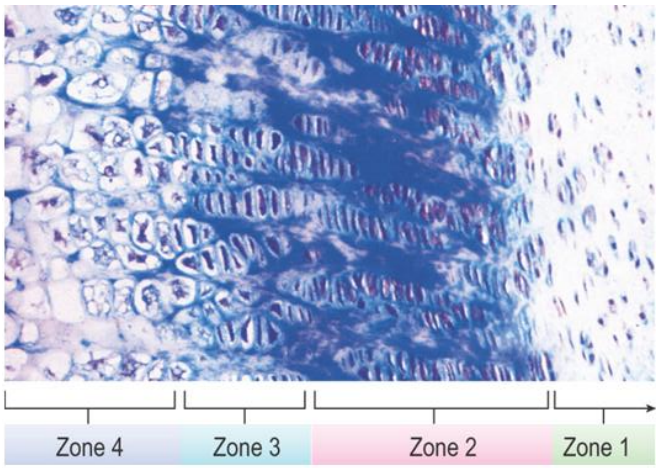
Finally, there are also periosteal veins that drain blood from the periosteum. Nerves accompany the blood vessels of bone. The periosteum has a rich supply of sensory nerves, some of which transmit pain sensations. These nerves are sensitive to tearing or tension and explain the severe pain from a fracture or a bone tumour.

Bone growth

During childhood the bones grow both in thickness and in length until around 25 years of age, although they may still increase in thickness after this time. Bone growth in length, especially the long bones, is by the addition of new bone on the diaphyseal side of the epiphyseal growth plates. The epiphyseal growth plate is composed of hyaline cartilage and separates the epiphyses from the diaphysis of the growing bones. It can be divided into four zones.

- Zone 1 is resting cartilage. It is closest to the epiphysis and is made up of small, scattered chondrocytes that have a low rate of proliferation. These cells do not have a function in the growth of the bone; their role is to anchor the epiphyseal growth plate to the bone of the epiphysis, to provide the supplies for the developing cartilage cells, and to store the necessary materials (lipids, glycogen, proteoglycan aggregates) for growth.

- Zone 2 is proliferating cartilage. The chondrocytes are slightly larger and are stacked like coins. The chondrocytes are dividing and replacing the ones that are dying at the diaphyseal side of the epiphyseal growth plate. These chondrocytes produce the necessary matrix and are responsible for longitudinal growth of the bone via active cell division.
- Zone 3 is hypertrophic cartilage. This zone can be further subdivided into maturation, degeneration, and provisional calcification zones. The chondrocytes increase in size, still in their columns, and they accumulate calcium within their mitochondria; this causes them to deteriorate, and ultimately leads to their cell death. Upon their death, calcium is released from matrix vesicles, impregnating the matrix with calcium salts. The calcification of the matrix is necessary for invasion of metaphyseal blood vessels, destruction of cartilage cells, and the formation of bone along the walls of the calcified cartilage matrix. No active growth occurs in this layer; columns of cells extending toward the metaphysis are at various stages of maturation. This is the weakest portion of the physis and is commonly a site of fracture or alteration.
- Zone 4 is calcified cartilage. This layer is only a few cells thick and is composed of mainly dead or dying chondrocytes, because they have become surrounded by a calcified matrix. The calcified matrix is removed by the action of osteoclasts and invaded by osteoblasts. The osteoblasts lay down new bone matrix and therefore result in the diaphyseal border being firmly attached to the epiphyseal growth plate. It is only by the action of the epiphyseal growth plate that the diaphysis can increase in length. Cartilage is replaced by bone at the diaphyseal end of the growth plate and new chondrocytes are added to the epiphyseal growth plate to maintain its size. Thus the thickness of the epiphyseal growth plate is maintained.



Between the ages of 18 and 25 the epiphyseal growth plates begin to close. The main stimulus for growth by the epiphyseal growth plate is growth hormone (GH, somatotrophin), which is secreted by the pituitary gland and promotes growth during childhood and adolescence. Growth hormone acts on the liver and other tissues to stimulate production of insulin-like growth factor 1 (IGF-1), which is responsible for the growth-promoting effects of growth hormone and also reflects the amount produced. The amount of GH, and so IGF-1, declines with age.

When the levels of GH and IGF-1 begin to decline the chondrocytes in Zone 2 stop dividing and so the thickness of the epiphyseal growth plate gets thinner as bone gradually replaces the cartilage. Eventually only the epiphyseal line remains as a bony feature on the bones, indicating that the bones have stopped growing - the last bone to finish growing is the clavicle. On X-rays of children and young adults, the epiphyseal growth plates are visible as a radiolucent area between the bone of the epiphysis and the diaphysis, as cartilage is radiolucent. If a fracture damages the epiphyseal growth plate while it is still open, then the fractured bone may be shorter than normal. This is because the epiphyseal growth plate is an avascular structure and damage to it accelerates the closure of the plate and so growth of the bone is

reduced. If the rate of bone formation is reduced then the affected bone will be shorter and may cause misalignment of joint surfaces, and in severe cases shorter stature.

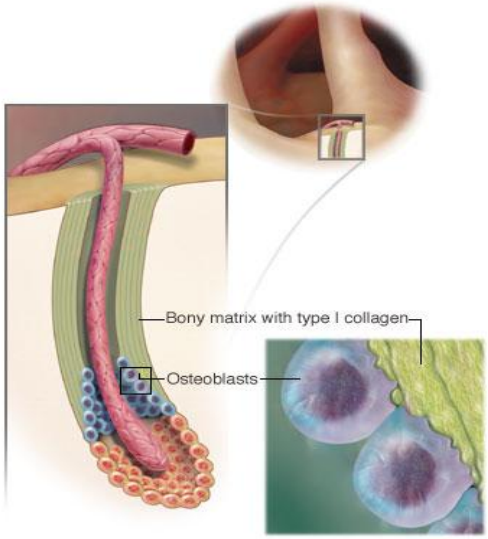
Bone can increase in thickness by appositional growth. The periosteal cells at the bone surface differentiate into osteoblasts and components secrete collagen fibres and other organic forming the bone matrix. The osteoblasts become surrounded by the matrix and develop into osteocytes. This forms bone ridges along the bone on either side of a periosteal blood vessel. As more bone matrix is produced the ridges grow and create a groove for the blood vessel. The ridges eventually fuse together and form a tunnel for the blood vessel. The former periosteum now becomes endosteum that lines the tunnel.

Bone deposition continues from the osteoblasts in the endosteum forming concentric lamellae that proceed towards the centre of the tunnel. Once the tunnel is filled in with bone it is a new osteon. As an osteon is forming, osteoblasts under the periosteum deposit a new circumferential lamella, which further increases the thickness of the bone. This process continues as new periosteal blood vessels become enclosed. As new bone is being added on the outer surface of the bone, the bone lining the medullary cavity is being destroyed by osteoclasts in the endosteum. Therefore, the medullary cavity gets larger as the bone increases in diameter.

Bone remodelling

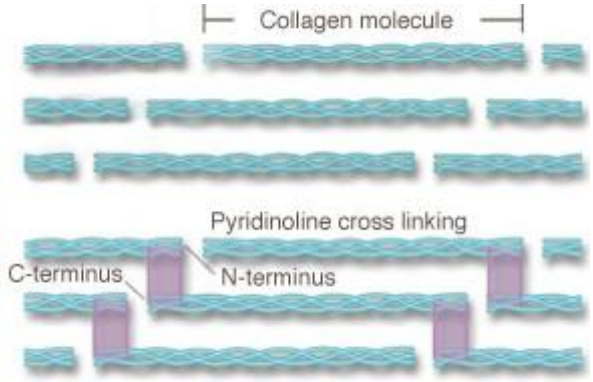
At the cellular level, bone remodelling can be conceptualised as consisting of approximately 1 million bone remodelling units. These remodelling units are approximately 1-2 mm long and 0.2-0.4 mm wide, and are comprised of a population of osteoclastic cells in front and a group of osteoblastic cells in the rear. The remodelling units are also composed of a central vascular capillary, a nerve supply and associated connective tissue.

Osteoclasts adhere to the bone and subsequently remove it by acidification and proteolytic digestion. As the remodelling unit advances, osteoclasts leave the resorption site and osteoblasts move in to cover the excavated area and begin the process of new bone formation by secreting osteoid which is eventually mineralized into new bone. Thus, bone remodelling units combine the sequential action of osteoclasts, which resorb bone leaving a lacuna or cavity, and the subsequent action of osteoblasts which synthesize new bone. The lifespan of an individual bone remodelling unit is 6 to 9 months

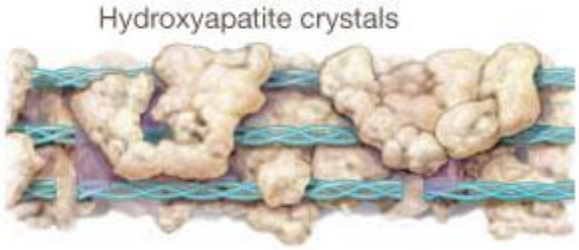


In a coupled process, bone is resorbed through the action of osteoclasts -large multinucleated cells derived from monocyte/macrophages. The most characteristic feature of monocytes is the ruffled border of finger-shaped projections of cell membrane that mediate the resorption of calcified bone matrix. The mineral content of the matrix is first dissolved in the acidic environment of the ruffled border and the remaining protein components of the matrix (primarily collagen) are then degraded by proteolytic enzymes secreted into the resorption space. The result is the dissolution of the bony matrix of the trabeculae to form a cavity or lacuna.

Once osteoclasts withdraw from the resorptive cavity, osteoblasts which are derived from multipotent mesenchymal stem cells synthesize new bone by first laying down a new protein matrix, principally composed of type I collagen. Type I collagen is initially secreted in the form of a precursor (tropocollagen), which contains peptide extensions at both the amino-terminal and carboxyl ends of the molecule. Individual collagen molecules become interconnected by the formation of pyridinoline cross-links which provide extra strength and are unique to bone. Osteoblasts also secrete other proteins that are incorporated into the bone matrix, including osteocalcin and osteonectin.



Two stages of mineralisation then follow which are mediated by osteoblasts, involving the deposition of hydroxyapatite. Osteoblasts are thought to regulate the local concentrations of calcium and phosphate in such a way to promote the formation of hydroxyapatite. First, hydroxyapatite crystals are deposited between the collagen fibrils. Alkaline phosphatase located on the membrane of osteoblasts is thought to play a role in this mineralization. The second stage occurs over the course of several months as additional mineral is added to the resorption cavity.



Bone healing

The process involved in bony healing is basically the same as that of connective tissue. In effect, the tissue defect created by a fracture is made good by well-vascularised connective tissue, such as happens in the healing of open wounds. However, after this stage is complete, different phases begin because bone, unlike soft tissue, requires mechanical and weight-bearing efficiency. These needs are met through the work of osteoblasts and osteoclasts.

There are three major phases of fracture healing, two of which can be further sub-divided to make a total of five phases;

1. Reactive Phase

    i. Fracture and inflammatory phase

    ii. Granulation tissue formation

2. Reparative Phase

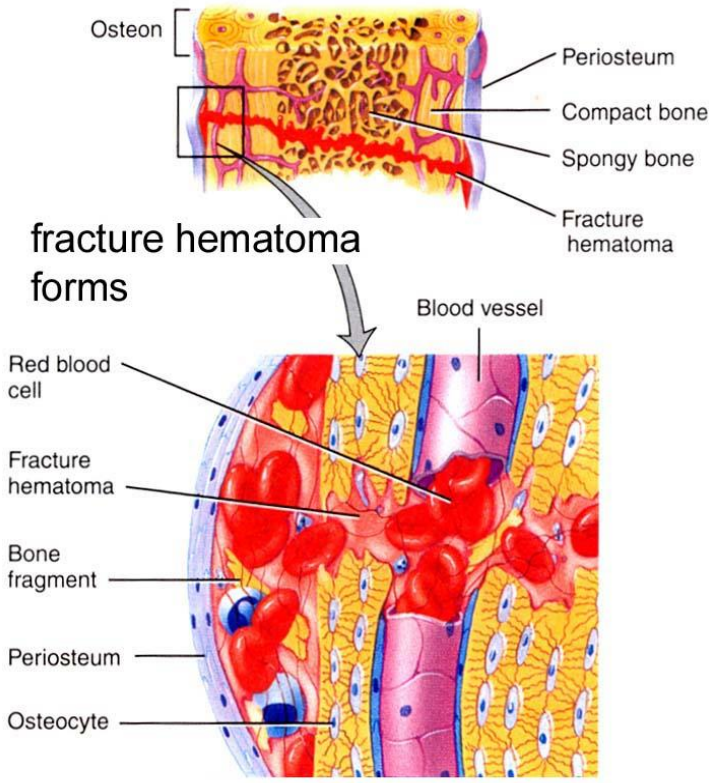
    iii. Callus formation

    iv. Lamellar bone deposition

3. Remodeling Phase

    v. Remodeling to original bone contour

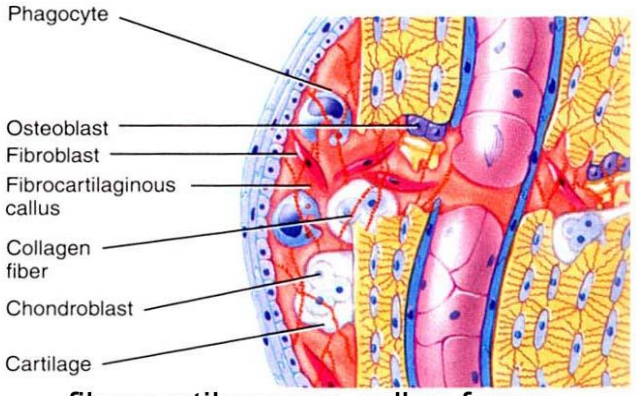
Reactive phase –



When a fracture occurs, blood vessels are damaged and blood leaks into the defect between the fractured ends on bone, and a clot forms (fracture haematoma). Other plasma-derived proteins also collect in the area. As with other tissues, the injury begins an acute inflammatory reaction, although there is less neutrophil infiltration. The combination of the haemorrhage and inflammatory odema causes loosening of the periosteum from the underlying bone ends, resulting in a fusiform swelling at the fracture site. Some degree of bone necrosis will occur, usually within 24-48 hours, the marrow being the site of the first changes.

Macrophages now invade the fracture site and begin to demolish tissues. About 4 days after injury the mass of blood clot is replaced by granulation tissue, which extends upwards and downwards within the marrow cavity away from the fracture site. Within the granulation tissue small groups of cartilage cells begin to differentiate from connective tissue stem cells.

Reparative phase –



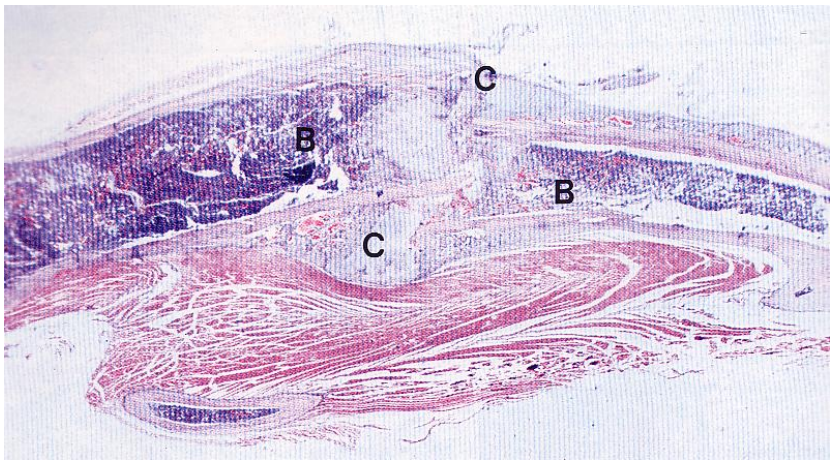
fibrocartilageous callus forms

Provisional callus is the term used to describe a cuff of woven bone admixed with islands of cartilage. This unites the severed portions of bone on the outside but not across the gap between the bone ends.

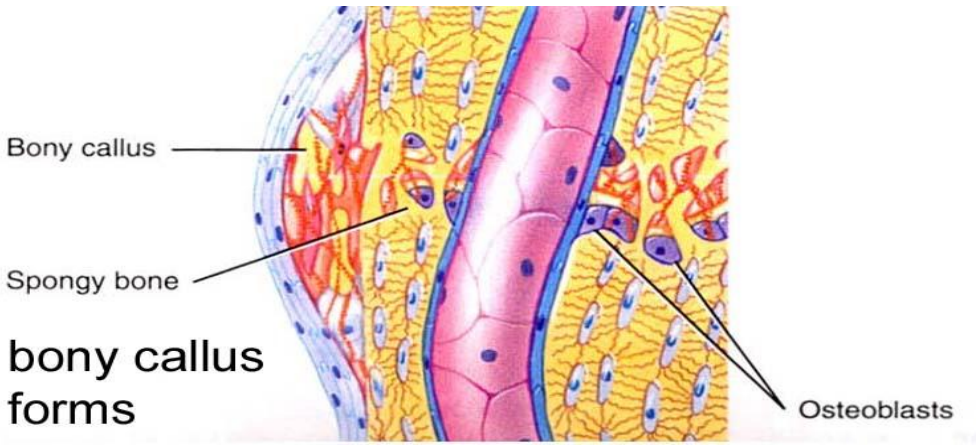
The callus originates from two sources :

a. *periosteum* – the cells on it's inner aspect proliferate and begin to lay down woven bone. When the perisoteum has been raised from the external surface of the bone the new woven bone fills the gap so that there are two cuffs of new bone around the periosteal aspect of the two fragments. These cuffs extend upwards and downwards until they meet, although there is no direct union across the gap between the separated bone ends.
b. *Medullary cavity* – following the formation of granulation tissue, fibroblasts and osteoblasts start to proliferate and lay down bone matrix. Some if this is deposited on trabecula of dead bone, while the remainder forms new trabeculae.

The provisional callus extends around the separated ends of the fractured bone (C), but does not bridge the gap between the separated portions of bone (B)



After the provisional callus has been formed the clot that fills the gap between the fragments is invaded, first by granulation tissue capillaries and then by osteoblasts. Ossification within this gap may occur as a primary event, the osteoblasts being derived from provisional callus.



In some cases the bone ends are united by fibrous tissue and over a long period of time this is replaced by woven bone. Occasionally the fibrous tissue is not replaced by bone (non-union) and weight-bearing in the affected limb is impossible. In some cases of delayed or non-union an improvement may be brought about by electrical stimulation, which can accelerate ossification at fracture sites.

Remodelling phase -

Once union has occurred and the patient is bearing weight, the lumpy new cortical bone gradually becomes resorbed and smoothed out, and excess medullary new bone is removed with the restoration of a normal medullary cavity. Woven bone is resorbed completely and is replaced by lamellar bone. This is a lengthy process and may take over a year.

In summary, bone is a dynamic tissue, constantly being remodelled in response to external stress, trauma, and metabolic status. It begins to develop in fetal life, and continues this process throughout childhood, until ossification is complete at around 20 years of age. Skeletal bone mass then remains relatively constant until later in life, when bone mass begins to decrease in both males and females (although frequently more rapidly in females), usually in response to hormonal changes, the ageing process, and co-morbidities. Future articles will explore the regeneration and healing process in bone, as well as the role of bone in calcium homeostasis.

KEY LEARNING POINTS.

1. Bone consists of a matrix, which contains different types of cells as well as water, collagen fibres, and mineral salts.
2. The four types of cells in the matrix are osteoprogenitor cells, osteoblasts, osteocytes, and osteoclasts.
3. The overall architecture of bone is divided into cancellous bone (also referred to as trabecular bone or spongy bone) and cortical bone (also referred to as compact bone).
4. Intramembranous ossification is the formation of bone directly on or within loose connective tissues. These bones form directly from the embryonic mesenchyme without first being cartilaginous.
5. Endochondral ossification is the formation of bone within hyaline cartilage. Here, mesenchyme is transformed into chondroblasts which then produce cartilage, which is eventually replaced by bone.
6. During childhood the bones grow both in thickness and in length until around 25 years of age, although they may still increase in thickness after this time. Between the ages of 18 and 25 the epiphyseal growth plates begin to close.
7. Bone remodelling units are comprised of a population of osteoclastic cells in front and a group of osteoblastic cells in the rear. The remodelling units are also composed of a central vascular capillary, a nerve supply and associated connective tissue.
8. Bony healing consists of a reactive, reparative, and remodelling phase.