Tissue Repair

Lecture 9,10 Healing by Regeneration and Fibrosis

Tissue repair

It is restoration of tissue architecture and function after an injury, involving the proliferation of various cells, and close interactions between cells and the extra-cellular matrix (ECM).

Types of tissue repair: The type of repair is determined by the tissue capacity for proliferation, and the severity of damage to the supporting structures of the tissue. Accordingly we have two types of repair:

1- **Regeneration**: Repair by replacement of the damaged components by the same original tissue so returning to a normal state.
2- **Fibrosis**: Repair by extensive deposition of collagen fibres, ending with scar formation.

THE CELL CYCLE

The cell cycle: represents the sequence of events that control DNA replication & mitosis in the proliferation of cells.

It consists of a series of steps at which the cell checks for the accuracy of the process and instructs itself to proceed to the next step. The cycle consists of the

1-presynthetic growth phase 1 (G1)
2-the DNA synthesis phase (S)
3-the premitotic growth phase 2 (G2)
4-and the mitotic phase (M) Non-dividing cells are either in cell cycle arrest in G1 or they exit the cycle to enter a phase called G0.

Any stimulus that initiates cell proliferation, such as exposure to growth factors, needs to promote the G0/G1 transition and the entry of cells into the G1.

Further progression is determined by the ability of the cell to pass through an intrinsic quality control mechanism for cell integrity, known as **checkpoint control**.

Checkpoint controls: prevent DNA replication or mitosis of damaged cells and either transiently stop the cell cycle to allow for DNA repair or eliminate irreversibly damaged cells by apoptosis. Progression through the cell cycle from G1 is regulated by proteins called cyclins, which form complexes with enzymes called cyclin-dependent kinases (CDKs).
These complexes regulate the phosphorylation of proteins involved in cell cycle progression leading to DNA replication and mitosis, and thus are required for cell cycle progression.

Proliferative Capacities of Tissues:
The ability of tissues to repair themselves is influenced by their intrinsic proliferative capacity. According to this capacity, tissues are divided into three groups:

1-**Labile tissues (Continuously Dividing Tissue** Cells of these tissues are continuously being lost and replaced by proliferation and maturation of stem cells. These tissues can readily regenerate after injury as long as the pool of stem cells is preserved. **Stem cells**: are the original embryonic cells that have the inherent property of proliferation. Stem cells are characterized by:

1- Prolonged self-renewal capacity.
2- Asymmetric replication i.e. after each cell division, one cell will be differentiated while other cell remain undifferentiated, retaining their self-renewal capacity.
3- They have very broad differentiation capabilities, being able to generate any cell like fat, cartilage, bone, endothelium, and muscle.

Examples: in the multi-layered epithelium of the skin and the gastrointestinal tract, in which stem cell positions have been identified near the basal layer of the epithelium. Cells differentiate progressively as they migrate to the upper layers of the epithelium; they die and are shed from the surface of the tissue.

Tissues contain labile cells:

1- **Hematopoietic cells in the bone marrow.**
2- **The majority of surface epithelia** (the stratified squamous Surfaces of the skin, oral cavity, vagina, and cervix).
3- **The cuboidal epithelia of the ducts draining exocrine organs**

(e.g., salivary glands, pancreas, and biliary tract).

4- **The columnar epithelium of the gastrointestinal tract, uterus, and fallopian tubes and the transitional epithelium of the urinary tract.**

Homeostasis:

Homeostasis means keeping a constant number of cells and tissue size to get normal shape and function. The normal size of cell populations is determined by a balance between cell proliferation, cell death by apoptosis, and emergence of new differentiated cells from stem cells.

In these tissues the mature cells are terminally differentiated and short-lived. As mature cells die the tissue is replaced by the differentiation of cells generated from stem cells. Thus, in these tissues there is a homeostatic equilibrium between the replication and differentiation of stem cells and the death of the mature, fully differentiated cells. The normal size of cell populations in any given tissue is determined by a balance of **1-**cell proliferation **2-**cell death by apoptosis **3-**The emergence of new differentiated cells from stem cells

2-**Stable Tissues: (Quiescent cells)**

Cells of these tissues are quiescent and have only minimal proliferative activity in their normal state. However, these cells are capable of proliferating in response to injury or loss of tissue mass. Stable cells constitute:-

1. **The parenchyma of most solid tissues (liver, kidney, and pancreas).**
2. **The endothelial cells.**
3. **The fibroblasts.**
4. **The smooth muscle cells.**

With the exception of liver, stable tissues have a limited capacity to regenerate after injury.

3-**Permanent Tissues: (Non dividing cells)** The cells of these tissues are considered to be terminally differentiated and non-proliferative in postnatal life ex. **neurons** and **cardiac muscle** cells. Thus, injury to the brain or the heart is irreversible and results in a scar formation.

Skeletal muscle is usually classified as a permanent tissue, **but** satellite cells attached to the endomysial sheath provide some regenerative capacity for this tissue

Cells involved in repair:-

1- **The remnants of the injured tissue**
2- **Vascular endothelial cells** to create new vessels (nutrition).
3- **Fibroblasts** which are the source of the fibrous tissue that forms the scar

Control of cell proliferation

Cell proliferation can be triggered by:

1-**Chemical mediators;** such as **growth factors**, hormones, and cytokines. These are involved in the stimulation or inhibition of cell growth. **2-Signals from the extracellular matrix (ECM)**

Growth factors:

Growth factor is a protein that expands cell populations by stimulating cell division usually accompanied by increased cell size and by promoting cell survival. Most growth factors, in addition to stimulating cellular proliferation, they stimulate migration, differentiation, and enhance the synthesis of specialized proteins (such as collagen in fibroblasts).

Growth factors that are involved in repair are produced by:

1-Leukocytes that are recruited to the site of injury or are activated at this site, as part of the inflammatory process.

2- Parenchymal cells or connective tissue cells in response to cell injury or loss.

Mechanism of action of growth factors:

1- They stimulate the function of growth control genes, many of which are called **proto-oncogenes** because mutations in these genes lead to uncontrolled cell proliferation that is the characteristic of cancer (oncogenesis).
2- They stimulate proliferation of some cells and inhibit proliferation of others.
3- They can have opposite effects on the same cell depending on its concentration. An example of such a growth factor is transforming Growth factor-β (TGF-β)

Growth factors act as extracellular signals that will bind receptors located on the cell surface or intracellularly. The activation of the receptor will

triggers a series of events leading to stimulation or repression of gene expression and cell division.

Extracellular matrix (ECM) and cell-matrix interactions:

Tissue repair depends not only on growth factor activity but also on interactions between the cells and ECM components. The ECM is a dynamic, constantly remodeling macromolecular complex synthesized locally, which assembles into a network that surrounds the cells. ECM occurs in two basic forms which are interstitial matrix and basement membrane.

Interstitial Matrix

This is present in the spaces between cells in connective tissue, and between epithelium and supportive vascular and smooth muscle structures; it is synthesized by mesenchymal cells (e.g., fibroblasts). Its major constituents are collagens, as well as fibronectin, elastin, proteoglycans, hyaluronate, and other elements.

Basement Membrane

The basement membrane lies beneath the epithelium and is synthesized by overlying epithelium and underlying mesenchymal cells; it tends to form a platelike "chicken wire" mesh. Its major constituents are amorphous type IV collagen and laminin.

Roles of the Extracellular Matrix

The ECM is much more than space filler around the cells. It has various functions include:

1- Mechanical support for cell anchorage and cell migration.
2- Control of cell growth. ECM components can regulate cell proliferation by signaling through cellular receptors.
3- Maintenance of cell differentiation. The type of ECM proteins can affect the degree of differentiation of the cells in the tissue.
4- Framework for tissue renewal. The maintenance of normal tissue structure requires a basement membrane or stromal frame. Damage of these structures leads to collagen deposition and scar formation.
5- Establishment of tissue microenvironments. Basement membrane acts as a boundary between epithelium and underlying connective tissue
6- Storage and presentation of growth factors like FGF. This allows the rapid deployment of growth factors after local injury, or during regeneration.

Components of the Extracellular Matrix

There are **three basic** components of ECM:

1. Fibrous structural proteins (collagens and elastins).
2. Water-hydrated gels (proteoglycans and hyaluronan).
3. Adhesive glycoproteins (fibronectin), and adhesive receptors (selectins, integrins and cadherins

Wound healing: Healing process in general involve:

(1) Inflammation

(2) Formation of granulation tissue

(3) ECM deposition and remodeling

Healing of any wound can occur by one of two type of repair:

1-Healing by First Intention (primary union).

2-Healing by Second Intention (secondary union).

1-Healing by First Intention (primary union):

It is the healing of a clean, uninfected small wound or surgical incision approximated by surgical sutures. The incision causes only **focal disruption** of epithelial basement membrane continuity and death of a relatively **few** epithelial and connective tissue cells. As a result, epithelial **regeneration** predominates over fibrosis. A very small scar is formed, but there is minimal wound contraction.

Steps of primary union:

1- The narrow incisional space first fills with fibrin-clotted blood, which is rapidly invaded by granulation tissue and covered by new epithelium.

2- Within 24 hours, neutrophils are seen at the incision margin, migrating toward the fibrin clot.

3- Basal cells at the cut edge of the epidermis show increased mitotic activity.

4- Within 24 to 48 hours, epithelial cells from both edges have begun to migrate and proliferate along the dermis, depositing basement membrane components as they progress. The cells meet in the midline beneath the surface cut, yielding a thin but continuous epithelial layer.

5- By 3rd day, neutrophils have been largely replaced by macrophages, and granulation tissue progressively invades the incision space. Collagen fibers are now evident at the incision margins, but these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, yielding a thickened epidermal covering layer.

6- By 5th day, neovascularization reaches its peak as granulation tissue fills the incisional space. Collagen fibrils become more abundant and begin to bridge the incision. The epidermis recovers its normal thickness as differentiation of surface cells yields a mature epidermal architecture with surface keratinization.

7- During the second week, there is continued collagen accumulation and fibroblast proliferation. The leukocytes infiltrate, edema, and increased

vascularity are substantially diminished. The long process of "blanching" begins, accomplished by increasing collagen deposition within the incisional scar and the regression of vascular channels.

8- By the end of the first month, the scar comprises a cellular connective tissue largely devoid of inflammatory cells and covered by an essentially normal epidermis. However, the dermal appendages destroyed in the line of the incision are permanently lost.

1. Healing by Second Intention (secondary union):

When tissue loss is more extensive, such as in large wounds, abscess formation, and ulceration, the repair process is more complex. In second-intention healing, the inflammatory reaction is more intense, there is abundant development of granulation tissue, and the wound contracts by the action of myofibroblasts. This is followed by accumulation of ECM and formation of a large scar.

Repair begins within 24 hours of injury by the emigration of fibroblasts and the induction of fibroblast and endothelial cell proliferation.

By 3 - 5 days, a specialized type of tissue that is characteristic of healing, called **granulation tissue** is apparent.

The term granulation tissue derives from the pink, soft, granular gross appearance, such as that seen beneath skin wound. Its **histologic appearance** is characterized by

1- proliferation of fibroblasts .
2- new thin-walled, delicate capillaries (angiogenesis).
3- with a loose ECM.

Granulation tissue then progressively accumulates connective tissue matrix, eventually resulting in the formation of a scar which may remodel over time.

Steps of repair by connective tissue deposition:

1- Formation of new blood vessels (angiogenesis).

2- Migration and proliferation of fibroblasts and deposition of ECM (scar formation). 3-Maturation and reorganization of the fibrous tissue (remodeling). 4-Wound contraction

1. Formation of new blood vessels (Angiogenesis):

Angiogenesis, or neovascularization,is a process of formation of new blood vessels in which preexisting vessels send out capillary sprouts to produce new vessels. Endothelial precursor cells may migrate from the bone marrow to areas of injury and participate in angiogenesis at these sites. Several growth factors induce angiogenesis, but the most important are vascular endothelial growth factor VEGF and fibroblast growth factor (FGF).

2. Migration of Fibroblasts and ECM Deposition (Scar Formation)

Scar formation occurs in two steps:

(1)-Migration and proliferation of fibroblasts into the site of injury.

** The granulation tissue is started by formation of a framework of **new vessels and loose ECM** that develop early at the repair site. ** Then the recruitment and stimulation of **fibroblasts i**s driven by many growth factors, including PDGF, FGF-2 and TGF-β. (Derived from activated endothelium and inflammatory cells). ** **Macrophages**, in particular, are important cellular constituents of granulation tissue, and besides clearing extracellular debris and fibrin at the site of injury, they elaborate a host of mediators that induce fibroblast proliferation and ECM production. ** Sites of inflammation are also rich in **mast cells**, and **lymphocytes**. These can contribute directly or indirectly to fibroblast proliferation and activation.

(2)-Deposition of ECM by fibroblasts:

As healing progresses, the number of proliferating fibroblasts and new vessels decreases; however, the fibroblasts progressively assume a more synthetic phenotype, and hence there is increased deposition of ECM. Collagen synthesis by fibroblasts begins early in wound healing (days 3 to 5) and continues for several weeks, depending on the size of the wound.

Many of the same growth factors that regulate fibroblast proliferation also participate in stimulating ECM synthesis and scar formation including **TGF-β, PDGF, and FGF**. These are potent fibrogenic agent stimulating the production of collagen, fibronectin and proteoglycans, causes migration and proliferation of fibroblasts, smooth muscle cells, and macrophages.

Cytokines as mediators of inflammation and may also function as growth factors and participate in ECM deposition and scar formation. **IL-1 and TNF**, for example, induce fibroblast proliferation and can have a fibrogenic effect. They are also chemotactic for fibroblasts and stimulate the synthesis of collagen by these cells.

The granulation tissue formed will evolves into a scar composed of largely **inactive,** spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components. Matured scar has vascular regression, which eventually transforms the highly vascularized granulation tissue into a **pale,** largely **avascular scar.**

3-Maturation and reorganization of the fibrous tissue (remodeling)

The transition from granulation tissue to scar involves shifts in the composition of the ECM; even after its synthesis and deposition, scar ECM continues to be modified and remodeled. The outcome of the repair process is, in part, a balance between ECM synthesis and degradation.

The degradation of collagens and other ECM components is accomplished by a family of matrix metalloproteinases (MMPs). MMPs are produced by a variety of cell types (fibroblasts, macrophages, neutrophils, synovial cells, and some epithelial cells), and their synthesis and secretion are regulated by growth factors, cytokines, and other agents.

4-Wound contraction:

Secondary healing involves wound contraction. Within 6 weeks, for example, large skin defects may be reduced to 5% to 10% of their original size, largely by contraction. This occurs due to the presence of **myofibroblasts,** which are modified fibroblasts exhibiting many of the ultrastructural and functional features of contractile smooth muscle cells.

Differences between secondary healing and primary healing:

1- A larger clot forms at the surface of the wound.
2- Inflammation is more intense because large tissue defects have a greater volume of necrotic debris, exudate, and fibrin that must be removed.
3- Large defects have a greater potential for secondary infections.
4- Much larger amounts of granulation tissue are formed to fill the gaps and provide the underlying framework for the regrowth of epithelium.
5- A greater volume of granulation tissue results in a greater mass of scar tissue.

Types of repair according to the type of the tissue:

1- Repair by regeneration:

Cell renewal occurs continuously in **labile tissues,** such as the bone marrow, gut epithelium, and the skin. Damage to epithelia or an increased loss of blood cells can be corrected by the proliferation and differentiation of stem cells and, in the bone marrow, by proliferation of more differentiated progenitors.

The renewal of hematopoietic cells is driven by growth factors called colony-stimulative factors (CSFs), which are produced in response to increased consumption or loss of blood cells.

Tissue regeneration can occur in parenchymal organs with **stable cell** populations, but with the exception of the liver, this is usually a limited process. Pancreas, adrenal, thyroid, and lung tissues have some regenerative capacity. Extensive regeneration or compensatory hyperplasia can occur only if the residual tissue is structurally and functionally intact, as after partial surgical resection. By contrast, if the tissue is damaged by infection or inflammation, regeneration is incomplete and is accompanied by scarring.

2- **Repair by connective tissue:**

Repair occurs by replacement of the nonregenerated cells with connective tissue, or by a combination of regeneration of some cells and scar formation:

1- If tissue injury is severe or chronic, and results in damage to parenchymal cells and epithelia as well as the stromal framework. 2 -If **nondividing cells** are injured, repair cannot be accomplished by regeneration alone.

Aberrations of cell growth and ECM production

This may occur even in what begins as normal wound healing.

1**. Keloid**: refers to the accumulation of exuberant amounts of collagen that give rise to prominent, raised scars. There appears to be a heritable predisposition to keloid formation, and the condition is more common in blacks.

2. **Exuberant granulation**: healing wounds may also generate excessive granulation tissue that protrudes above the level of the surrounding skin and hinders re-epithelialization. The restoration of epithelial continuity requires cautery or surgical resection of the granulation tissue.

3. **Disabling fibrosis** associated with chronic inflammatory diseases such as rheumatoid arthritis, pulmonary fibrosis, and cirrhosis have many similarities to those involved in normal wound healing. In these diseases, however, persistent stimulation of fibrogenesis results from chronic immune reactions that sustain the synthesis and secretion of growth factors, fibrogenic cytokines, and proteases.

Delay healing: Factors causes

delay healing:

1- **Infection** is the single most important cause of delay in healing; it prolongs the Inflammatory phase of the process and increases the local tissue injury.

2- **Nutritional deficiency** like vitamin C deficiency, inhibits collagen synthesis and retards healing.

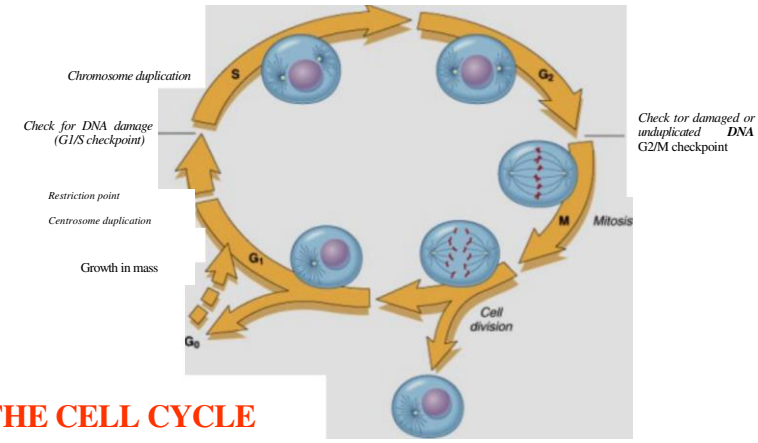
3- **Glucocorticoids** (steroids) have well-documented anti-inflammatory effects, and their administration may result in poor wound strength due to

diminished fibrosis.

4- **Mechanical injuries** such as increased local pressure or torsion may cause wounds to pull apart, or opended..

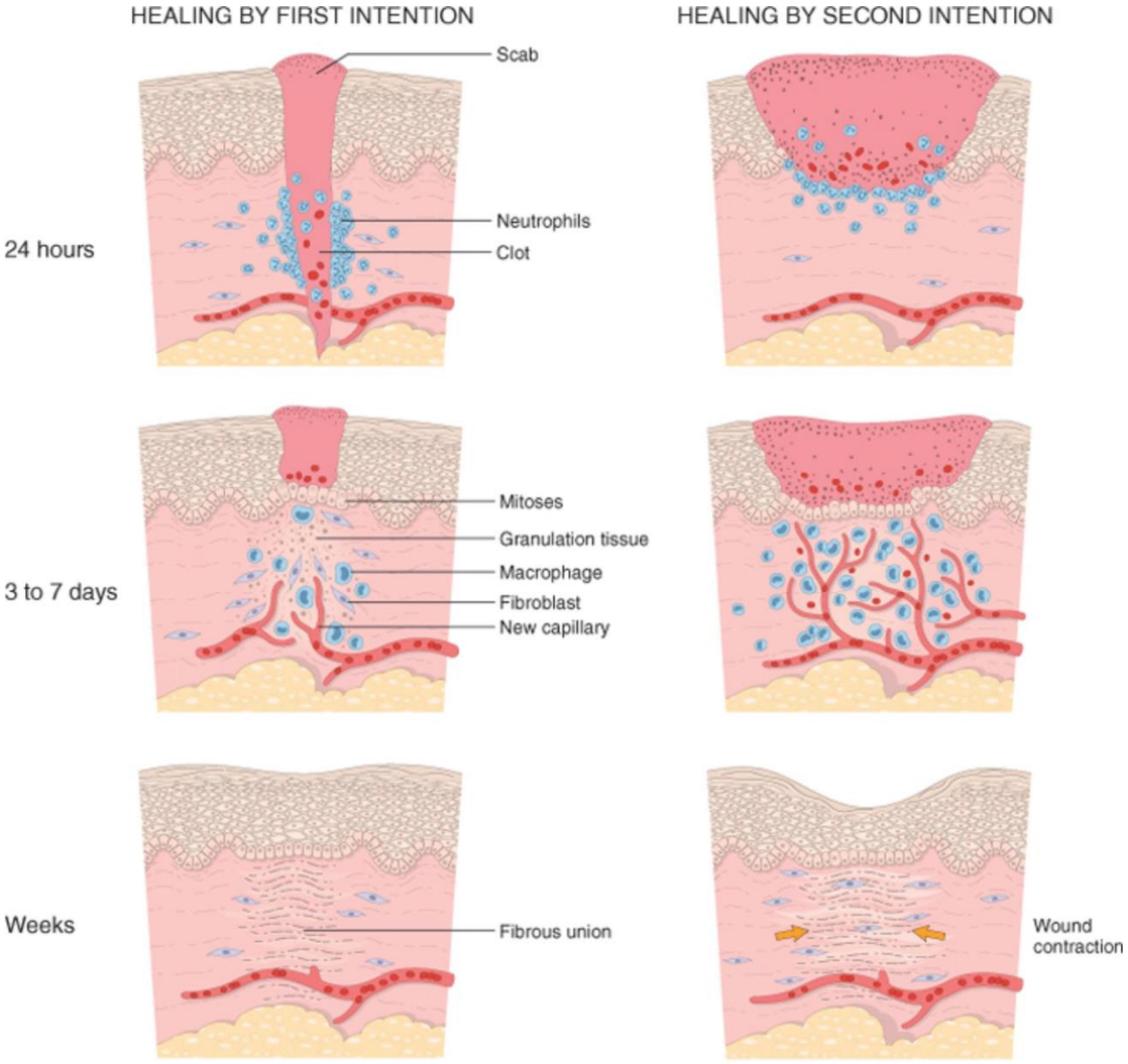
5- **Diminished blood supply**, due either to arteriosclerosis and diabetes or to obstructed venous drainage (e.g. in varicose veins).

6- **Foreign bodies** such as fragments of steel, glass, or fractured bone.



THE CELL CYCLE

G0: quiescent stage , G1: growth presynthetic, S: DNA synthesis
G2: growth premitotic, M: mitotic
The G1 and S stages generally constitute the majority of the time of the cell cycle; the mitotic (M) phase is typically brief.
Note the G1 restriction point, and the G1/S and G2/M checkpoints.



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HEALING OF BONE FRACTURE

Bone fracture is caused by physical trauma, leading to discontinuity of the bone. The separation of fractured ends may be **complete** or **incomplete**. The latter is common in young children and called **greenstick fracture**. The fracture may be a **closed** one i.e. with an intact overlying skin or **open** i.e. the overlying skin is also injured so that the fractured bone is exposed through a gaping wound. A **comminuted** fracture is the one in which the bone is divided into multiple fragments.

Fracture healing

Due to tearing of blood vessels in the medullary cavity, cortex and periosteum, **a hematoma** forms at the site of fracture. The periosteum is stripped off form the bone surfaces. The bone with haemopoietic marrow around the fracture site undergoes **ischemic necrosis**. Bone death is recognized histologically by loss of osteocytes from lacunae (empty lacunae).

Organization of the hematoma is associated with a local inflammatory response, with hyperemia, exudation of protein rich fluid, & migration of neutrophils & macrophages. These cells phagocytose the hematoma & necrotic debris. This is followed by in-growth of capillaries & fibroblasts, producing **fibrovascular granulation tissue.**

At the end of the 1st week, osteoblasts derived from osteoprogenitor cells of the inner layer of the periosteum will migrate into the granulation tissue and proceed to deposit larger quantities of osteoid in a haphazard way, producing a **woven bone** pattern. **External callus** is thus formed by the periosteum and tends to immobilize the bone fracture site. The two enlarging cuffs of callus advance towards each other until finally unite to bridge the fracture gap outside externally.

If there is a significant gap between the bone ends, it may induce cartilage formation.

The **internal callus derived from endosteal osteoprogenitor cells** bridges the fracture from within the medullary cavity, and rarely contains cartilage due to better vascularization.

The cartilaginous component of callus is converted to bone by endochondral ossification Callus is usually formed by the 3rd week after the incident of fracture, but the **initial bony union is by woven bone, which is mechanically weak.**

The amount of periosteal callus formed (external callus) depends on the site of fracture & the degree of immobilization. It tends to be abundant in poorly immobilized fracture e.g. clavicle & ribs. **Remodeling** of callus occurs once the defect between the two bone ends is bridged by bony callus, so the newly laid down bone is reconstructed to restore full mechanical strength. The newly formed woven bone is resorbed and gradually replaced by **lamellar bone** (compact).

The cortex is re-formed across the fracture gap & gradually the medullary callus is removed with restoration of the marrow cavity. Remodeling is done by the osteoblasts & osteoclastes. The whole reparative process may take about a year, although the time varies from site to site. It is also more rapid and more complete in children.

Factors Affecting Fracture Healing

These are basically similar to those of affecting healing in general. However, mobility of fracture ends and mal-alignment play a detrimental role in interfering fracture healing. Vitamin D deficiency leads to abundant callus, which fails to calcify & remains soft.

Complications of fractures

1. **Delayed union,** after fibrous union, bony conversion is slow.
2. **Non-union,** in which the fractured bone ends do not join by bone. This occurs if the fibrous tissue becomes very dense. The latter is then is converted to fibrocartilage.
3. **Fat embolism,** which may follow damage to the bone marrow. In such cases globules of fat embolize to such sites as the lungs, brain, and kidneys with the ultimate result of ischemic necrosis (infarction).
4. **Osteonecrosis;** this refers to local bone necrosis after fracture. It may occur depending on local peculiarities of the blood supply, e.g. fracture of femoral neck is often followed by osteonecrosis of the femoral head.
5. **Osteoarthritis (osteoarthosis)**; this degenerative joint disease may occur when the fracture line has involved the articular surface that result in the production of an incontinuity of the articular cartilage.

Pathological Fracture

For fracture of normal bone to occur, the causative trauma has to be severe enough.In contrast, trivial trauma may cause fracture when the underlying bone is abnormal e.g**. presence of osteoporosis** (reduced bone mass) that occurs in the elderly may be associated with pathological fractures particularly in the femur & vertebral column. **Osteomalacia** (vitamin D deficiency lead to inadequate bone mineralization→ soft weak bone**), Primary or metastatic tumors** (from carcinoma of breast bronchus, thyroid & kidney) may be associated with pathological fractures.