REPAIR

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- The term “repair” refers to all processes directed at replacing the damaged cells and tissues by new healthy ones.
- “Repair” refers to the restoration of tissue architecture and function after an injury.
- It occurs by two reactions: “regeneration” and “healing” by fibrosis.

- The term “regeneration” is reserved for replacement of damaged cells with identical new ones.
- The term “healing” refers to replacement of the damaged cells with fibrous connective tissue (CT).

1. Regeneration

- It is defined as replacement of the diseased or damaged tissue by proliferation of **parenchymal** cells.
- It results in complete restoration of the original tissues.

2. Repair by fibrosis

- Repair by laying down of connective tissue **(fibrosis).**
- It occurs if the injured tissue are incapable of proliferation to complete restoration, or if supporting structures of the tissue are severely damaged.

- If fibrosis develops in a tissue space occupied by an inflammatory exudate it is called **organization,** as in organizing pneumonia, and in serofibrinous inflammation of the pleura.

The cell cycle

- The cells of the body divided into 3 groups on the basis of their proliferation capacity and their relationship to the cell cycle.
- Some mature cells do not divide at all, whereas others complete a cycle every 16 to 24 hours.

- **Cell cycle is divided into 4 unequal phases:**
- **G1 (gap1) phase:** stages of synthesis of messenger RNA and proteins; called pre-synthetic phase.
- **S (synthesis phase):** stage of synthesis and replication of DNA.
- **G2 (gap2) phase:** stage of correction of synthesized DNA, also called pre-mitotic phase.

- **M (mitosis phase):** stage of formation of daughter cells: It occurs in 4 stages, namely; prophase, metaphase, anaphase and telophase.
- **G0 (gap 0) phase:** is a resting phase.

- **Mitosis** is controlled by genes which encode the release of specific proteins that promote or inhibit mitosis at different steps.
- Mitosis promoting proteins are **cyclins.**
- These cyclins activate cycline dependant kinases **(CDKs)** which act in conjunction with cyclins.
- After the mitosis is competed, cyclin and CDKs are degraded.
- Period between two successive mitosis is called **interphase.**

Classification of cells according to the proliferative potential

- **Labile cells:** these cells continue to multiply throughout life and in a constant state of renewal. They include:
- Surface epithelial cells of the epidermis, cornea, alimentary tract, respiratory tract, urinary tract, and reproductive tract.
- The epithelium of the ducts draining exocrine organs (e.g., salivary glands, pancreas, and biliary tract).

- Hematopoietic cells of the bone marrow, lymph node and spleen.
- Under appropriate conditions, tissues composed of labile cells regenerate after injury, provided that enough stem cells remain.

- **Stable cells:** are quiescent cells (in stage G0 phase of the cell cycle), and have minimal replicative activity in their normal state.
- However, they are capable of multiplying and regenerating in response to injury. They include:
- The parenchymal cells of organs, such as liver, kidney (proximal tubules), pancreas and endocrine glands.
- Mesenchymal cells like smooth muscle cells, fibroblasts, vascular endothelium, bone and cartilage cells.

- **Permanent cells:** are terminally differentiated cells and have lost their ability to proliferate around the time of birth. They include:
- Neurons of nervous system, cardiac myocytes, skeletal muscle cell and cells of lens of the eye.
- Thus, injury to the brain or heart is irreversible, and results in scar.

Stem Cells

- Normal cells carry out specialized functions like producing keratin or secreting substances as mucin.
- The term “differentiated cells” refers to cells with specialized functions.
- Differentiated cells only have limited ability to undergo division and to regenerate.
- Their replacement come from nearby less differentiated *“stem cells”*, or “reserve cells”.

Stem cells

- **Stem cells:** are unspecialized cells that can divide through mitosis and differentiate into various specialized cell types. Moreover they have the ability to renew themselves to produce more stem cells.
- They are found in all multi-cellular organisms.
- The classical definition of a stem cell requires that it possess two properties:

1. ***Self-renewal***: The ability to go through numerous cycles of cell division while maintaining the undifferentiated state.
2. ***Potency***: The capacity to differentiate into specialized cell types.

- This requires stem cells to be either totipotent or pluripotent to be able to give rise to any mature cell type, although multipotent or unipotent progenitor cells are sometimes referred to as stem cells.

- **Self-renewal:**
- Two mechanisms exist to ensure that the stem cell population is maintained:

1. **Obligatory asymmetric replication**: stem cell divides into one daughter cell that is identical to the original stem cell, and another daughter cell that is differentiated
2. **Stochastic differentiation**: when one stem cell develops into two differentiated daughter cells, another stem cell undergoes mitosis and produces two stem cells identical to the original.

- **Stem cell lineage:** To ensure self-renewal, stem cells undergo two types of cell division.

1. **Symmetric division** gives rise to two identical daughter cells both have stem cell properties.
2. **Asymmetric division** produces only one stem cell and a progenitor cell with limited self-renewal potential.

- Progenitors can go through several cell divisions before terminally differentiating into a mature cell.

- The distinction between symmetric and asymmetric divisions lies in differential segregation of cell membrane proteins between the daughter cells.
- Stem cells remain undifferentiated due to environmental factors in their particular niche.
- Stem cells differentiate when they leave that niche or no longer receive signals from the surrounding tissues.

- ***Potency:*** Specifies the differentiation potential (the potential to differentiate into different cell types). This includes:

I. **Totipotency:** is the ability of a single cell to divide and produce all the differentiated cells in an organism, including extra-embryonic tissues (complete living organisms).

- Human development begins when a sperm fertilizes an egg and creates a single totipotent cell (zygote).

- In the first hours after fertilization, this cell divides into identical totipotent cells, which can later develop into any of the three germ layers of a human (endoderm, mesoderm, or ectoderm) and into cells of the cytotrophoblast and syncytiotrophoblast layers of the placenta.
- After reaching the 16-cell stage, the totipotent cells of the morula differentiate into cells that will eventually become either the blastocyst's inner cell mass or outer trophoblasts.

- Approximately four days after fertilization and after several cycles of cell division, these totipotent cells begin to specialize.

II. **Pluripotency** refers to the potential to differentiate into any *fetal* or *adult* cell type of the three germ layers: endoderm (lining of GIT & bronchi), mesoderm (muscle, bone, blood vessels), or ectoderm (epidermal tissues and nervous system).

- **Pluripotent stem cells:** a stem cell that alone cannot develop into a complete fetal or adult animal because they lack the potential to differentiate into extra-embryonic tissues, such as the placenta.

- **Types of stem cells:**
- There are two basic types of stem cells: embryonic stem cells and adult stem cells.

A. Embryonic stem (ES) cells:

- These cells are pluripotent cells found in early embryonic life.
- They are isolated from inner cell mass of blastocyst.
- The inner cell mass is pluripotent, not totipotent.
- They have the ability to virtually become any type of cells.
- They are not found in adults and therefore are not responsible for cell regeneration.

- **ES cell differentiation potential:**
- The potential of the ES cells to differentiate into all lineages diminishes with advancing stages of embryo development.

III. Multipotent cells:

- **These are progenitor cells** that have the potential to give rise to cells from multiple, but a limited number of lineages.
- An example is a hematopoietic stem cell that can develop into several types of blood cells, but cannot develop into brain cells or tissue.

IV. Oligopotent cells:

- **Oligopotency:** cells that have the ability to differentiate into a few cell.
- Examples of **Oligopotent stem cells:**
- The lymphoid or myeloid stem cells.
- The vascular stem cells which have the capacity to become both endothelial or smooth muscle cells.

IV. Unipotent (precursor cells): cells that have the capacity to differentiate into only one type of cells.

- **Unipotent cell:**
- The most common of these in humans are skin cells.
- These cells have a unique property of self-renewal.
- This property distinguishes it from most other terminally differentiated non-stem cells.

- Hepatocytes, which constitute most of the cytoplasmic mass of the liver.
- The liver's ability to regenerate from as little as 25% of its original mass is attributed to this property.
- A close synonym for *unipotent cell* is *precursor cell*.

B. Adult stem cells:

- Stem cells can be found in children, as well as adults.
- They are known as somatic (Latin, body) or germline stem cells (giving rise to gametes).
- They are more differentiated than embryonic stem cells.
- They possess a more restricted range of cell differentiation than the ES cells.
- They have the ability to divide forever, without terminally differentiating.

- These cells are found in various tissues.
- Most adult stem cells are lineage-restricted (multipotent).
- They have limited capacity to become different cell types.
- They are generally referred to by their tissue origin (mesenchymal stem cell, adipose-derived stem cell, endothelial stem cell, etc.).
- They are programmed to become cells with a particular set of functions.

- They are found in “niches” close to functioning differentiated cells.
- Adult stem cell treatments have been successfully used for many years to treat leukemia and related bone/blood cancers through bone marrow transplants.
- Pluripotent adult stem cells are rare and generally small in number but can be found in a number of tissues including umbilical cord blood.

- **Other stem cells:**

C. Fetal stem cells: are primitive cell types found in the organs of fetuses.

d. Amniotic stem cells: Multipotent stem cells found in amniotic fluid.

- These stem cells are very active, expand extensively without feeders and are not tumorogenic.
- They are multipotent and can differentiate into cells of adipogenic, osteogenic, myogenic, endothelial, hepatic and also neuronal lines.

E. Induced pluripotent stem cells:

- These are not adult stem cells, but rather reprogrammed cells (e.g. epithelial cells) given pluripotent capabilities.
- Using genetic reprogramming with protein transcription factors, pluripotent stem cells equivalent to embryonic stem cells have been derived from human adult skin tissue.
- As a result of success of these experiments, Ian Wilmut; who helped create the first cloned animal Dolly Sheep.

Factors affecting tissue repair

- Cell proliferation and tissue repair can be triggered by many chemical mediators, such as growth factors, hormones, and cytokines, as well as interactions between cells and ECM components.

A. Growth factors:

- A growth factor is a protein that has the following effects:

➢ Stimulate cellular proliferations.

- Stimulate migration, differentiation and contractility.
- Enhance the synthesis of specialized proteins (such as collagen in fibroblasts).
- Stimulate the function of growth control genes (proto-oncogenes).

- **Mechanisms of action:**

- They induce cell proliferation by binding to specific receptors.
- Affecting the expression of genes whose products typically promote replication.
- Prevent apoptosis and enhance the synthesis of cellular proteins involved in mitosis.

Growth factors and cytokines involved in regeneration and wound healing

Cytokine	Source	Functions
Epidermal growth factor (EGF)	• Activated macrophages, keratinocytes, and other cells	• Mitogenic for keratinocytes and fibroblasts.
Transfoming growth factor β (TGF-β)	• Platelets, T-lymphocytes, macrophages, endothelial cells, keratinocytes, smooth muscle cells and fibroblasts.	• Chemotactic to PMNs. macrophages, lymphocytes, fibroblasts, and smooth muscle cells. • Stimulate TIMP synthesis, angiogenesis, and fibroplasia. • Inhibits production of MMPs and keratinocytes proliferation. • Regulates integrin expression and other cytokines.

Cytokine	Source	Functions
Vascular endothelial growth factor (VEGF)	• Mesenchymal cells	• Increased vascular permeability. • Mitogenic for endothelial cells.
Platelet derived growth factor (PDGF)	• Platelets, macrophages, endothelial cells, keratinocytes, smooth muscle cells.	• Chemotactic for and activating to PMNs, macrophages, fibroblasts. • Mitogenic for fibroblasts, endothelial cells, smooth muscle cells. • Stimulate angiogenesis and wound remodeling. • Regulates integrin expression.

- HA: hyaluronic acid, MMPs: matrix metaloproteinase, PMNs: polymorphnuclear leucocytes, TIMP: tissue inhibitor of matrix metaloproteinase.

B. Extracellular matrix (ECM):

- The ECM is dynamic component, synthesized locally and assembles into a network that surrounds cells.

- **Synthesis and degradation of ECM accompanies:**
  - Morphogenesis.
  - Wound healing.
  - Chronic fibrotic processes.
  - Tumor invasion and metastasis.

- **Forms of ECM:**

1. Interstitial matrix: this is present in the spaces between cells; in the connective tissues, between epithelium, and supportive vascular and smooth muscle structure.

- It is synthesized by mesenchymal cells (e.g, fibroblasts).
- Its major constituent is fibrillar and non-fibrillar collagens as well as fibronectin, elastin, proteoglycans, and hyaluronate.

2. Basement membrane: the basement membrane lies beneath the epithelium.

- It is synthesized by the overlying epithelium and the underlying mesenchymal cells.
- The major constituents are amorphus non-fibrillar type IV collagen and laminin.

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

1. Fibrous structural proteins such as collagen and elastins, which give tensile strength and recoil.
2. Water-hydrated gel such as proteoglycans and hyaluronan, which permit flexibility and lubrications.
3. Adhesive glycoproteins that connect matrix elements to one another and to cells.

- **Role of ECM:**

1. It provides mechanical support to tissues: this is the role of **collagen** and **elastin.**
2. Control of cell growth and cell proliferation: ECM control growth and differentiation by signaling through cellular receptors of the **integrin** family.
3. Maintenance of cellular differentiation: ECM proteins can affect the degree of differentiation of the cells via cell surface **integrins**.

4. Scaffolding for tissue renewal: The integrity of the basement membrane and the stroma is critical for regeneration of tissues, whose disruption leads to scar formation.
5. Establishment of tissue microenvironments: Basement membrane acts as a boundary between epithelium and underlying connective tissue and also forms part of the filtration apparatus of the kidney.

6. Storage and presentation of regulatory molecules: e.g. growth factors are excreted and stored in ECM like fibroblast GF (FGF), and hepatocyte GF (HGF).

- This allows for the rapid use of growth factors after local injury, or during regeneration.

Cell and tissue regeneration

- It occurs in tissues formed of labile and stable cells such as bone marrow, gut epithelium and skin.
- Damage of the epithelia can be corrected by proliferation and differentiation of stem cells.
- The renewal of hematopoietic cells is driven by growth factors called colony stimulating factors (CSFs), which are produced in response to increasing consumption or loss of blood cells.

- Regeneration occurs after surgical removal of 40% to 60% of the liver in liver-donner transplantation.
- In this situation, the tissue resection triggers a proliferative response of the remaining hepatocytes (stable cells), and the subsequent replication of the hepatic non-parenchymal cells.

- Extensive regeneration can occur only if the residual tissue is structurally and functionally intact, as after surgical excision.
- But, if the tissue is damaged by inflammation, regeneration is incomplete and is accompanied by scarring.

- **Repair by connective tissue:**
- This occurs if:
- Non-dividing (permanent cells) are injured.
- Tissue injury is severe or chronic, and results in damage to parenchymal cells and epithelium as well as the stromal framework.
- Repair takes place by participation of mesenchymal cells (C.T. stem cells), endothelial cells, platelets and parenchymal cells of the injured organ.

- Repair by connective tissue deposition consists of four sequential processes:

a. Formation of new blood vessels **(angiogenesis)**.
b. Migration and production of fibroblasts.
c. Deposition of ECM **(scar formation)**.
d. Maturation and reorganization of the fibrous tissue **(remodeling)**.

I. Angiogenesis

- Angiogenesis or neo-vascularization is a critical process in:

➢ Healing at sites of injury.

➢ The development of collateral circulation at sites of ischemia.

➢ Allowing tumors to increase in size and metastasize.

- **Mechanism of angiogenesis:**
- Angiogenesis results from migration of endothelial precursor cells (EPCs) from the bone marrow or from pre-existing vessels in areas of injury where they differentiate and form a mature network by linking with pre-existing vessels.
- **Steps of angiogenesis:**

1. Vasodilatation in response to nitric oxide and increased permeability of pre-existing vessel induced by vascular endothelial growth factor (VEGF).

2. Migration of endothelial cells towards the area of tissue injury.
3. Proliferation of endothelial cells just behind the leading front of migrating cells.
4. Inhibition of endothelial cell proliferation and remodeling into capillary tubes.
5. Recruitment of peri-endothelial cells (pericytes for small capillaries and smooth muscle cells for large vessels).

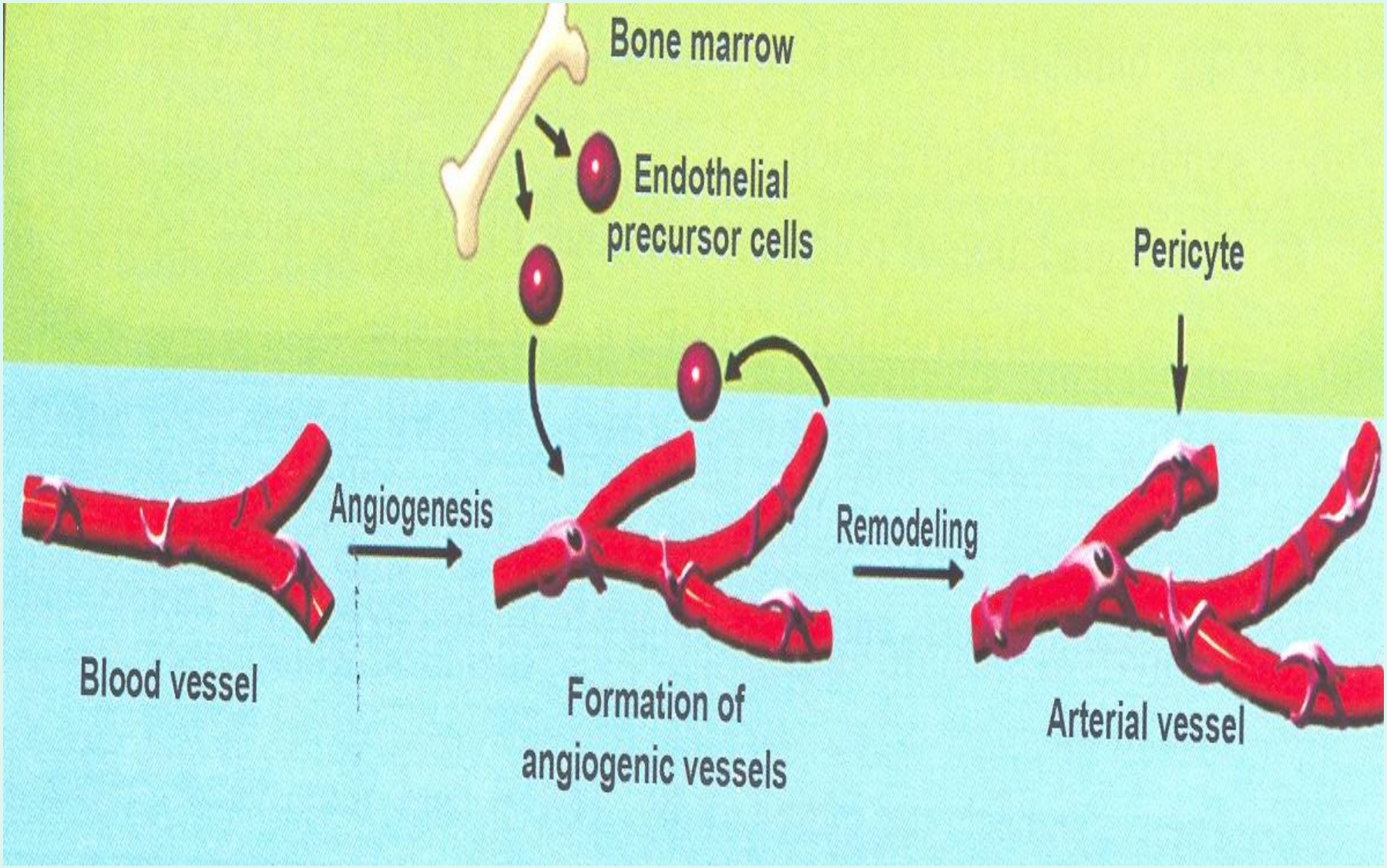
- New vessels formed during angiogenesis are leaky because of:

a) Incompletely formed inter-endothelial junctions

b) VEGF increases vessel permeability.

- The leakiness explains why granulation tissue is often edematous, and accounts in part for the edema that may persist in healing wounds long after the acute inflammatory response has resolved.

- **Growth factors involved in angiogenesis:**
- Several growth factors induce angiogenesis, but the most important are VEGF and basic fibroblast growth factor (FGF-2).


Bone marrow
Endothelial
precursor cells
Pericyte
Angiogenesis
Remodeling
Blood vessel
Formation of
angiogenic vessels
Arterial vessel

II. Migration of fibroblasts

- The recruitment and stimulation of fibroblasts is driven by many growth factors, including PDGF, FGF-2, and TGF-β.
- The sources of these factors are the activated endothelium and inflammatory cells as macrophages, mast cells and lymphocytes.

III. ECM deposition (scar formation)

- As healing progresses, the number of proliferating fibroblasts and new vessels decreases.
- However the fibroblasts progressively increase ECM deposition.
- Collagen synthesis by fibroblasts begins early in wound healing (3-5 days) and continues for several weeks, depending on the size of the wound.

- It is critical to the development of strength in a healing site.
- New collagen accumulation depends on:

a) Increased synthesis

b) Diminished degradation.

- As the scar matures, there is progressive vascular regression, so the highly vascularized granulation tissue is transformed into a pale, avascular scar.
- Many growth factors are involved in ECM deposition and scar formation, e.g., FGF, TGF-β, PDGF, and cytokines (TNF, IL-1).

IV. ECM and tissue remodeling

- After its synthesis and deposition, ECM of the scar tissue continues to be modified and remodeled.
- The outcome of the repair process is a balance between ECM synthesis and degradation.
- The degradation of collagen and other ECM components occurs by collagenases and matrix metalloproteinases (MMPs).

- MMPs are produced by a variety of cell types (fibroblasts, macrophages, neutrophils, synovial cells, and some epithelial cells).
- MMPs synthesis and secretion are regulated by growth factors, and cytokines.
- Their synthesis is inhibited by TGF-β and may be suppressed pharmacologically by steroids.

Cutaneous wound healing

- This is process that involves both epithelial regeneration and the formation of connective tissue scar.
- Based on the nature of the wound, the healing of cutaneous wounds can occur by:
  - First intension. or
  - Second intension.

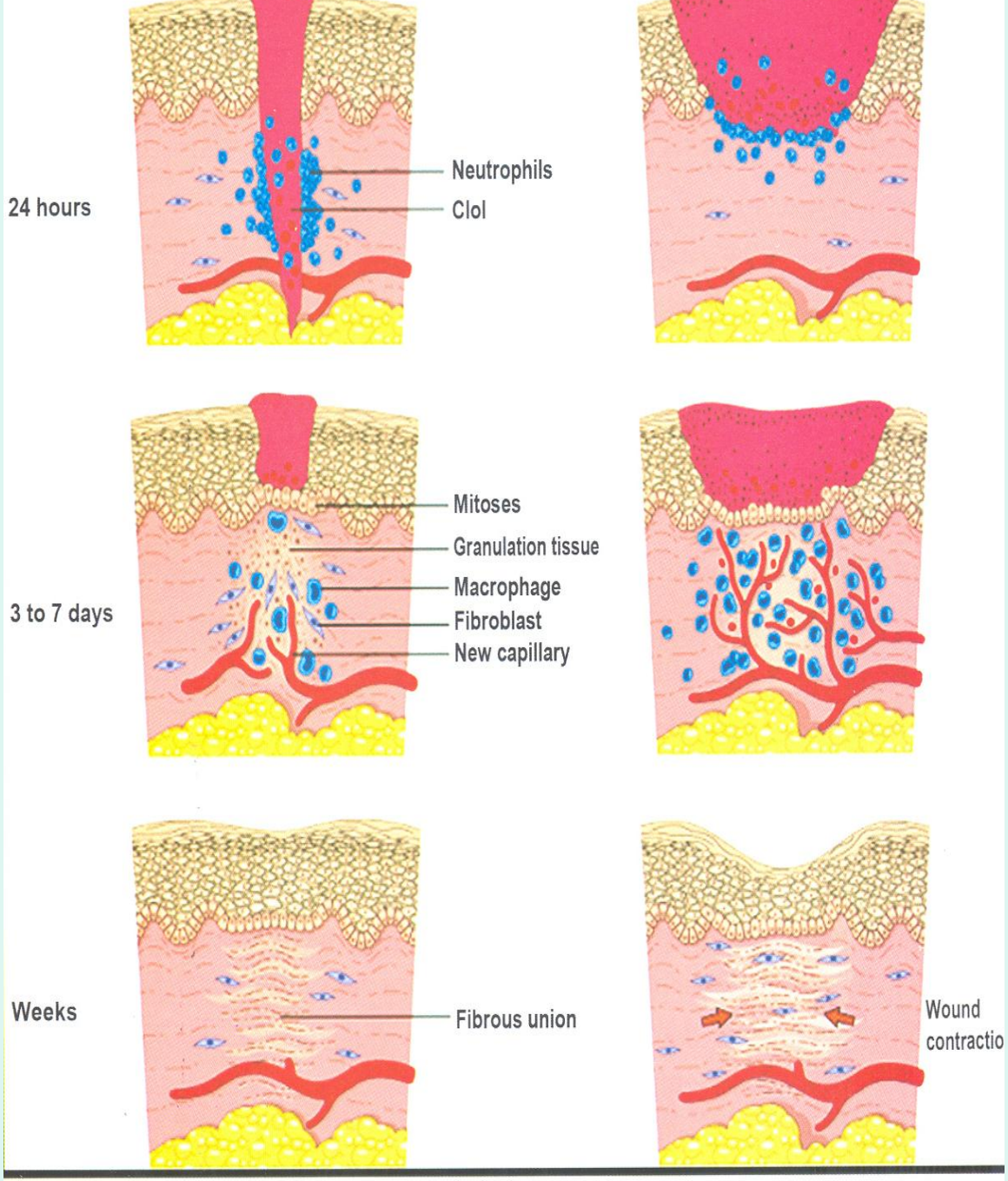


Fig.4.5: Steps in wound healing by first intention (left) and second intention (right).

Healing by first intention (primary union)

- This is defined as healing of a wound which have the following characteristics:

1. Clean and uninfected.
2. Surgically incised.
3. Without much loss of cells and tissues.
4. Edges of the wound are approximated by surgical sutures.

- **The sequence of events in primary union:**
- **Initial hemorrhage:**
- Immediately after injury, the spaces between the approximated surfaces of incised wound is filled with blood, which then clots and seals the wound against dehydration and infection.

- **Within 24 hours:**
- Neutrophils are seen at the incision margin, migrating toward the fibrin clot.
- Basal cells at the cut edges of the epidermis begin to show increased mitotic activity.

- **Within 24-48 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 as a thin but continuous epithelial layer separating the underlying viable dermis from the overlying scab.

- **By day 3:**
- Neutrophils has been largely replaced by macrophages, and granulation tissue progressively invades the incision space.

- **Morphology of granulation tissue:**
- **Grossly:** granulation tissue appears granular soft, moist, pink, and bleeds on touch.
- It is insensitive and resistant to bacterial infection.
- **Microscopy:** characterized by proliferation of fibroblasts and new thin walled, delicate capillaries (angiogenesis), in a loose ECM.
- Collagen fibers start to appear at the incision margins.
- They are vertically oriented and do not bridge the incision.

- Epithelial cell proliferation continues as a thickened epidermal covering layer.
- **By day 5:**
- 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 producing a mature epidermal architecture with surface keratinization.

- **During the second week:**
- There is fibroblast proliferation and continued collagen accumulation.
- The leucocytic infiltrate, edema, and increased vascularity diminished.
- **By the end of the first month:** the scar comprises of vascular connective tissue devoid of inflammatory cells and covered by a normal epidermis.

- The dermal appendages destroyed in the line of the incision are permanently lost.
- The tensile strength of the wound increases with time.

Healing by second intention (secondary union)

- Healing of a wound having a following characteristics:

1. Large infected or open wounds not approximated by surgical sutures e.g., large wound, abscess or ulceration.
2. Extensive loss of cells and tissues.

- The basic events are similar to primary union but differ in: having larger tissue defect which has to be bridged.
- Hence, healing takes place from the base upwards as well as from the margins inwards.
- The inflammatory reaction is more intense.
- There is abundant development of granulation tissue.

- The wound contacts within 6 weeks.
- It may be reduced to 5-10% of its original size, due to presence of myofibroblasts (modified fibroblasts).
- This may be followed by accumulation of ECM and formation of a large scar.

Differences between primary and secondary union of wounds

Feature	Primary union	Secondary union
cleanliness of wound	Clean	Unclean
Infection	Generally uninfected	May be infected
inflammation	Mild	More intense
Granulation tissue	Scanty	Much larger amount
Wound contraction	Not present	Present
Outcome	Neat linear scar	Contracted irregular wound
Complications	Infrequent; epidermal inclusion cyst.	More frequent; the most important is suppuration.

Wound strength

- Carefully sutured wounds have approximately 70% of the strength of normal skin.
- When sutures are removed, usually at one week, wound strength is approximately 10% of that of normal skin, but this increases rapidly over the next 4 weeks.

- The recovery of tensile strength results because:
  - The collagen synthesis exceeds degradation during the first 2 months, and
  - Structural modifications of collagen (e.g., cross linking and increased fiber size).
- Wound strength reaches approximately 70-80% of normal by 3 months but usually does not improve beyond that point.

Factors affecting healing

1. **Infection** is the most important cause of delayed wound healing.
2. **Nutrition** has profound effects on wound healing: protein and vitamin C deficiency inhibit collagen synthesis and delayed healing.
3. **Glucocorticoids (steroids):**

- Have anti-inflammatory effects. And their administration may result in poor wound strength due to diminished fibrosis.

- In some instances, however, in corneal infections glucocorticoids are sometimes prescribed (along with antibiotics) to reduce the likelihood of opacity that may result from collagen deposition.

4. **Poor perfusion** due to arteriosclerosis, diabetes, or obstructed venous drainage results in impaired healing.
5. **Foreign body:** such as fragments of steel, glass or even bone impair healing.

6. **The type of tissue injured:** complete restoration can occur only in tissues composed of labile and stable cells.

- Injury to tissues composed of permanent cells must inevitably result in scarring e.g., myocardial infarct.

7. **The site of injury:** inflammation arising in some spaces (e.g., pleural, peritoneal, and synovial cavities) develops extensive exudate.

- Repair may occur by digestion of the exudate, initiated by proteolytic enzymes of leucocytes and resorption of liquefied exudate.
- This is called resolution in which normal tissue architecture is restored.
- However, in the settings of large accumulations, the exudate may undergo organization.

Complications of wound healing

1. **Infection** of wound.
2. **Implantation (epidermal) cyst,** due to persistence of epithelial cells in the wound after healing.
3. **Deficient scar formation:** inadequate formation of granulation tissue may lead to wound splitting, incisional hernia, or ulceration.

4. **Excessive scar formation (keloid):** mass formed of excessive collagen that tends to progress beyond the site of initial injury, covered by stretched epidermis and recures after excision. It is more common in blacks and shows hereditary tendency.
5. **Exurberante granulation tissue:** excessive granulation tissue that protrudes above the level of the surrounding skin and hinders re-epithelialization.

6. Excessive contraction:

- Exaggeration of wound contraction is termed contracure or cicatrization. It results in severe deformity of the wound and surrounding tissue.
- Contractures may complicate healing of severe burns and may compromise the movements of joints.

7. **Pigmentation:** healed wounds may have rust-like color due to presence of hemosiderin.

8. **Malignant change:** scar may be the site for development of carcinoma later, e.g., squamous cell carcinoma in Marjolin's ulcer.