!! JAY AMBE !!

2. INFLAMMATION AND REPAIR/WOUND HEALING

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INFLAMMATION AND REPAIR/WOUND HEALING

INTRODUCTION:

- Inflammation is a protective mechanism of the body to remove the injurious stimuli or complex biological response of vascular tissue against the harmful stimuli like pathogen, damaged cells or irritants.
- Inflammation is not the synonyms of infection. But sometimes inflammation is caused by the infections.
- Inflammation is characterized by 5 signs & symptoms **(Clinical Sign of inflammation)**
  - ✓ **Rubor** (*redness*): Dilatation of small blood vessels in damaged area
  - ✓ **Calor** (*heat*): Increased blood flow to area (*hyperaemia*)
  - ✓ **Tumour** (*swelling*): Accumulation of fluid in extracellular space (*edema*, British: *Oedema*)
  - ✓ **Dolor** (*pain*): Stretching/distortion of tissue from oedema (esp. from pus), chemical mediators can induce pain
  - ✓ **Loss of function**: Movement hindered etc.
- Under normal circumstances inflammation is a protective response, the goal of which is to eliminate both the initial cause of injury, such as bacteria or toxins, and the consequences of such injury, such as dead cells and tissues. However, if triggered or directed inappropriately, the inflammatory response can itself become harmful, leading to cell, tissue and organ destruction.

There are two basic types of inflammation:

1. **Acute inflammation:**
   - Acute inflammation consists of the initial response of the body to tissue injury.
   - Acute inflammation is of short duration, which could be anything from a few minutes to a few days.

- **Pathogenesis of acute inflammation:**

Pathogenesis of acute inflammation can be explained by two events;

1. **Vascular Event**

Vascular Event

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Tissue Injury

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Release of chemical for local effect (Histamine, Prostaglandins, Kinins, Leukotriens, Complement Etc)

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Immediate Response is Vascular Contraction

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It may persist for 3-5 second or last for 5 minutes depending on the severity of injury

↓

It is known as transient vasoconstriction

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After this phase arterioles get dilate known as persistent progressive vasodilatation

↓

It increase the blood flow and this type of vascular expansion produce **redness and heat known as erythema**

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When the volume of blood flow increases, it increases intravascular hydrostatic pressure

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So fluid from the capillary get migrate in to the tissue and this fluid is know as **transudate**

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When the volume of blood flow may raise more and permeability gets increase

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Fluid with the protein migrate from capillary to tissue known as **exudates**

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Loss of the Protein rich fluid from the capillary reduces the intravascular osmotic pressure and increase the osmotic pressure of the interstitial fluid (Fluid present in to the tissue due to migration from capillary)

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So it produces out flow of the water and ions into the extra-vascular

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These fluids accumulate in to the extravascular space and produce **edema.**

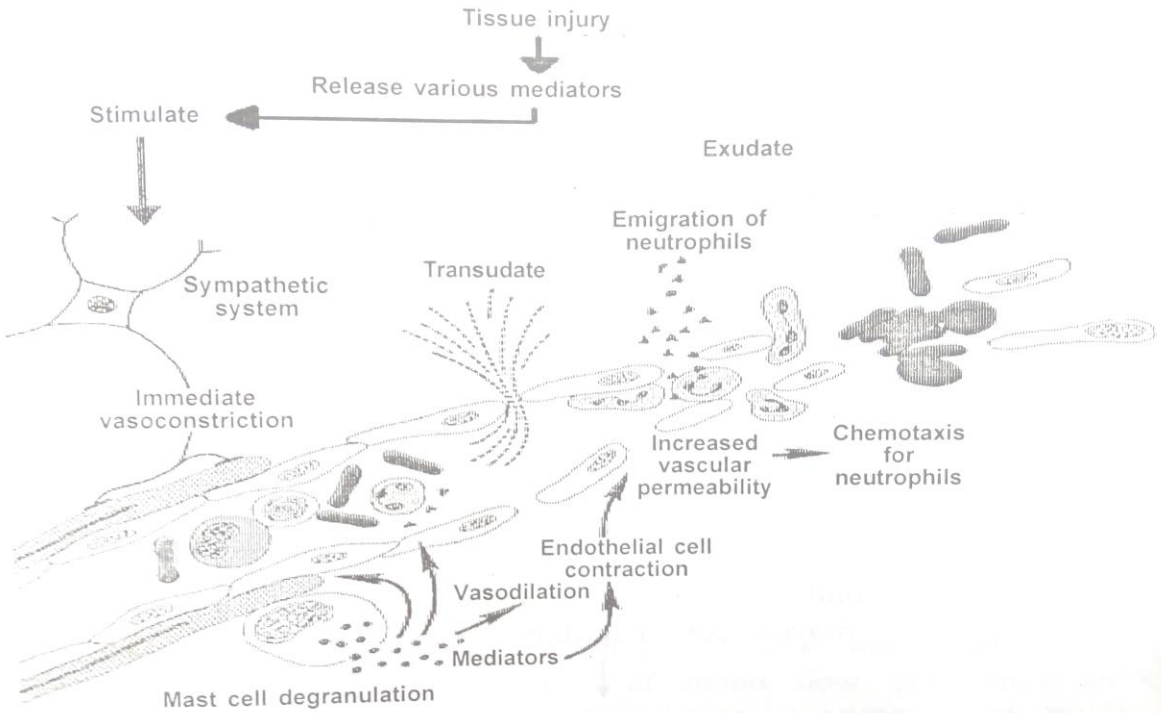

Figure 2.1: Mechanism of vascular events

Due to the tissue injury Histamine, Prostaglandins, Kinins, Leukotriens, Complement likes chemical mediators are released for the local effect. These chemical mediators produce immediate vasoconstriction which may persist for 3-5 second or last for 5 minutes depending on the severity of injury. This phase is known as transient vasoconstriction. After these phase arterioles get dilate known as persistent progressive vasodilatation. This kind of vasodilatation increase the volume of blood flow in to the capillary and produce redness and heat due to the vascular expansion. Vascular expansion increases the gap between endothelial cells of the capillary and decreases their thickness due to the expansion. So fluid in to the capillary move into the tissue and this migrated fluid is known as transudate. If the volume of blood flow may rise more and permeability of capillary further increases that time capillary protein also migrate with the fluid in to the tissue known as exudates. Now, the intravascular osmotic pressure get decreases and osmotic pressure of the interstitial fluid get increase due to the migration of protein from capillary to tissue. So it causes out flow of water and ions in to the extravascular and it get accumulate in to the extravascular and produces edema.

2. Cellular Event

Cellular event of the inflammation is described by the two processes;

1. Leukocytes (WBC) movement process

Tissue injury

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Produce vasodilation due to increases the volume of blood flow

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So the permeability of capillary get increases and fluid (Plasma) get move from capillary to tissue

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Plasma or fluid gets discharge from the capillary so the ratio of WBCs and RBCs get increases, plasma discharge due to the exudates known as **migration**

↓

So it increases the viscosity of blood and slowing the flow of blood is known as stasis.

↓

Due to the increasing the viscosity, the leukocyte roll on the endothelial of the capillary

↓

The leukocyte on their surface consist integrin protein and endothelial cell of blood capillary consist selectin (E-Selectin & P-Selectin) protein on their surface.

↓

During the leukocyte **rolling** on to the endothelial cell, integrin and selectin get activated and produce **adhesion** of leukocyte on endothelial cell of capillary.

↓

After the adhesion or sticking leukocyte moves in to the extravascular space from the gap between endothelial cells or from the capillary

↓

These phenomenons is known as **emigration**

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At the site of injury, several mediators get released which attract the leukocytes

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That kind of mediators at a site of injury s known as chemokines (Histamine, Leukotrines, Platelet activating factors, Prostaglandins, Cytokines, Complementary proteins etc) and the attraction process of the leukocyte towards the injury is known as **chemotaxis process**

Tissue injury produce persistent progressive vasodilatation, due to the vasodilatation volume of blood flow gets increased. Increased volume of blood flows contains WBCs and RBCs but because of the plasma or fluid part of blood migration from capillary to tissue their viscosity gets increased. So the blood flow gets decreased. Because of that reason leukocyte of white blood cells roll on the endothelial cell of blood capillary. Leukocyte on their surface consist integrin protein molecules and blood capillary on that surface consist selectin (E-Selectin & P-Selectin) protein molecules. During the rolling

time integrin and selectin get activated and produce adhesion of leukocyte on endothelial cell of capillary. Then after leukocyte get migrate from capillary to extravascular space known as emigration. The emigrated leukocyte get attracted towards the injured part because the injury release several mediators known as chemokines(Histamine, Leukotrines, Platelet activating factors, Prostaglandins, Cytokines, Complementary proteins etc). This attraction process of the leukocyte towards the injury is known as chemotaxis process.

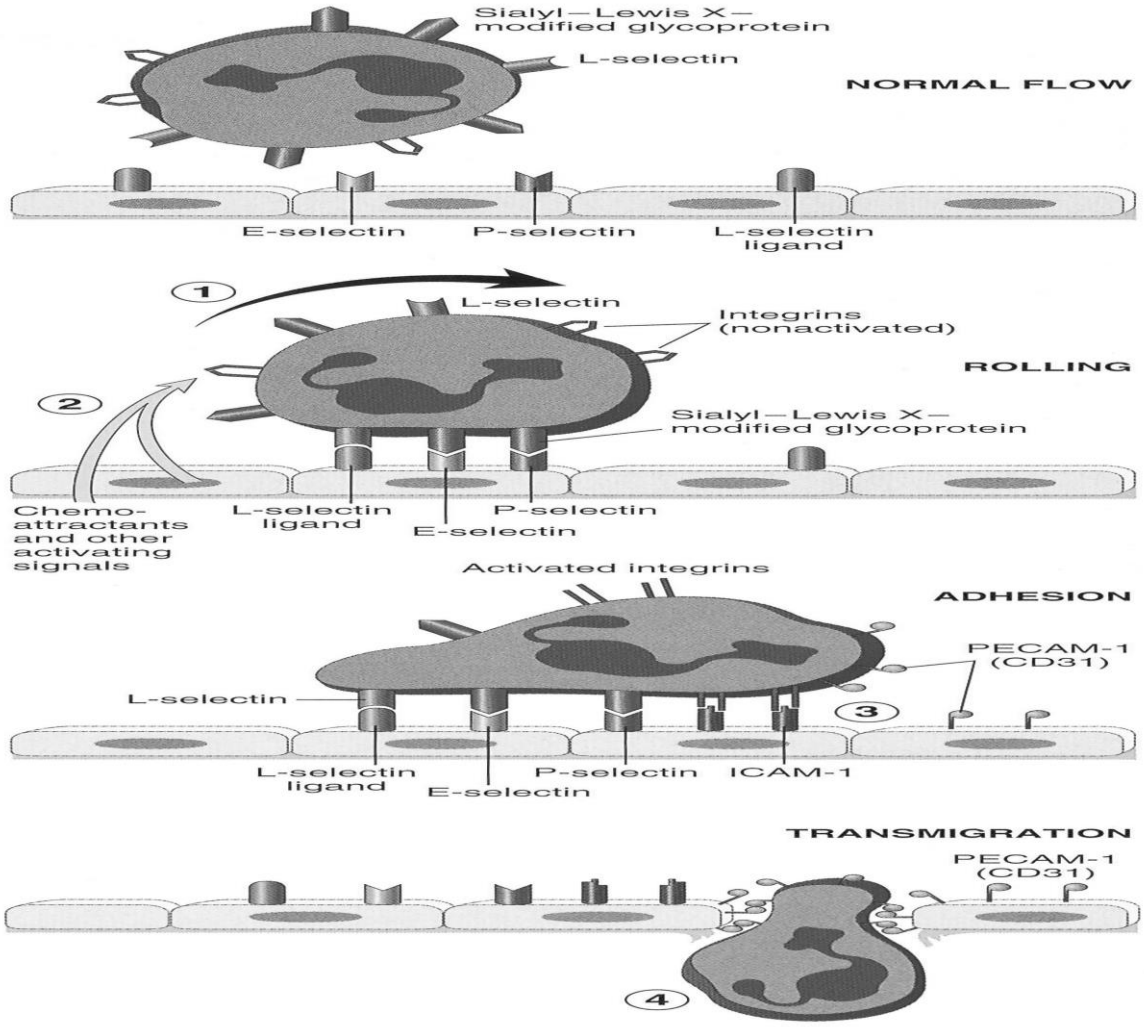

Figure 2.2: Leukocyte (WBC) movement process

In short this process includes the following steps:

- Migration
- Rolling and Adhesion
- Emigration and
- Chemotaxis

2. Phagocytosis:

- Phagocytosis means engulfment of solid particle or agent means cell eating.
- The process perform by the agent is known as phagocyte**.**
- This process involves the various stapes;

a) Identification and attachment:

- At the site of injury several chemotatic factor get released from the infected bacteria
- It attracts the phagocytic cells.
- It identifies or recognizes the micro-organisms at the site of injury and coat the micro-organism by opsonin proteins which are naturally occurring substance from the serum and it gives help for the attachment of phagocyte to micro-organism.

b) Engulfment:

- Now the phagocyte is ready to engulf the micro-organisms.

c) Degranulations:

- After the engulfment phagocyte release the lysosome the degradating enzyme.

d) Digestions or degradations:

- These degradating enzymes kill the micro-organism and digest it.
- How ever this mechanisms gets fail to kill bacteria like mycobacterium tuberculosis.

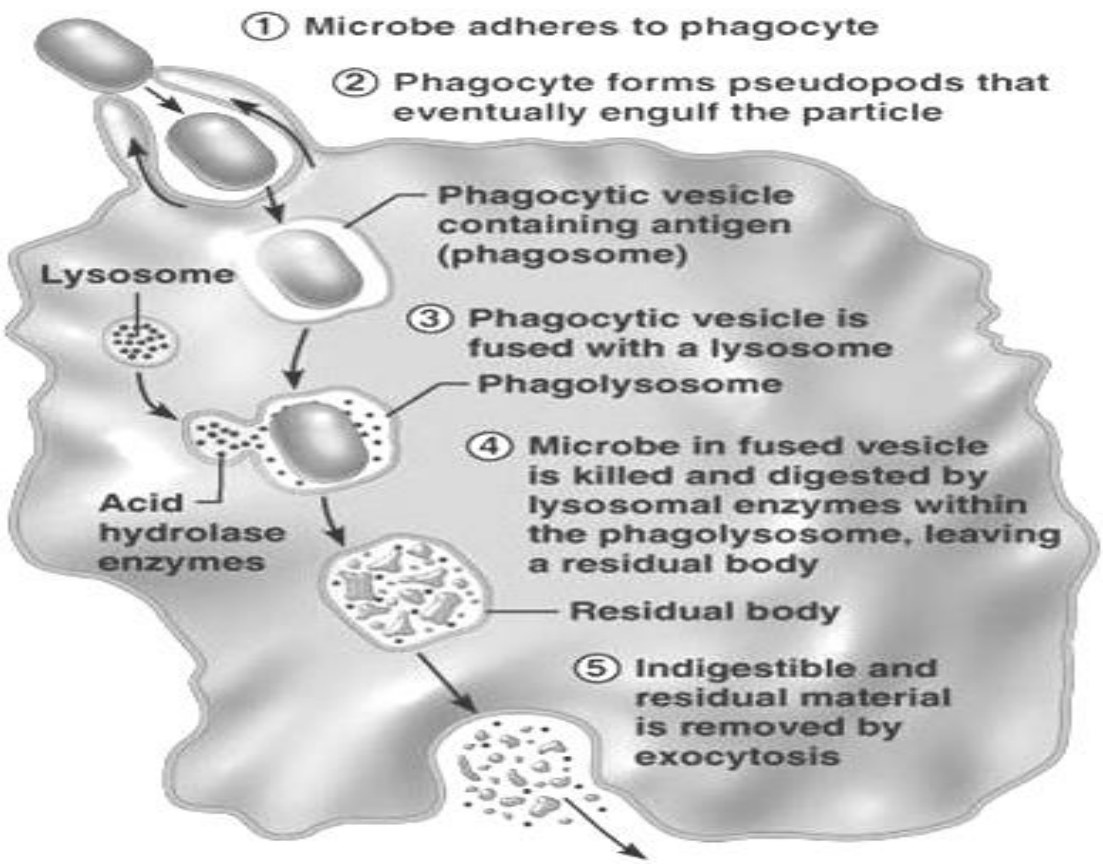

Figure 2.3: Process of phagocytosis

2. Chronic inflammation

- Chronic inflammation is the prolonged tissue reactions following the initial response.
- It is long lasting. It may persist for weeks, months or even years.
- The symptoms are not as severe as with acute inflammation, but the condition is insidious and persistent.
- Chronic inflammation may follow on from acute inflammation or exist by itself.
- An acute inflammation will become chronic if the immune system is unable to rid the body of the offending foreign agent or if the agent is constantly able to re-enter the body, such as tuberculosis.
- The main cells involved in chronic infection are macrophages and lymphocytes.

- **Pathogenesis of chronic inflammation:**

When acute phase cannot be resolved
Persistent injury or infection
Prolonged toxic agent exposure
Autoimmune disease states
Repeated acute inflamations

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Activates the mononuclear cells like phagocyte (Tissue macrophages and circulating monocytes) and lymphoid cells

↓

It get migrate towards the injured area

↓

Engulf the microorganism

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Digestion of microorganism or their particle release bacterial endotoxin or various cytokines

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It stimulate the immune response against the dead microbes or dead cells

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It activates the T-Cells

↓

Activated T-cell ($CD4^+$) release lymphokinase IL-1, IL-2, growth factor IFN-γ and INF-α

↓

It produces tissue injury and inflamation or fibrosis

Because of the various reasons like acute phase cannot be resolved, persistent injury or infection, prolonged toxic agent exposure, Autoimmune disease states, Repeated acute inflammations activates the mononuclear cells like phagocyte and lymphocyte. Phagocytic cells like tissue macrophages and circulating monocyte migrate toward the injured area and it identify the microorganism and attach with them and engulf the microorganism. Tissue phagocyte after the engulfing digest them by releasing several lysis enzyme like lysozyme. It produce distruction of bacteria. Due to the destruction of bacteria, the dead component or bacterial enotoxin and varous cytokines get released. These are the harmful producet or foreign particle for us so immune system get activated and produce activation of T-cell ($CD4^+$) which release lymphokinase IL-1, IL-2, growth factor IFN-γ and INF-α. It produces tissue injury and inflamation or fibrosis.

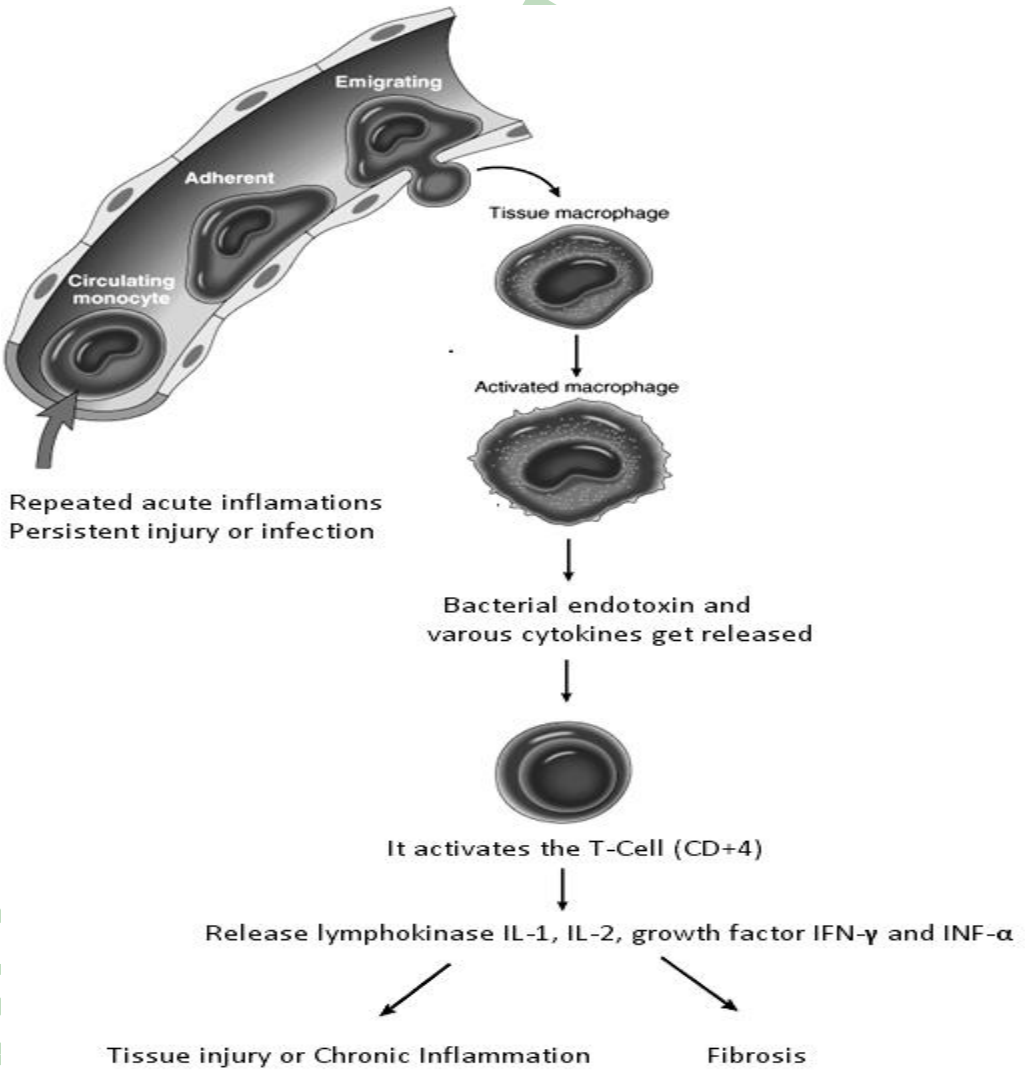


Figure 2.4: Pathogenesis of chronic inflammation

GRANULOMATOUS INFLAMMATION:

- It is the distinct process than the chronic inflammation.
- In this pattern aggregation of activated macrophages are arises
- It assume an epitheliod appearance
- The fusion of adjacent epitheliod cell and may have more than 20 nuclei are known as giant cell.
- It produces the granuloma, granules means granule like lesion and oma means inflammatory mass or bunch of macrophages.
- This type of reaction is very common in tuberculosis, leprosy, fungal infection.

- **Types of Granulomatous Inflammation**

1. Immune granulomas:

- Caused by insoluble particles that are capable of inducing a cell-mediated immune response
- Macrophages are transformed into Epitheloid cells and multinucleate giant cells
- Examples:
  a) Bacteria
     - ✓ Tuberculosis (high incidence due to drug resistant stains)
     - ✓ Leprosy

  b) Parasites
     - ✓ Schistosomiasis

  c) Fungi
     - ✓ Histoplasmosis
     - ✓ Blastomycosis

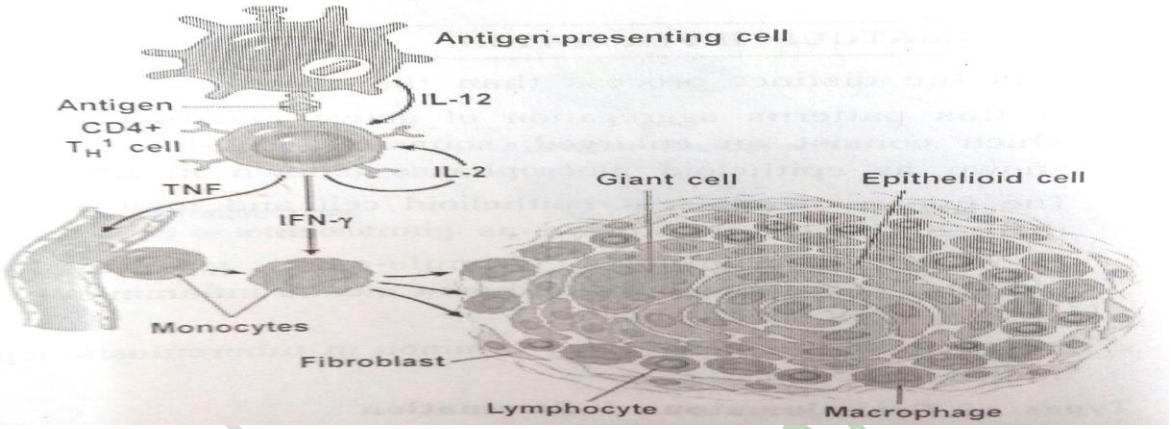


Figure 2.5: Immune granulomas

2. Foreign Body Granulomas

- Don't incite either an inflammatory or immune response.
- Epitheloid cells and giant cells are apposed to the surface and encompass the foreign body.
- The foreign body is usually found in the center of the granuloma.
- Examples:
  - ✓ Metal/Dust
  - ✓ Silicosis
  - ✓ Splinter
  - ✓ Berylliosis
  - ✓ Foreign body
  - ✓ Suture

3. Sarcoidosis

- Bad systemic disease, probably autoimmune disease
- Etiologic agent is unknown

CHEMICAL MEDIATORS OF INFLAMMATION:

1. **Vasoactive Amines:**
   - **a) Histamine:**
     - Stored in connective tissue mast cells, blood basophils and platelet granules.
     - Promotes contraction of smooth muscles (as in asthma), dilatation of arterioles, and contraction of endothelial cells.
     - The main action of histamine are vasodilatation, increase vascular permeability, itching and pain.
     - Many drugs act to block this amine's action (anti-histamines for common cold).
   - **b)** Serotonin:
     - It is not present in human mast cells, present in platelets.
2. **Plasma Proteases**:
   - a) **Complement system:**
     - The system contains 20 component proteins and cleavage products which mediate biologic reactions against microbial invasion. This is primarily a plasma system.

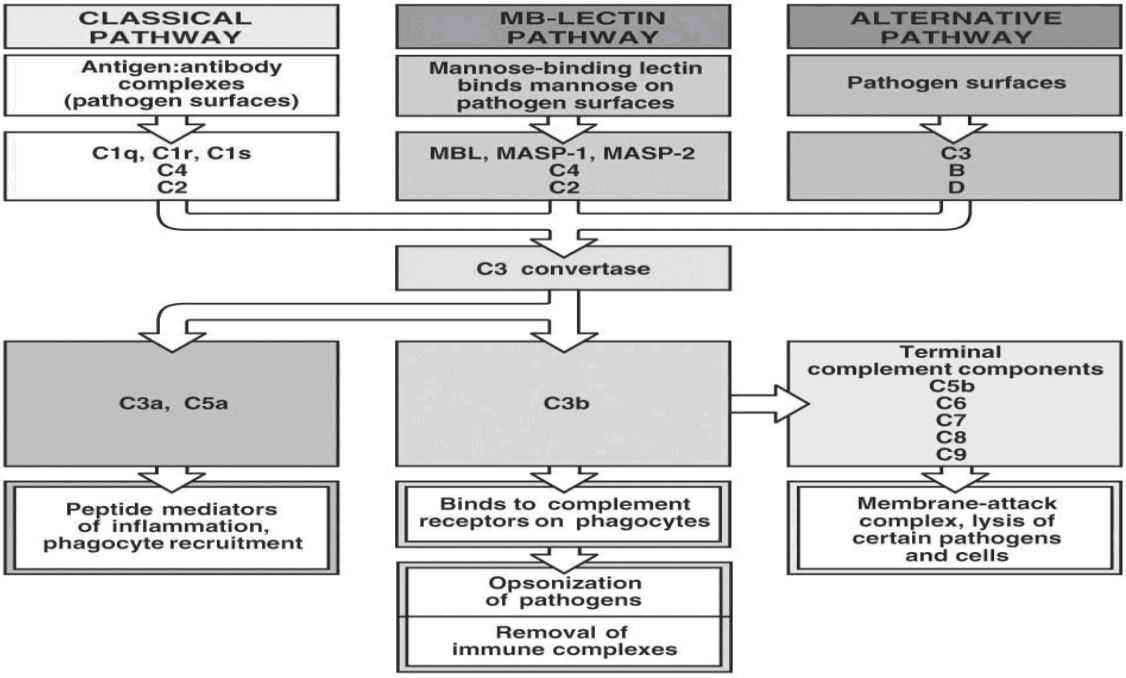


Figure 2.6: Complement protein system

The reactions include vascular permeability (C3a, C5a), chemotaxis (C5a), opsonization (C3b) and cell lysis (C5b-9, membrane attack complex).

b) Kinin system:

- It is a plasma protein cascade.
- It activated by Factor XII of the coagulation system (Hageman factor).
- Its end product is bradykinin (a polypeptide) which increases vascular permeability, causes contraction of smooth muscles and dilatation of vessels.
- It may directly stimulate pain fibers.

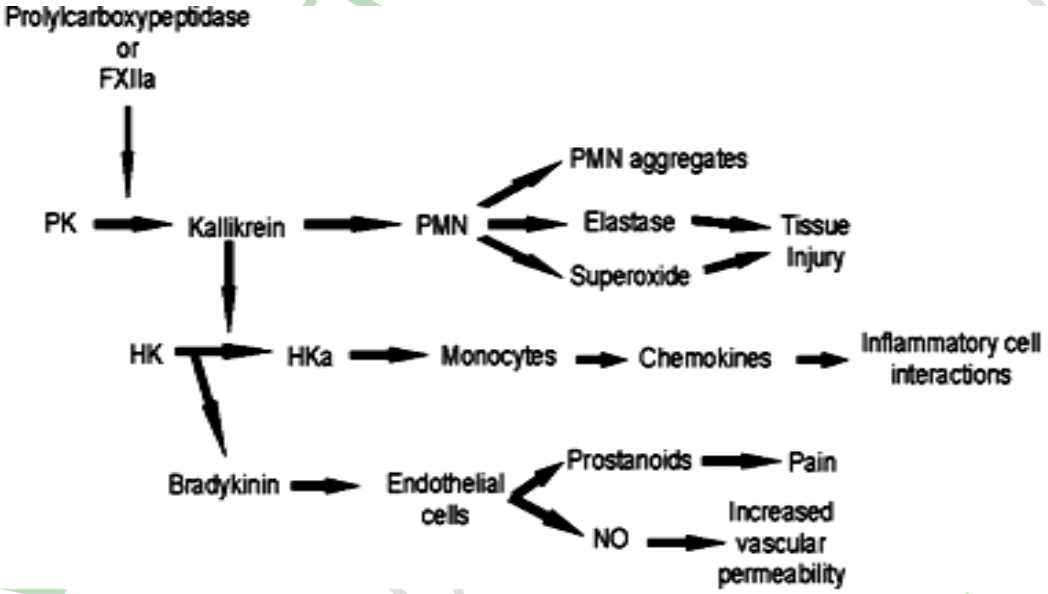


Figure 2.7: Kinin pathway

c) Clotting system:

- It is another plasma protein cascade system activated by Factor XII.
- The result of activation of the coagulation cascade is the generation of fibrin. Fibrinopeptides induce vascular permeability and are chemotactic for leukocytes. The coagulation cascade is actually a dynamic balance between forming fibrin clots and breaking down fibrin clots.
- The fibrinolytic system generates plasmin which in turn activates Factor XII (producing bradykinin and activating complement).

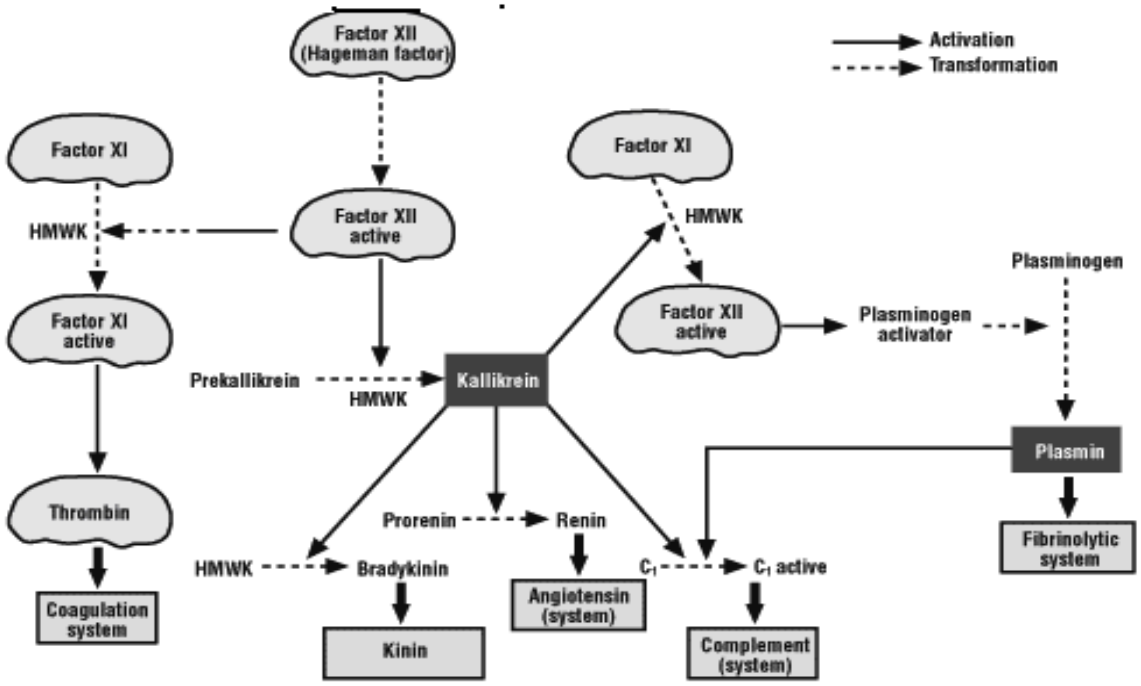


Figure 2.8: Plasma protein cascade system

3. Arachidonic Acid Metabolites :

Prostaglandins and leukotrienes (including thromboxane): These "local, short-range hormones" (autocoids) act primarily as effectors of vascular permeability, vascular constriction or leukocyte chemotaxis.

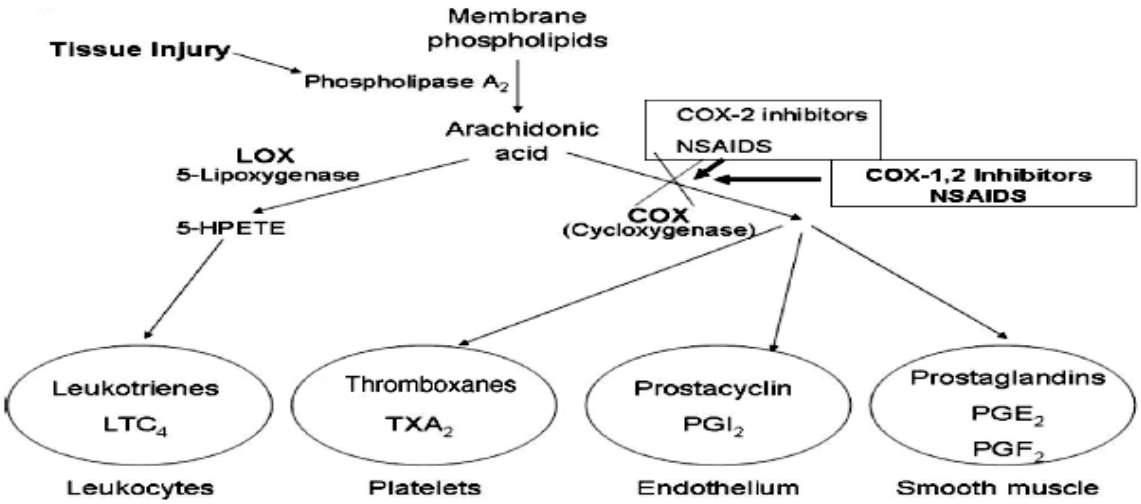
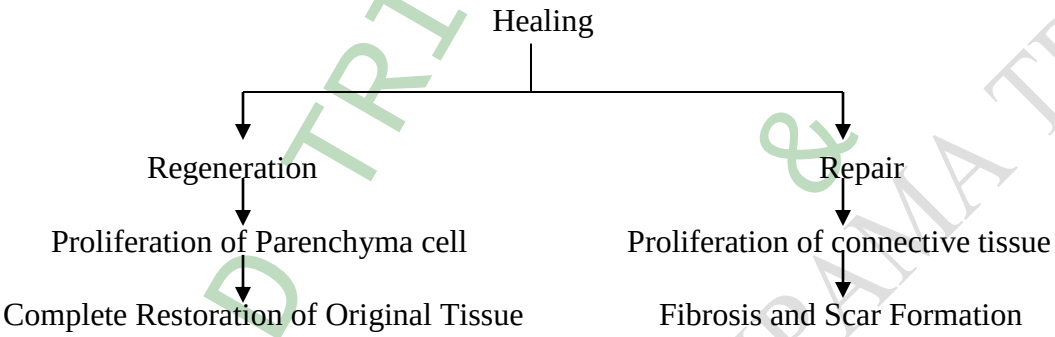


Figure 2.9: Arachidonic acid pathway

4. **Cytokines**
   - These polypeptide products were initially described in immunologic responses but have since been shown to play a role in the inflammatory response.
   - The two principle players in inflammation are IL-1 (interleukin-1) and TNF (tumor necrosis factor). Among other things, they also stimulate both collagen and collagenase production by fibroblasts.
   - The cytokines play important roles in neoplasia as well via autocrine (act on same cell), paracrine (act on cell in proximity) and endocrine (act on distant cell) effects.
5. **Nitric Oxide**
   - Nitric oxide synthetase (NOS) produces nitric oxide (NO) from L-arginine in the presence of oxygen and NADPH.
   - It is a free radical produced by endothelial cells and macrophages. It causes smooth muscle relaxation in the vessel wall (vasodilation) and reduces platelet activation and aggregation.
   - In macrophages, the free radical nature of NO is toxic to microbes.
6. **Growth Factors**
   - The major growth factors of importance are EGF (epidermal growth factor), PDGF (platelet derived growth factor), FGF (fibroblast growth factor), VEGF (vascular endothelial growth factor), TGFbeta (transforming growth factor, a growth inhibitor), and the cytokines (TNF and IL-1).
   - These mediators are involved in the proliferation and production of vessels and collagen (granulation tissue) during the repair phase of inflammation.

CELL AND TISSUE REGENERATION:

Regeneration and Repair are the body response against the injury to restore normal function and structure. These both are the distinct process of healing and under the constant control of their cell cycle and these include growth factor such as epidermal growth factor, platelet derived growth factor, endothelial growth factor, transforming growth factor –β.



Regenerating or dividing cells under goes in three groups

1) **Labile Cells:** these cells continue multiply throughout life, remain in cell cycle from one mitosis to the next.

Eg. Epithelial cells of the epidermis, alimentary tract, respiratory tract, urinary tract, vagina, cervix, uterine endometrium, haematopoietic cells of bone marrow and cells of lymph nodes and spleen.

2) **Stable Cells**: Decrease or Loss their proliferation capacity after adolescent but retain the capacity during the injury or in response to certain stimuli, are in G0 Phase enter in to cell cycle by any stimuli.

Eg. Paranchymal cells of organ like liver, Pancreas, Kidney, adrenal gland;Masenchymal cells like Bone, Cartilage Cells, Smoth Muscles Cells.

3) **Permanent Cells:** After the birth these cells lose their ability to proliferate, Non-dividing cells and die after injury.

Eg. Neurons of nervous system, Skeletal Muscles and Cardiac Muscles Cells.

REPAIRS OF WOUND IN SKIN:

Wound healing is a classical example of combination of regeneration and repair. Wound healing, or wound repair, is an intricate process in which the skin (or another organ) repairs itself after injury. In normal skin, the epidermis (outermost layer) and dermis (inner or deeper layer) exists in steady-state equilibrium, forming a protective barrier against the

external environment. Once the protective barrier is broken, the normal (physiologic) process of wound healing is immediately set in motion.

The classic model of wound healing is divided into three sequential phases:

1) Inflammatory phase:

- It is the body's natural response to injury. After initial wounding, the blood vessels in the wound bed contract and a clot is formed.
- Once haemostasis has been achieved, blood vessels then dilate to allow essential cells; antibodies, white blood cells, growth factors, enzymes and nutrients to reach the wounded area. This leads to a rise in exudate levels so the surrounding skin needs to be monitored for signs of maceration.
- It is at this stage that the characteristic signs of inflammation can be seen; erythema, heat, oedema, pain and functional disturbance.
- The predominant cells at work here are the phagocytic cells; 'neutrophils and macrophages'; mounting a host response and autolysing any devitalised 'necrotic / sloughy' tissue.

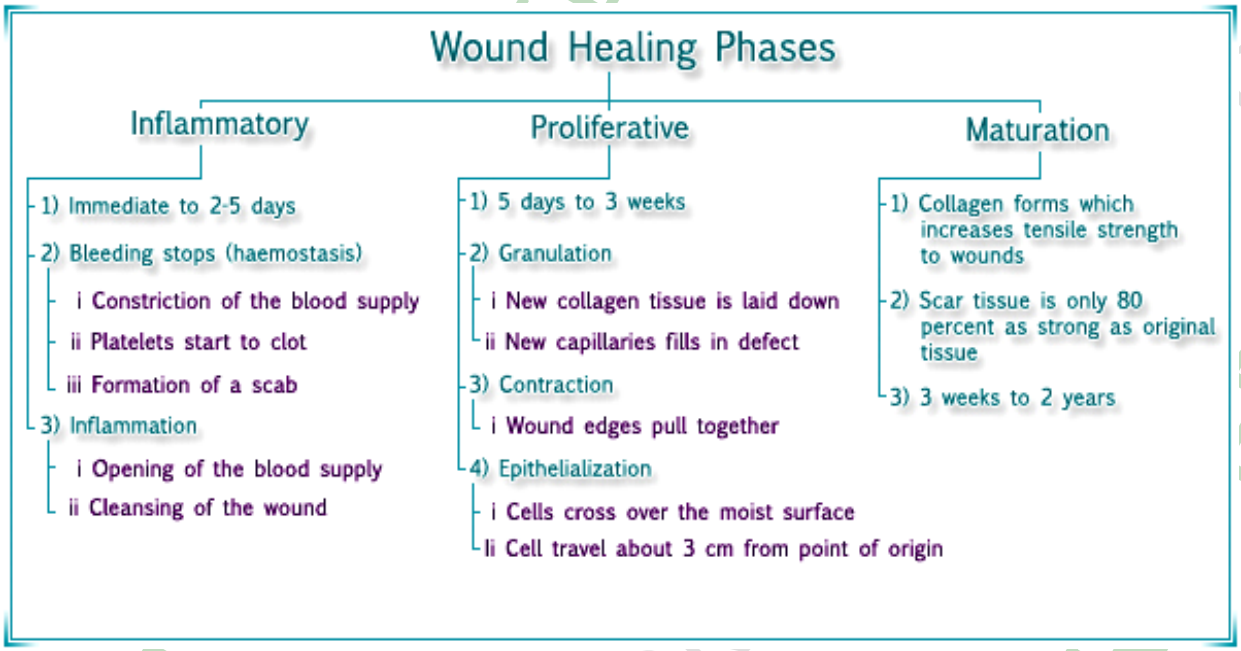
2) Proliferation:

- The wound is 'rebuilt' with new granulation tissue which is comprised of collagen and extracellular matrix and into which a new network of blood vessels develop, a process known as 'angiogenesis'.
- Healthy granulation tissue is dependent upon the fibroblast receiving sufficient levels of oxygen and nutrients supplied by the blood vessels. Healthy granulation tissue is granular and uneven in texture; it does not bleed easily and is pink / red in colour.
- The colour and condition of the granulation tissue is often an indicator of how the wound is healing. Dark granulation tissue can be indicative of poor perfusion, ischaemia and / or infection.
- Epithelial cells finally resurface the wound, a process known as 'epithelialisation'.

3) Maturation or remodeling:

- It is the final phase and occurs once the wound has closed. This phase involves remodelling of collagen from type III to type I.

- Cellular activity reduces and the number of blood vessels in the wounded area regress and decrease.



Wound healing accomplished in one of the following two ways.

1. Healing by first intention (Primary Union)
2. Healing by second intention (Secondary union)

1. Healing by first intention (Primary Union):

These types of healing have following characteristics

- Clean and uninfected
- Surgically incised
- With out much loss of cells and tissue
- Edge of wound is approximated by surgical sutures.

The sequence of primary union is described as under;

1) Initial Hemorrhage:

After the injury the blood is clot in wound space for the prevention of dehydration and infection.

2) Acute Inflammatory Response:

This occurs within 24 hours with appearance of polymorphs from the margins of incision. By, 3rd day, polymorphs are replaced by macrophages.

3) Epithelial Changes:

From the both side of cut Epithelial cell starts proliferating and migrating

↓

They form epithelial spurs in incisional space

↓

Epithelium layer is form who covers most of part of wound

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Now the viable dermis cells separate from the overlying necrotic material and clots

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Scab is formed

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In this process basal cell continue divided so superficial and deeper layer is formed

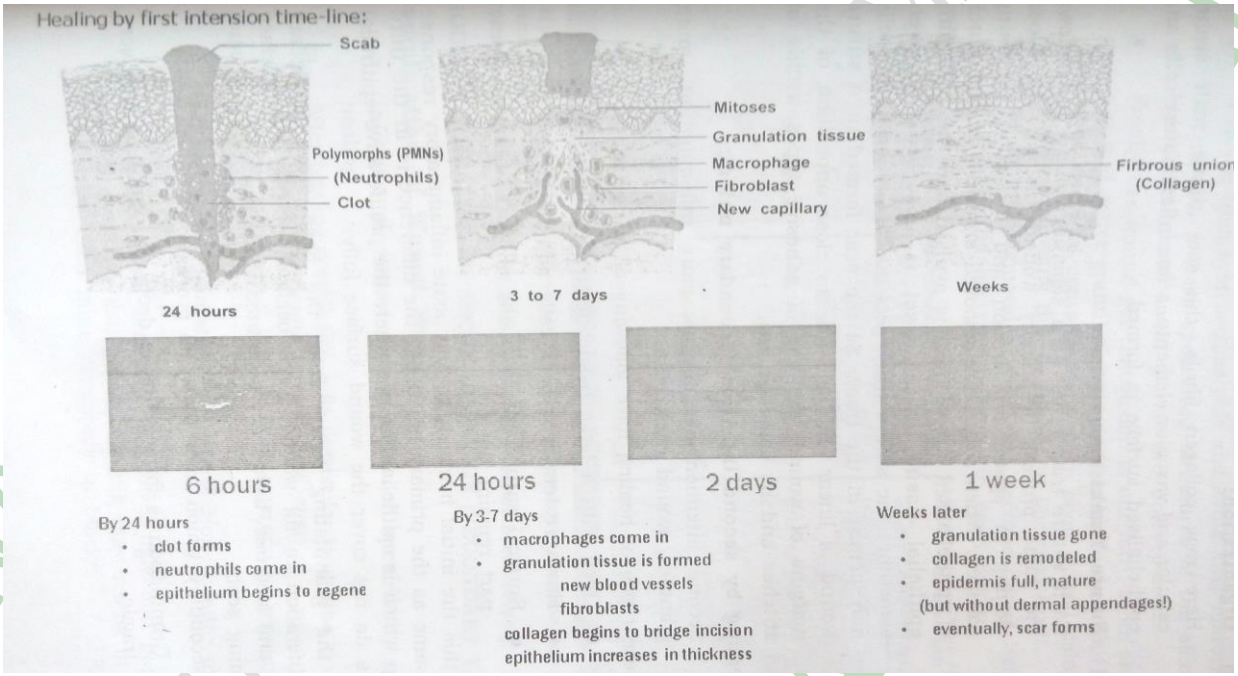

Figure 2.10: Healing by first intention (Primary union)

4) **Organization:**

Here new collagen fibrils, the scar tissue with scanty cellular and vascular elements, a few inflammatory cells and epithelialised surface is formed

5) **Suture Tracks:**

Each suture tract is act as a separate wound and follows the same process of primary wound healing like blood clotting in space, proliferation and formation of young collagen. When suture are removed around 7th day, much of epithelialised suture track is avulsed and the remaining epithelial tissue in the tract is absorbed. However, sometimes the suture track gets infected or epithelial cells may persist in the track. So

the scar formed in a suture wound is linear and neat due to close apposition of the margins of wound; the use of adhesive tapes avoids of stitches and its complications.

2. Healing by second intention (Secondary union):

These types of healing have following characteristics

- Open with a large Tissue defect, at times infected
- Having extensive loss of cells and tissue
- Surgical suture not cover the all part of injury, some part remains open.

In this the initial hemorrhage and acute inflammatory response is same as the primary union but the different is in the third step which is epithelial changes where the proliferative epithelial cells do not cover the wound surface fully.

So the granulating tissue from the base start secondary proliferation to fill wound space because of that the surface clot and necrotic tissue get separated from the viable tissue, forming scab.

In secondary union after these all process;

- Granulation is formed this is deep red, granular and very fragile.
- With the time scar become pale and white after maturation.
- But in this process specialized structures of the skin like hair follicles and sweat glands are not replaced.
- Finally the wound get contraction to one forth of its original size and active granulating tissue is being formed. But some times bacterial contamination of an open wound delay the process of healing due to release of bacterial toxin.
- But surgical removal of dead and necrotic tissue helps in preventing the bacterial infection in open wound.

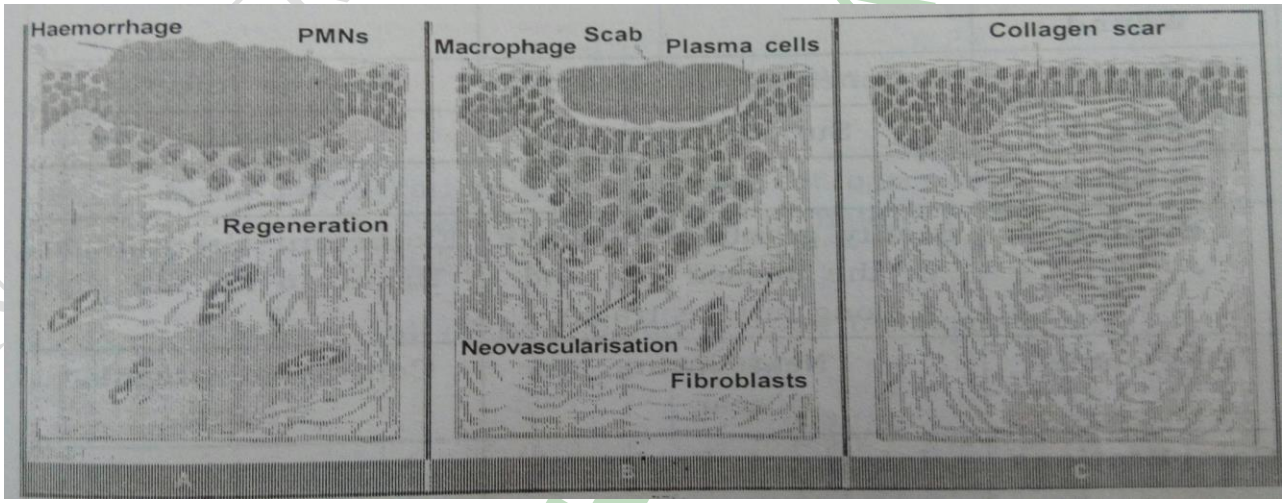

Figure 2.11: Healing by second intention (Secondary union)

Complication of Wound Healing:

a) Infection of wound due to entry of bacteria delays the healing.

b) After healing if epithelial cells persist for long time in wound it cause implantation or epidermal cyst.

c) Due to the effect of haemosiderin it produces pigmentation on skin.

d) In adequate formation of granulation tissue produce deficient scar.

e) T times scar formed is excessive, ugly and painful it formed excessive collagen in healing may result in keloid (claw like) seen more commonly in blacks.

f) Excessive contraction of wound creates plantar cavernous and peyronie's disease (contraction of the cavernous tissue of the penis) etc.

g) Rarely scar may leads to carcinoma which is known as neoplasia.

Different between primary union and Secondary Union

Features	Primary Union	Secondary Union
Cleanliness of Wound	Clean	Unclean
Infection	Generally Uninfected	May be infected
Margin	Surgical Clean	Irregular
Sutures	Used	Not Used
Healing	Scanty granulation tissue at the incised gap and along suture tracks	Exuberant Granulation Tissue to fill the gap
Out come	Neat linear scar	Contracted irregular wound
Complications	Infrequent, Epidermal inclusion cyst formation	Suppuration, may required debridement

FACTOR INFLUENCING HEALING

Local Factors

i) Infection: Delay the healin

ii) Poor blood supply: Delay healing

iii) Movement delay healing.

iv) Ionizing Radiation: Delay healing.

v) Ultraviolet light: Facilitates healings.

Systemic Factors

i) Age: Rapid in young slow in aged due to poor blood supply.

ii) Nutrition: Deficiency of vitamin C, protein and zinc delay healing.

iii) Systemic infection delay healing.

iv) Uncontrolled Diabetes: Develop infections and hence delay healing.

v) Haematologic abnormalities: Slows the healing process

PATHOLOGICAL ASPECTS OF REPAIR

Repair is the replacement of injured tissue by fibrous tissue. It is a complex system and consist series of events;

Due to the Injury

↓

For the removal of damage and dead tissue

↓

Inflammation is occur

↓

Then Proliferation and migration of parenchymal and connective tissue is held.

↓

Formation of new blood vessels (Angiogenesis) and granulation

↓

Synthesis of ECM proteins and collagen deposition

↓

Tissue remodeling

↓

Wound Contraction

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Acquisition of wound strength

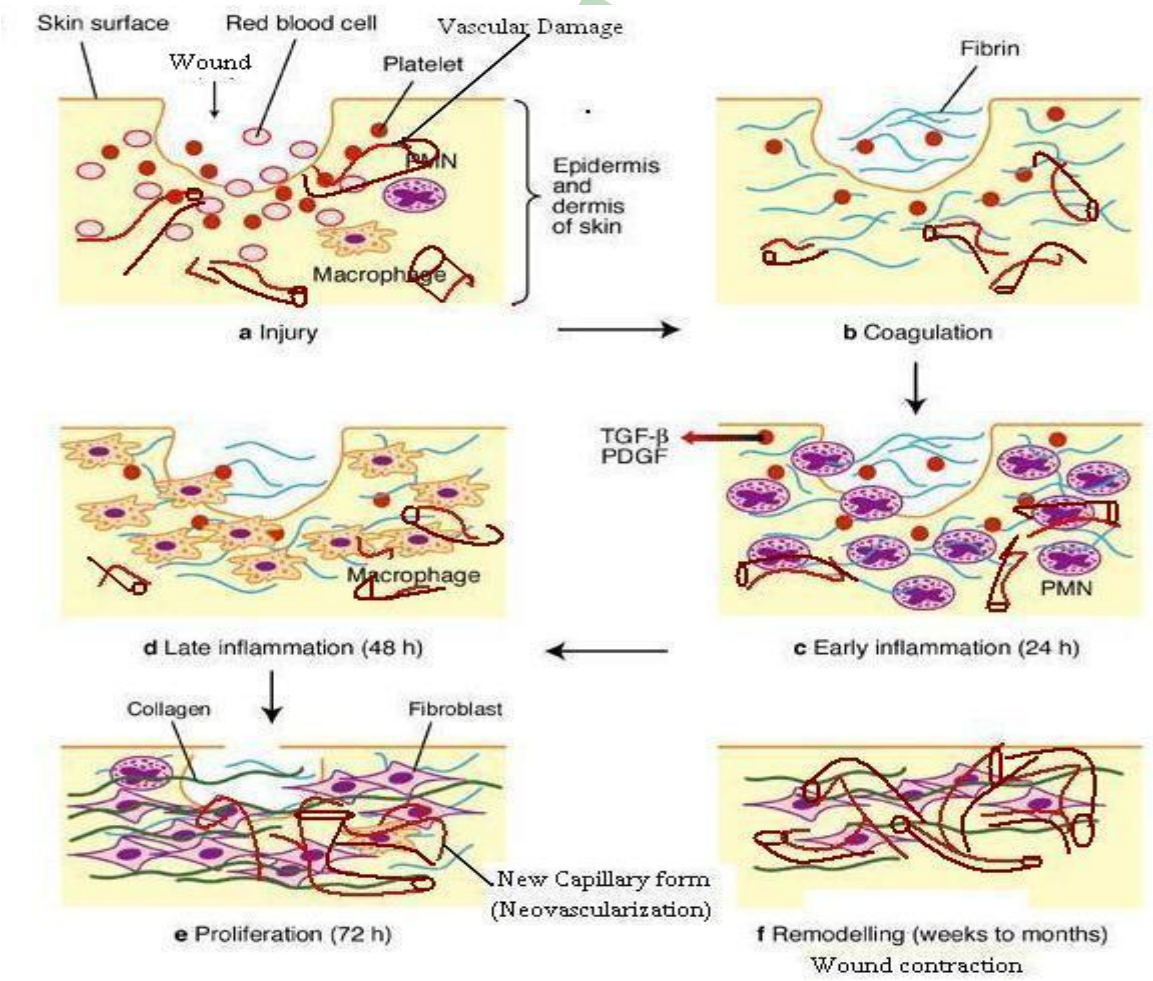

Figure 2.12: Tissue repair mechanism

This all process is very well explained by two processes:

1. Granulation tissue formation.
2. Contraction of wound.

1. **Granulation Tissue Formation:** Granulation tissue indicates granular and pink appearance of the tissue. In this,
   - Each granule proliferates and forms new small blood vessels
   - Which are slightly lifted on the surface by thin covering of fibroblast and young collagen

There are three phase are observe in the formation of Granulation Tissue

A) Phase of Inflammation: In this Phase at the site of injury trauma and blood clot is take place. This period is also known as acute inflammation in which exudation of plasma, neutrophils and some monocytes arise within 24 hours.

B) Phase of Clearance: In this phase neutrophils librates proteolytic enzymes, Dead tissue Cells liberates Autolytic enzymes and macrophages remove necrotic tissue, debris and red blood cell at site of injury by phagocytosis.

C) Phase of Ingrowths of Granulation Tissue : This Phase consist two main process

i) Angiogenesis ii) fibro genesis

i) Angiogenesis: This process is stimulated by proteolytic destruction of basement membrane. In which Vascular Growth Factor, Platelet Derived growth factor play major role. This process is also known as neovascularisation. In which proliferation of endothelial cell is arise. At starting they form solid buds but within a few hours develop lumen and start carrying blood. But here the newly formed blood vessels are more leaky later these blood vessels differentiate in to muscular arterioles, thin walled venules and true capillaries.

ii) Fibrogenesis: The newly formed blood vessels are present in an amorphous ground substance or matrix. The new fibroblast originates from fibrocytes as well as by mitotic division of fibroblasts. Some of these have morphological and functional characteristics of smooth muscle cell.

Collagen fibrils begin to appear by about 6^{th} day. At maturation process more and more collagen formed while the number of fibroblast and new blood vessels decreases. This results in inactive looking scar known as cicatrisation.

2. Contraction of Wound:

Wounds can contract at a speed of up to 0.75 mm per day, depending on how loose the tissue in the wounded area is. A large wound can become 40 to 80% smaller after contraction.

- At first, contraction occurs without myofibroblast involvement.
- Later, fibroblasts, stimulated by growth factors, differentiate into myofibroblasts. Myofibroblasts, which are similar to smooth muscle cells, are responsible for contraction. Myofibroblasts contain the same kind of actin as that found in smooth muscle cells.
- Myofibroblasts are attracted by fibronectin and growth factors and they move along fibronectin linked to fibrin in the provisional ECM in order to reach the wound edges.

- They form connections to the ECM at the wound edges, and they attach to each other and to the wound edges by desmosomes. Also, at an adhesion called the fibronexus, actin in the myofibroblast is linked across the cell membrane to molecules in the extracellular matrix like fibronectin and collagen. Myofibroblasts have many such adhesions, which allow them to pull the ECM when they contract, reducing the wound size. In this part of contraction, closure occurs more quickly than in the first, myofibroblast-independent part.
- As the actin in myofibroblasts contracts, the wound edges are pulled together. Fibroblasts lay down collagen to reinforce the wound as myofibroblasts contract the contraction stage in proliferation ends as myofibroblasts stop contracting and commit apoptosis.

GROWTH FACTORS:

Definition: "A **growth factor** is a naturally occurring substance capable of stimulating cellular growth, proliferation and cellular differentiation." Usually it is a protein or a steroid hormone.

- Growth factors are important for regulating a variety of cellular processes.
- Growth factors typically act as signaling molecules between cells. Examples are cytokines and hormones that bind to specific receptors on the surface of their target cells.
- They often promote cell differentiation and maturation, which varies between growth factors. For example, bone morphogenic proteins stimulate bone cell differentiation, while fibroblast growth factors and vascular endothelial growth factors stimulate blood vessel differentiation (angiogenesis).
- Growth factors have been increasingly used in the treatment of hematologic and oncologic diseases and cardiovascular diseases like:

✓ neutropenia
✓ myelodysplastic syndrome (MDS)
✓ leukemias
✓ aplastic anaemia
✓ bone marrow transplantation
✓ angiogenesis for cardiovascular diseases

Following are the main growth factors involved in wound healing

Growth factor	Main origins	Effects
Epidermal growth factor (EGF)	▪ Activated macrophages ▪ Salivary glands	▪ Keratinocyte and fibroblast mitogen ▪ Keratinocyte migration ▪ Granulation tissue formation
Transforming growth factor-α (TGF-α)	▪ Activated macrophages ▪ T-lymphocytes ▪ Keratinocytes	▪ Hepatocyte and epithelial cell proliferation ▪ Expression of antimicrobial peptides ▪ Expression of chemotactic cytokines
Hepatocyte growth factor (HGF)	▪ Mesenchymal cells	▪ Epithelial and endothelial cell proliferation ▪ Hepatocyte motility
Vascular endothelial growth factor (VEGF)	▪ Mesenchymal cells	▪ Vascular permeability ▪ Endothelial cell proliferation
Platelet derived growth factor (PDGF)	▪ Platelets ▪ Macrophages ▪ Endothelial cells ▪ Smooth muscle cells ▪ Keratinocytes	▪ Granulocyte, macrophage, fibroblast and smooth muscle cell chemotaxis ▪ Granulocyte, macrophage and fibroblast activation ▪ Fibroblast, endothelial cell and smooth muscle cell proliferation ▪ Matrix metalloproteinase, fibronectin and hyaluronan production ▪ Angiogenesis ▪ Wound remodeling ▪ Integrin expression regulation
Fibroblast growth factor 1 and 2 (FGF-1, -2)	▪ Macrophages ▪ Mast cells ▪ T-lymphocytes ▪ Endothelial cells ▪ Fibroblasts	▪ Fibroblast chemotaxis ▪ Fibroblast and keratinocyte proliferation ▪ Keratinocyte migration ▪ Angiogenesis ▪ Wound contraction ▪ matrix deposition
Transforming growth factor-β (TGF-β)	▪ Platelets ▪ T-lymphocytes ▪ Macrophages ▪ Endothelial cells ▪ Keratinocytes ▪ Smooth muscle cells ▪ Fibroblasts	▪ Granulocyte, macrophage, lymphocyte, fibroblast and smooth muscle cell chemotaxis ▪ TIMP synthesis ▪ Angiogenesis ▪ Fibroplasia ▪ Matrix metalloproteinase production inhibition ▪ Keratinocyte proliferation
Keratinocyte growth factor (KGF)	▪ Keratinocytes	▪ Keratinocyte migration, proliferation and differentiation

EXTRACELLULAR MATRIX:

Definition: "The **extracellular matrix** (**ECM**) is the extracellular part of tissue that usually provides structural support to the cells in addition to performing various other important functions. The extracellular matrix is the defining feature of connective tissue." Extracellular matrix includes the interstitial matrix and the basement membrane. Interstitial matrix is present between various cells (i.e., in the intercellular spaces). Gels of polysaccharides and fibrous proteins fill the interstitial space and act as a compression buffer against the stress placed on the ECM. Basement membranes are sheet-like depositions of ECM on which various epithelial cells rest.

Function:

- It gives structural support to Proliferative cells.
- Segregating tissues from one another and regulating intercellular communication.
- Regulates a cell's dynamic behavior.
- Formation of the extracellular matrix is essential for processes like growth, wound healing and fibrosis.

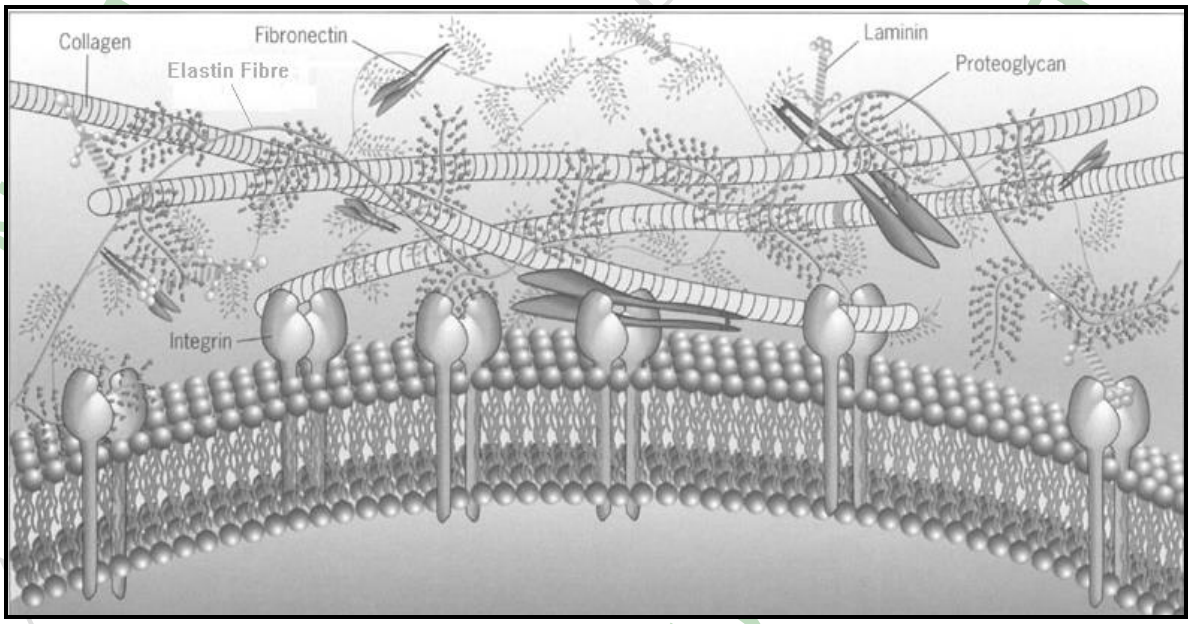

Figure 2.13: Extracellular matrix

ECM has five main components.

1. Collagen 2. Adhesive Glycoprotein (Fibronectin, Tenascin or Cytotactin, Trombospondin) 3. Basement membrane 4. Elastic Fibre 5. Proteoglycans

1) Collagen:

It is a synthesized and secreted from the ribosome. These are protein and provide structural support to multi cellular organism. Collagen synthesis and degradation is regulated by collagenase enzyme. Depending upon the biochemical compositions, 18 types of collagen have been identified.

- Out of Type I to XVIII many of them unique for specific tissue.
- Type I, III and V are true fibrillar collagen which forms the main portion of the connective tissue during healing of wounds in scar.
- Other types are Non-fibrillar and amorphous material seen as component of the basement membranes.
- Morphologically the smallest units of Collagens are collagen fibrils, which align together in parallel bundles to form collagen fibers and then collagen bundles.

2) Adhesive Glycoprotein:

It acts as Glue for the ECM. It consists

I. Fibronectins: Means to bind something. It is of two types

a. Plasma Fibronectin: Synthesized by Liver Cells and trapped in basement membrane.

Eg. : Filtration through the renal glomerules.

b. Tissue Fibronectine: Formed by Fibroblasts, Endothelial Cells and other mesenchymal cells. It is responsible for the primitive matrix such as in the foetus.

II. Tenascin or Cytotacin: it is appeared after the 48 hour of injury. It is a glycoprotein associated with fibroblasts. It is disappear after maturation of scar.

III. Thrombospondin: Manly synthesized from platelets granules. It act as adhesive protein for keratinocytes and platelets but is inhibitory attachment of fibroblasts and endothelial cells.

3) Basement membrane:

These are periodic acid-Schiff (PAS) positive amorphous structure. It consists collagen type IV and laminin. Reside underneath epithelia or endothelium of different organs.

4) Elastic Fibers:

It gives the tensile strength. It consist two components;

a) Elastin Glycoprotein b) Elastic Fibrils.

5) Proteoglycans:

These are groups of molecules having 2 components essential carbohydrates components (Polysaccharides or glycosaminoglycan) and proteins.

So it is known as proteoglycan. Which are distributed in various tissues as under:

i) Chondroitin Sulphate: Abudent in cartilage, dermis

ii) Heparan Sulphate: In basement membrane.

iii) Dermatan Sulphate: In Dermis.

iv) Keratan Sulphate: In cartilage.

v) Hyaluronic acid: In cartilage, Dermis.