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Tissue repair and regeneration

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Abstract
Tissue Repair and Regeneration is the process of regaining normal architecture as well as function of cell or tissue following an injury. Extracellular matrix components and their interaction with cell play critical role in development and healing. Growth factors are the one which plays key role in cellular signaling mechanism thereby regulate the wound healing process. Stem cell is self renewing, undifferentiated and can differentiate into any type of cell. Mesenchymal stem cells secretions by paracrine action are called as conditioned media helps in healing process. Scaffolds are support matrices made of biocompatible materials that enable cell attachment, growth and proliferation. Some of the tissue engineering techniques like use of stem cells and scaffolds helps to create artificial organs and transplantation into the patients.

Keywords: Stem cell, scaffolds, growth factors

Introduction
Life requires functional regeneration of tissues that have been destroyed or injured by trauma or illness and this process occurs in many different species. Salamanders, for example, have the rare capacity to regrow a whole leg in a matter of weeks. Even complete bodies can regenerate in some animals, such as Planarians and Cnidarians.
Most vertebrates have developed the ability to repair damage quickly before it exposes the organism to dangers outside which may leave behind non-functional tissue replacements and a fibrous matrix known as a scar (Eming *et al*., 2014) [4].
Mammals tissues with high stem cell numbers and proliferative capacity, such as the liver, blood, and intestinal epithelium, are where regeneration is most frequently seen. Moreover, resident stem cell pool's decline or depletion (caused, for example, by age) impacts cellular turnover (Spehar *et al*., 2020) [16].

What is healing?
Healing by definition is the response of a body to any injury in order to regain normal structure and function.
Healing involves two distinct processes:
a) Healing by regeneration
b) Healing by repair

Healing by Regeneration
Regeneration occurs by proliferation of parenchymal cells and restoration of the normal tissues. e.g., Regeneration by stem cells.
The cells are under the constant regulatory control of their cell cycle.
Cell cycle is the time gap between two cell divisions and is divided into 5 unequal phases (Mohan, 2018) a [12] (Figure 1)
1. M (mitosis) phase: Phase of mitosis
2. G1 (gap 1) phase: Daughter cell enters G1 phase after mitosis
3. S (synthesis) phase: it is the phase of synthesis of nuclear DNA
4. G2 (gap 2) phase: When the nuclear DNA duplication completes, the cell enters into this phase
5. G0 (gap 0) phase: After M phase if there is no nuclear DNA synthesis, the cell enters into quiescent or resting phase

Cells of the body can be divided into 3 groups based on their capacity to divide

Labile cells: Cells which divides throughout their lifespan, e.g., Epithelial cells of the skin, gastrointestinal tract etc.

Healing by repair: Repair occurs by proliferation of connective tissue which usually results in fibrosis and scar formation.

Molecular events in cell growth

It involves signalling pathways, signalling is the method of communication of cells through signals.

Mainly three pathways of signalling are there:

1. Autocrine signalling
2. Paracrine signalling
3. Endocrine signalling

Autocrine signalling: When signalling substance acts on the same cell that secretes it, therefore it plays a role in compensatory epithelial hyperplasia

Paracrine signalling: When a cell produces signalling substances that only acts on surrounding target cells. It is important in the connective tissue repair of wounds.

Endocrine signalling: Signalling substances which are produced in the endocrine organs act on target cells away from their site of synthesis by blood as medium of transportation e.g., endocrine hormones (Vegad, 1995) [19].

Cell surface receptor

Growth of a cell begins by binding of signalling molecule to specific receptor.

Three major types of cell surface receptors (Figure 2) as follows:

1. **Receptors with intrinsic kinase activity:** Contain two regions, an extracellular ligand- binding domain and intracellular cytosolic domain. e.g., Epidermal Growth Factor (EGF), Fibroblast Growth Factor (FGF) etc., acts as ligands and PI3-kinase pathway (Phosphatidyl-inositol-3 kinase), MAP kinase pathway etc., act through these receptors.
2. **G-protein-linked receptors:** It consists of seven loops that span across the cell membrane, it include receptors for chemokines, epinephrine (adrenaline) and glucagon e.g., cAMP pathway.
3. **Receptors without intrinsic kinase activity:** it includes single molecule that spread across the cell membrane, consists of extracellular domain for ligand binding and a cytosolic domain. Structural change in the cytosolic domain is induced by ligand binding and activate intracellular protein kinases which in turn, phosphorylate the receptor complex and stimulate a down-stream activation sequence involves janus kinases (JAKs) and signal transducers and activators of transcription (Vegad, 1995) [19].

Signal transduction system

Signal transduction is the process by which extracellular signals are recognized and transformed into intracellular signals, which in turn produce certain biological responses. The most important kinase pathway involved in the regulation of cell growth are as follows:

a) Phosphatidyl inositol 3-kinase (PI3-kinase)
b) Mitogen-activated protein kinase (MAP-kinase)
c) Inositol triphosphate (IP3)
d) Cyclic adenosine monophosphate (cAMP)
e) Janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling System

Phosphatidyl inositol 3-kinase (PI3- kinase) pathway

A group of intracellular kinases are activated by second messengers that are produced by kinase. These kinases' activity eventually causes biological reactions that are related to cell survival, like phosphorylation of glycogen synthase kinase 3 and enhanced glycogen production.

Mitogen-activated protein kinase (MAP) pathway

When a receptor with tyrosine kinase activity binds to a ligand, the receptor undergoes autophosphorylation, which in turn causes adaptor proteins to attach. This pathway is crucial for the production of growth factors. At some point, the RAS (Rat Sarcoma Protein) is activated by these proteins. RAS is a member of the group of proteins known as guanosine triphosphatases (GTPases). MAP-kinases are then energized by RAS. Nuclear transcription factors are activated by the distal MAP kinase as it enters the nucleus, and this in turn triggers gene expression. In the end, this causes dormant cells to be stimulated to begin the cell cycle. (Vegad, 1995) [19].

Inositol triphosphate pathway (IP3)

Both G-protein-linked receptors and tyrosine kinase receptors can be connected to the IP3 signaling mechanism. When a ligand is bound, a G-protein is activated, which then activates phospholipase C. Inositol triphosphate (IP3) and diacylglycerol (uAG) are produced when phospholipase C breaks down PIP2 (phosphatidyl inositol bisphosphate). The endoplasmic reticulum's IP3-sensitive calcium channels are contacted by the IP3 as it diffuses into the cytoplasm, releasing calcium ions and triggering cellular reactions (Vegad, 1995) [19].

Cyclic adenosine monophosphate (cAMP) pathway

Adenylate cyclase is activated and second messenger cAMP is produced when hormones like adrenaline, glucagon, and chemokines bind to G-protein-linked receptors. Increased cAMP levels trigger a cascade of events that activate protein kinase A and increase the expression of target genes (Vegad, 1995) [19].

Janus kinase/signal transducers and activators of transcription JAK/STAT pathway

After ligand binds to receptors devoid of tyrosine kinase activity, these receptors link up with and activate one or more Janus kinases (JAKs), which are protein kinases found in the cytoplasm. The downstream proteins known as signal transducers and activators of transcription (STATs) as well as the receptors are both phosphorylated by JAKs (Vegad, 1995) [19].

Extracellular matrix (ECM)

In order to maintain healthy tissue architecture and to promote growth and healing, cell interactions with the ECM are essential.

ECM performs the following crucial tasks:

- Mechanical aid for cell migration, cell anchoring, and cell polarity
- Provides a growth factor depot and promotes cell proliferation

- Establishing scaffolds for tissue microenvironments and tissue renewal

Components of ECM: (Figure 3 and 4)

Interstitial matrix

- Elastin
- Fibrillar collagens (type I, III, V)
- Proteoglycon and Hyaluronan
- Adhesive glycoprotein
- Fibronectin
- Laminin
- Integrins

Basement membrane

- Non fibrillar collagen (type IV)
- Proteoglycon
- Adhesive glycoprotein
- Fibronectin
- Laminin

Role of ECM components

- Collagen-fibrous structural proteins which gives tensile strength
- Elastin gives tissue elasticity
- Proteoglycans-act as GFs reservoirs(bFGF)
- Fibronectin-attachment, spreading and migration of cells
- Laminin-regulates proliferation, differentiation of cells
- Integrins – mediates attachment of cell to ECM

Growth factors in wound healing

Whenever there is wound, the blood platelets gather along with collagen to produce a clot to stop loss of blood (Mohan, 2018) [12]. The primary and dominant inflammatory cells are neutrophils, which are brought on by chemical signals. Elastase, protease and collagenase are a few of the enzymes that are released once they are activated and help to remove damaged tissues from the wound site. Inflammation is triggered by platelets, which also produce growth factors like epithelial growth factor (EGF), platellet derived growth factor (PDGF), transforming growth factor (TGF-b1), and interleukin-1 (IL-1). They use unique strategies to lure neutrophils to the site of the wound and into macrophages. Mast cells are drawn to the site of the wound by the monocyte chemotactic protein i.e., TGF-b1, which also causes them to release histamine, proteoglycans, proteases, and platelet activating factor. Inflammatory cells release TGFb1 and IL-4 as soon as they become active, which represses them and causes inflammation to decrease.

Macrophages production of TNF-a and IL-1 triggers and controls the proliferation of fibroblasts and endothelial cells. The granulation tissue is mainly composed of extracellular matrix, is formed by release of collagen and other glycosaminoglycans by activated fibroblasts. Angiogenesis, which is required for the granulation tissue to grow further, is started by basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) produced by endothelial cells, keratinocytes, and macrophages (Zachary *et al*., 2012) [21].

Increased blood vessel structural integrity is a result of PDGF, which is released by degranulating platelets. The connective tissue gradually replaces the granulation tissue during remodelling, adding to the material's elasticity and tensile strength. The connective tissue growth factors make up the majority of the contribution (Vaidyanathan, 2021) [20].

Epidermal growth factor (EGF)

EGF (epidermal growth factor) promotes division, proliferation, and growth of cell. EGF has a crucial role in controlling epithelial cell motility and promotes reepithelialization. By promoting fibroblast migration and proliferation, it assists in wound contraction. According to certain research, EGF and insulin-like growth factor-1 (IGF-1) work together to promote the proliferation of keratinocytes in culture (Kim *et al*., 2019) [7].

Fibroblast growth factor (FGF)

The expression of myofibroblast markers is considerably downregulated by basic fibroblast growth factor (bFGF), while epidermal stem cell markers and Notch1/Jagged1 signaling are significantly upregulated. Tissue remodeling occurs as a result of the bFGF's mitogenic and angiogenic characteristics.

Transforming growth factor (TGF)

The human TGF-b superfamily, which consists of over 33 different proteins, is crucial for the regulation of tissue homeostasis in multicellular organisms through cellular signaling. It is an essential cytokine that starts intracellular signaling pathways by activating type II serine threonine kinase receptors, which in turn affects the transcription of genes that regulate wound healing at all stages (Mahiddine *et al*., 2020) [10].

TGF-b1 plays a critical function in myofibroblast development and boosts angiogenesis by encouraging endothelial progenitor cells to facilitate blood supply to the wound site.

Platelet derived growth factor (PDGF)

Platelet derived growth factor (PDGF) helps in mitogenesis of fibroblasts and many cells, angiogenesis and chemotaxis. Activation of these receptors induce chemotaxis in certain cell types, however PDGF receptors are found to inhibit chemotaxis of only few selected cell types like the fibroblasts and smooth muscle cells (Pati *et al*., 2016) [13].

Vascular endothelial growth factor (VEGF)

Proangiogenic VEGF (vascular endothelial growth factor) stimulates angiogenesis. Activated platelets at the site of the lesion release VEGF-A, which attracts circulating neutrophils and monocytes when it binds to the VEGF receptor-1 on inflammatory cells. Other pro-inflammatory cytokines, such as IL-1 and TNF-a, are taken in by the neutrophils and enter the process.

It is crucial for fostering vascularity and healing in chronic hypoxic wounds. The expression of VEGF and its receptors may be regulated by hypoxia, a condition brought on by a traumatic wound or an ischemic injury (Singh *et al*., 2018) [17].

Granulocyte macrophage colony stimulating factor (GM-CSF)

The keratinocytes release GM-CSF (granulocyte macrophage colony stimulating factor), which has an autocrine effect to encourage epidermal growth. Lack of GM- CSF resulted in inappropriate collagenous matrix synthesis in the blood vessels, demonstrating the need of preserving vascular integrity.

Additionally, GM-CSF is chemotactic towards a variety of inflammatory mediators and cells, which indirectly promotes wound inflammation and healing (Mahiddine *et al*., 2020) [10].

Connective tissue growth factor (CTGF)
Many biological processes, including cell division, adhesion, and angiogenesis, are governed by the protein known as connective tissue growth factor. The amount of healing and the level of healing are both influenced by collagen manufacture and deposition. CTGF controls scarring in addition to acting as a chemotactic for fibroblasts (Reilly *et al*., 2021) [15].

Repair
Replacement of injured tissue by fibrous tissue is called as repair.
Mainly two processes are involved in repair:
1. Granulation tissue formation
2. Contraction of wounds.

Mesenchymal cells, including connective tissue stem cells, fibrocytes, and histiocytes, endothelial cells, macrophages, platelets, and the parenchymal cells of the wounded organ, all contribute to the repair response.

Granulation Tissue Formation
The phrase "granulation tissue" gets its name from the tissue's granular, pink appearance. According to histology, each granule represents the growth of new, small blood vessels that are superficially raised by a thin layer of fibroblasts and immature collagen.
The three phases which are observed in the formation of granulation tissue as follows:
1. **Phase of Inflammation**: Blood clots form at the site of damage after any trauma. Within 24 hours, there is an initial inflammatory response with the exudation of plasma, neutrophils, and a few monocytes.
2. **Phase of Clearance**: The necrotic tissue, debris, and red blood cells are removed by a combination of proteolytic enzymes released from neutrophils, autolytic enzymes from dead tissues cells, and phagocytic activity of macrophages.
3. **Phase of Ingrowth of Granulation tissue**: This phase consists of two main processes:

a) Angiogenesis or neovascularization phase
b) Fibrogenesis phase

Angiogenesis phase (Figure 5)
Endothelial cell proliferation from the edges of blood vessel fragments causes the formation of new blood vessels at the site of injury.
Initially solid buds, the proliferating endothelial cells soon acquire a lumen and begin to convey blood. The oedematous look of fresh granulation tissue is caused by the newly created blood vessels, which are more leaky. These blood arteries soon separate into muscular arterioles, venules with thin walls, and genuine capillaries.
The process of angiogenesis is stimulated with proteolytic destruction of basement membrane.
Angiogenesis takes place under the influence of following factors as follows:
a) Vascular endothelial growth factor: Mesenchymal cells produce vascular endothelial growth factor (VEGF), however only endothelial cells have its receptors.
b) Cellular proliferation is correlated with surface integrins, platelet-derived growth factor (PDGF), transforming growth factor- beta (TGF-beta), and basic fibroblast growth factor (bFGF).

Fibrogenesis phase: Blood vessels which are newly formed are present in an amorphous ground substance or matrix. The new fibroblasts originate from fibrocytes as well as by mitotic division of fibroblasts. Some of these fibroblasts have combination of morphologic and functional characteristics of smooth muscle cells (Myofibroblasts). Collagen fibrils begin to appear by about sixth day. As maturation proceeds, more and more of collagen is formed while the number of active fibroblasts and new blood vessels decreases. This results in formation of inactive looking scar known as cicatrisation (Mohan, 2018) [12].

Wound Healing (Figure 6)
One of the following two methods can be used to repair wounds:
- Healing by first intention (primary union)
- Healing by second intention (Secondary union)

Healing by first intention
Healing of a wound with the following features is referred to as healing by first intention.
1. Clean and free from infection
2. Surgically incised
3. Little loss of cells and tissue
4. Surgical sutures are used to approximate the edges of the incision sutures

Sequence of healing by first intention
Initial haemorrhage: Blood immediately after injury fills the area between the approximated surfaces of the incised wound, clotting it to protect it from infection and dehydration.

Inflammatory response: Within 24 hours, there is an acute inflammatory response, and polymorphs start to develop around the incision margins. By the third day, macrophages replace polymorphs.

Epithelial changes: Both cut margins' basal epidermal cells begin to proliferate and migrate toward the incisional area in the form of epithelial spurs. A layer of epithelium covers a well-approximated wound in 48 hours. The migrating epidermal cells coagulate, generating a scab that is peeled off, and separate the necrotic material from the overlying, viable dermis. The margin-derived basal cells keep dividing. A multilayered new epidermis that is distinct into superficial and deeper layers is produced by the fifth day.

Organization: The wound region is also invaded by fibroblasts by the third day. By the fifth day, new collagen fibrils begin to grow and take over until the healing process is complete. Scar tissue with sparse cellular and vascular components, a few inflammatory cells, and an epithelialized surface forms in the fourth week.

Suture tracks: Each suture track is a distinct wound that undergoes the same healing processes as the underlying wound. (Mohan, 2018) [12].

Healing by Second Intention (Secondary Union)

This is characterized as the wound healing exhibiting the following traits:

1. Open with a significant tissue defect that is occasionally infected.
2. Extensive loss of cells and tissues
3. The wound is not closed with surgical stitches but is instead left open.

Similar to primary union in terms of the fundamental events, secondary union is distinguished by the bigger tissue defect that needs to be bridged. So, both from the edges inward and from the base up, healing occurs. When compared to the primary union's quick healing and tidy scar, the healing caused by the second purpose is gradual and leaves a noticeable, occasionally unattractive scar.

1. **Initial haemorrhage:** After an injury, a fibrin clot and blood fill the wound space, which then dries.
2. **Inflammatory phase:** After an initial acute inflammatory response, macrophages appear and remove the debris, similar like in primary union.
3. **Epithelial changes:** Similar to primary healing, epidermal cells from both wound margins multiply and move into the wound as epithelial spurs until they meet in the middle and completely reepithelialize the gap. However, the proliferating epithelial cells do not completely cover the surface until the wound space has begun to fill with granulation tissue from the base.
4. **Granulation tissue:** Fibroblast proliferation and neovascularization from the surrounding viable elements result in the formation of granulation tissue. The freshly created granulation tissue is granular, deep crimson, and extremely delicate. Due to an increase in collagen and a decrease in vascularity, the maturation scar eventually turns pale and white. Without their viable remnants, which may regenerate, specialized skin structures like hair follicles and sweat glands are not restored.
5. **Wound contraction:** A crucial aspect of secondary healing that is absent from primary healing is the contraction of the wound. The wound shrinks to a third to a fourth of its original size thanks to the activity of myofibroblasts found in granulation tissue. When active granulation tissue is forming, wound contraction takes place.
6. **Presence of infection:** Due to the production of bacterial toxins that cause necrosis, suppuration, and thrombosis, bacterial contamination of an open wound slows the healing process.

Factors Influencing Healing

Two types of factors influence the wound healing:

Local Factors

The primary localizing factor that slows the healing process is infection.

1. Wounds with inadequate blood flow heal more slowly, as in the case of leg injuries with varicose ulcers.
2. Foreign objects, such as sutures, impede healing and trigger a severe inflammatory response and infection.
3. Motion prevents a wound from healing
4. Granulation tissue development is delayed by ionizing radiation exposure.

Systemic Factors

1. Age: Young people heal wounds more quickly than elderly and disabled people, who have insufficient blood flow to the damaged area.
2. Nutrition: A lack of components like protein, vitamin C (scurvy), and zinc causes the healing of wounds to be delayed.
3. Systemic infection hinders the healing of wounds.
4. Glucocorticoid administration has an anti- inflammatory effect.
5. Uncontrolled diabetics are more likely to get infections, which causes their recuperation to take longer. (Vagad, 1995) [19].

Liver Regeneration

Regeneration of the liver occurs by two major mechanisms:

1. Proliferation of remaining hepatocytes
2. Repopulation from progenitor cells

Proliferation of remaining hepatocytes (Figure 7)

Hepatocyte proliferation in the regenerating liver is triggered by the combined actions of cytokines and polypeptide growth factors. The process occurs in distinct stages. In the first or priming phase, cytokines such as IL•6 are produced mainly by Kupffer cells and act on hepatocytes to make the parenchymal cells competent to receive and respond to growth factor signals. In the second or growth factor phase, growth factors such as HGF and TGF-α, produced by many cell types, act on In the regenerating liver, cytokines and polypeptide growth factors work together to promote hepatocyte proliferation. There are various phases to the procedure. In the first, or priming, phase, Kupffer cells release the majority of the cytokines, like IL•6, which operate on hepatocytes to prepare them to recognize and react to signals from growth factors. In the second phase, known as the growth factor phase, many different cell types release growth factors like HGF and TGF, which work on primed hepatocytes to increase cell metabolism and the entry of the cells into the cell cycle.

Hepatocytes require several hours to begin the cell cycle since they are dormant cells. Following the wave of hepatocyte replication, non-parenchymal cells (Kupffer cells, endothelial cells, and stellate cells) replicate. Hepatocytes return to quiescence in the last phase of the termination process (Kumar *et al*., 2014) [8].

Liver regeneration from progenitor cells (Figure 8)

Progenitor cells in the liver aid in repopulation when the ability of hepatocytes to proliferate is compromised, as is the case with chronic liver injury or inflammation. Due to the shape of their nuclei, these progenitor cells are known as oval cells in rodents. Some of these progenitor cells are found in the Canals of Hering, which are specialized bile canaliculi junctions with larger bile ducts.

Fracture Healing (Figure 9)

Whether a fracture can be healed by callus development relies on various clinical factors, including:

- Traumatic
- Pathological

1. Complete or incomplete like green-stick fracture
2. Simple (closed)
3. Comminuted (splintering of bone)
4. Compound (communicating to skin surface)

But the fundamental processes in the healing of any kind of fracture are the same. When the ends of the fracture are approximated, as is done by applying compression clamps, primary union of fractures happens in a select few specific circumstances. In these situations, bone union occurs together with medullary callus formation but not periosteal callus formation. The method of fracture healing known as secondary union is more prevalent. The three phases below describe secondary bone union:

1. Procallus formation
2. Osseous callus formation
3. Remodelling

Procallus Formation: The following steps are involved in procallus formation:

1. A haematoma arises around the fracture as a result of bleeding from blood vessels that have been ruptured.
2. Fibrin, polymorphs, and macrophages exude from the injury site as part of the local inflammatory response. Red blood cells, fibrin, inflammatory exudate, debris, and inflammatory exudate are removed by macrophages. Macrophages and osteoclasts scavenge fragments of necrosed bone.
3. Neovascularization and mesenchymal cell proliferation from the periosteum and endosteum are the first steps in the ingrowth of granulation tissue. Thus, a weakly joined soft tissue callus forms to unite the ends of shattered bone.

Within the first several days, a callus formed of braided bone and cartilage begins to form. In the granulation tissue, the cells of the inner layer of the periosteum, which have osteogenic potential, lay down both collagen and osteoid matrix. The osteoid becomes woven bone callus after going through calcification. Calluses momentarily immobilize the ends of the bones because they are made of braided bone and cartilage. External, intermediate, and internal procallus are arbitrary divisions of this stage, which is also known as provisional callus or procallus formation.

Osseous Callus Formation: The osseous callus made of lamellar bone is created on the procallus, which serves as a scaffolding. Arriving osteoclasts remove the woven bone, and the calcified cartilage breaks down. They are replaced by freshly created blood vessels and osteoblasts, which infiltrate and lay down calcified osteoid while forming lamellar bone by concentrating the development of the Haversian system around the blood vessels.

Remodelling: Osteoblastic laying and osteoclastic removal rebuild the connected bone ends during the creation of lamellar bone, which over time becomes identical to regular bone. The interior callus develops a bone marrow cavity, the intermediate callus is replaced by compact bone (cortex), and the external callus is removed (Mohan, 2018) [12].

Nervous system regeneration

Central nervous system

Once destroyed, the nerve cells in the brain, spinal cord, and ganglia are not regenerated. CNS axons do not exhibit any appreciable regeneration either. However, the injured neuroglial cells may exhibit gliosis, or astrocyte proliferation.

Peripheral nervous system (Figure 10)

The regeneration of peripheral nerves is mostly due to the growth of schwann cells and fibrils at the distal end. The undamaged distal nerve's axon and myelin sheath both go through wallerian degeneration until they reach the next Ranvier node at the proximal end. Macrophages remove deteriorated trash from the environment. From the viable end of the axon, regeneration takes the form of sprouting fibrils. The peripheral stump eventually develops into a tube packed with elongated Schwann cells in six to seven weeks as a result of these fibrils growing along the path of the degenerating nerve. A proximal stump fibril enters the old neural tube and grows into a new, functional axon.

Role of immunity (Figure 11)

A short time after damage, platelets are attracted in and release chemoattractants such thromboxane A2, serotonin, and ADP (adenosine diphosphate) to attract more platelets to the injury site. A fibrin-rich temporary matrix is entered by leukocytes and thrombocytes, where platelets degranulate and initiate the complement cascade. The resulting DAMPs (Damage -associated molecular patterns) help trigger the inflammatory immune system.

First, chemokines from the CXCL8 family facilitate neutrophils' migration to the site of the damage. Here, pathogens are captured by NETosis (NET-neutrophil extracellular traps), released ROS (Reactive oxygen species), and other antimicrobial compounds, and then phagocytosed. They expel a variety of cytokines and growth factors that encourage angiogenesis, the recruitment of inflammatory cells, and the growth of cells like fibroblasts and epithelial cells. These neutrophils also secrete MCP-1, which when in touch with additional pro-inflammatory stimuli in the injury microenvironment triggers circulating monocytes to quit surrounding blood vessels and change into macrophages. ILC1s and NK cells now release IFN, a cytokine that promotes inflammation and feeds the inflammatory milieu. Additionally, tissue- resident macrophages are encouraged by pro- inflammatory mediators to proliferate and specialize at the injury site. During this inflammatory stage of tissue repair, pro- inflammatory/M1-like macrophage subpopulations predominate. Inflammatory immune cell recruitment is sustained by the pro-inflammatory secretome of M1-like macrophages.

As the phases of inflammation and repair advance, the macrophage subpopulation changes from being mostly M1-like to M2- like (this can be fueled by IL-13 produced from ILC2) and secretes anti-inflammatory and pro-resolutory cytokines like TGF-1, as well as depositing ECM. Strong anti- inflammatory effects are produced by M2-like macrophages, which also suppress the M1-like phenotype by secreting IL-10 and mature The macrophage subpopulation shifts from being mostly M1-like to M2-like as the phases of inflammation and repair progress (this can be fuelled by IL-13 produced from ILC2), secretes anti-inflammatory and pro-resolutory cytokines including TGF-1, as well as depositing ECM. Strong anti-inflammatory effects are produced by M2-like macrophages, which additionally secrete IL-10 to decrease the M1-like phenotype and PDGF-BB to mature newly formed vessels. The two most crucial T cell subsets in tissue healing and remodeling are the Th2 and Treg subsets, both of which release molecules that help create the matrix, such as TGF-1, IL-4, IL-5, IL-13, and IL-21. The cytokines that macrophages release draw T lymphocytes to them. In the

later stages of healing, Amphiregulin produced by Tregs promotes immune cell growth, proliferation, and anti-inflammatory activity. (Alaribe *et al*., 2016) [1].

Stem cells

The specialized cells known as stem cells have the capacity to self-renew, multiply, and develop into a range of body tissues and organs.

Classification of stem cells:

On the basis of sources

1. Embryonic stem cells
2. Adult stem cells
3. Induced pluripotent stem cells

On the basis of differentiating ability

1. **Totipotent:** Able to differentiate into all cellular kinds inside the body, including placental cells
2. **Pluripotent:** Able to differentiate into any bodily tissue besides the placenta
3. **Multipotent:** Able to differentiate into only a few types of specialized cells

All distinct differentiated tissues are created by stem cells during development, and stem cells also replace injured cells and maintain tissue populations.

There are two basic ways MSCs can improve the healing of wounds (Figure 12):

1. Release of important cytokines and growth factors together with inflammatory mediators.
2. The cells themselves take part in the healing of wounds by differentiating into the cell types necessary for wound closure. Also by increasing angiogenesis, re-epithelialization, and granulation tissue formation (Isakson *et al.*, 2015) [6].

Recruit certain cell types to the wound, such as epidermal keratinocytes, dermal fibroblasts, and endothelial cells, by acting as chemo attractants (Lee *et al.*, 2012) [9].

Conditioned media (CM) (Figure 13)

Culture media that contains VEGF, SCF, EGF, IGF-1, IGF-2, and SDF-1 is known as CM rich in secretome.

This strategy is known as "stem cell-based cell-free therapy''. (Gunawardena *et al*., 2019) [5].

Effects of Conditioned media (CM)

- Increase cellular proliferation
- Decrease Apoptosis
- Reducing inflammation & immune reactions
- Helps in neovascularization
- Chemotactic signalling & activation of neighbouring cells

Whether directly from the cells or through their derivatives, mesenchymal stem cells have the ability to emit substances that change the biological processes of target tissues or cells.

Study conducted on how dog sperm cryopreservation was affected by conditioned Study on the effects of conditioned medium (CM) from MSCs made from canine amniotic membrane (cAMSCs) on dog sperm cryopreservation.

After 4-6 hours of cooling at 4 degrees Celsius, kinetic characteristics were used to determine the appropriate concentration before cryopreservation in the case of sperm samples treated with freezing fluid supplemented with 0%, 5%, 10%, and 15% of the CM.

The study of chromatin and acrosome integrity revealed no significant differences between the treatment and control groups, however the results showed that 10% of the CM considerably increased motility, viability, mitochondrial activity, and membrane integrity (p 0.05).

Concluded that 10% CM made from cAMSC added to the freezing media during the cryopreservation process preserved dog sperm. (Mahiddine *et al*., 2020) [10].

Scaffold (Figure 14)

Biocompatible support matrices known as scaffolds promote cell adhesion, growth, and proliferation. Biological scaffold is the one which composed of allogeneic or xenogeneic extracellular matrix

Case study (Figure 14.a to 14.d)

Buffalo calves with large umbilical hernias pose a clinical challenge to surgeons because to their enormous size rings and severe distortion of the hernial boundaries.

Hernioplasty with the implantation of biomaterials of synthetic or animal origin is necessary whenever the diameter of the hernial ring is greater than six cm in such circumstances. In order to herniate the umbilicus in buffalo calves, a biomaterial called extracellular matrix produced from bubaline rumen was created and tested. Hernias could be repaired without tension thanks to the bubaline rumen-derived extracellular matrix, which also significantly reduced postoperative pain, the length of the healing process, and recurrence (Singh *et al*., 2018) [17].

3D Bioprinted tissue/organ models (Figure 15)

In order to create complex living tissues and organs with the desired 3D cellular architecture and functions, 3D bioprinting is a computer- assisted technology that involves the rapid printing of biofunctional materials and their supporting components in a layer-by- layer fashion on a substrate or a tissue culture dish. Applications for 3D The creation of a cell-specific microenvironment, testing of drugs or chemicals on human-specific models, integration of microfluidics with 3D structures, fabrication of vascularized tissue constructs, extension of cell viability and functionality, potential for creating tissue- tissue interfaces, and automated and reproducible production of tissue constructs are all aspects of bioprinted tissue (Pati *et al*., 2016) [13].

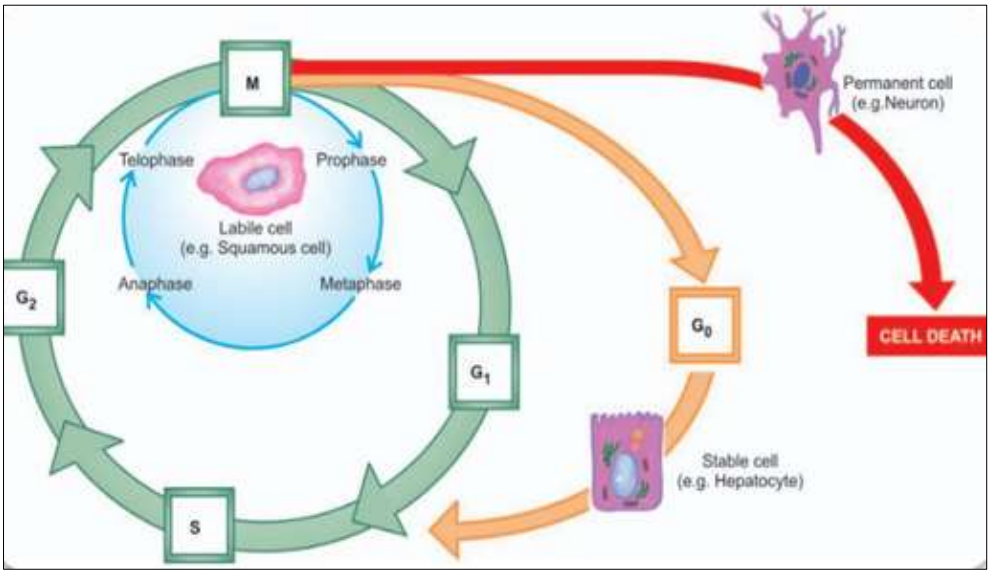

Fig 1: Schematic representation of Cell cycle (Mohan, 2018) [12]

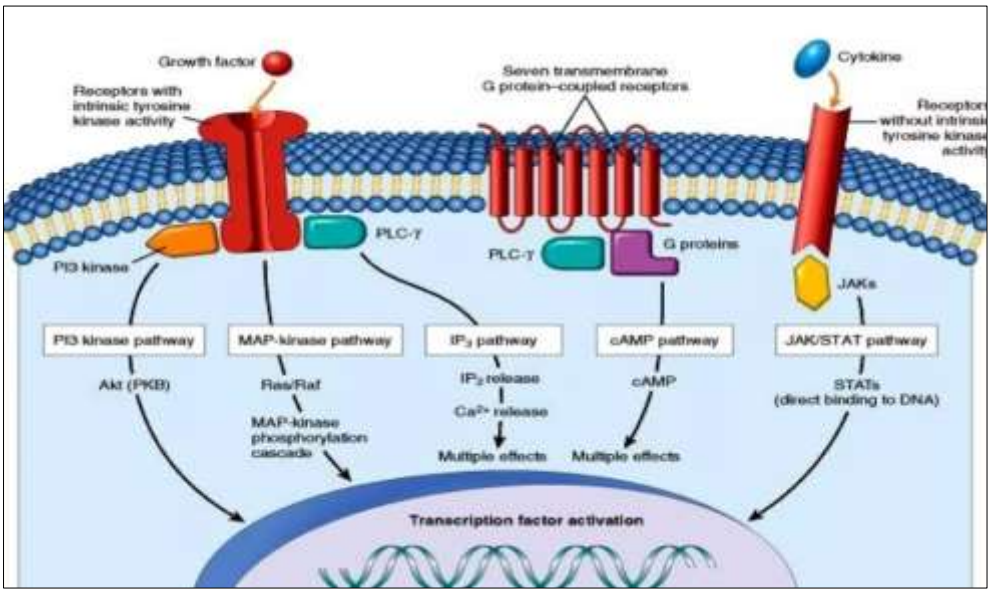

Fig 2: Cell surface receptors (Kumar *et al*., 2014) [8]

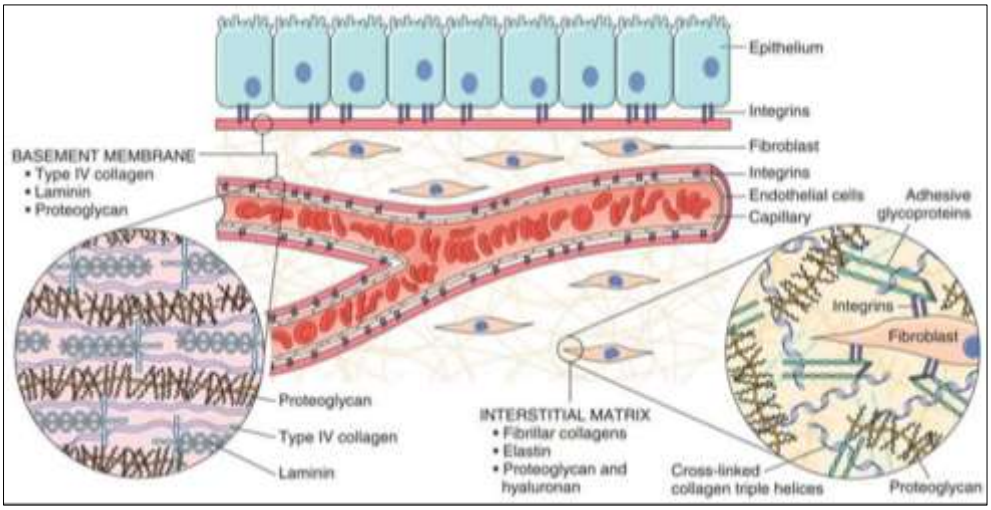

Fig 3: Schematic representation of Components of ECM (Kumar *et al*., 2014)[8]

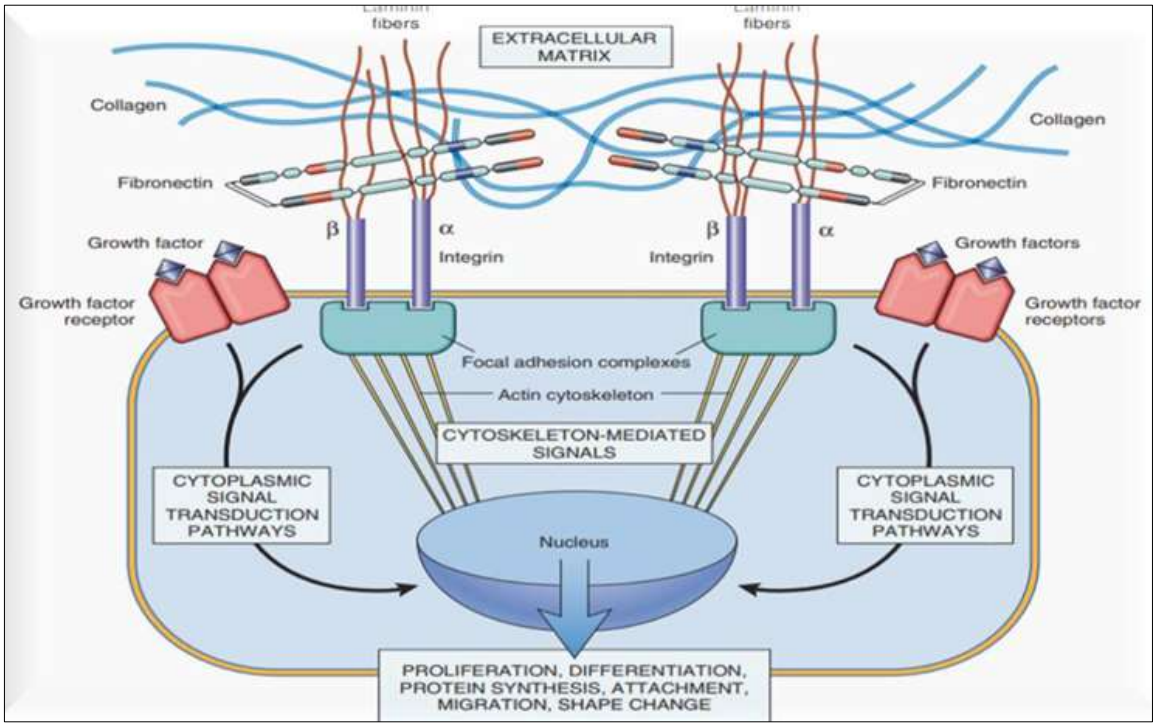

Fig 4: Schematic representation of Interaction of ECM with cell (Zachary, 2012) [21]

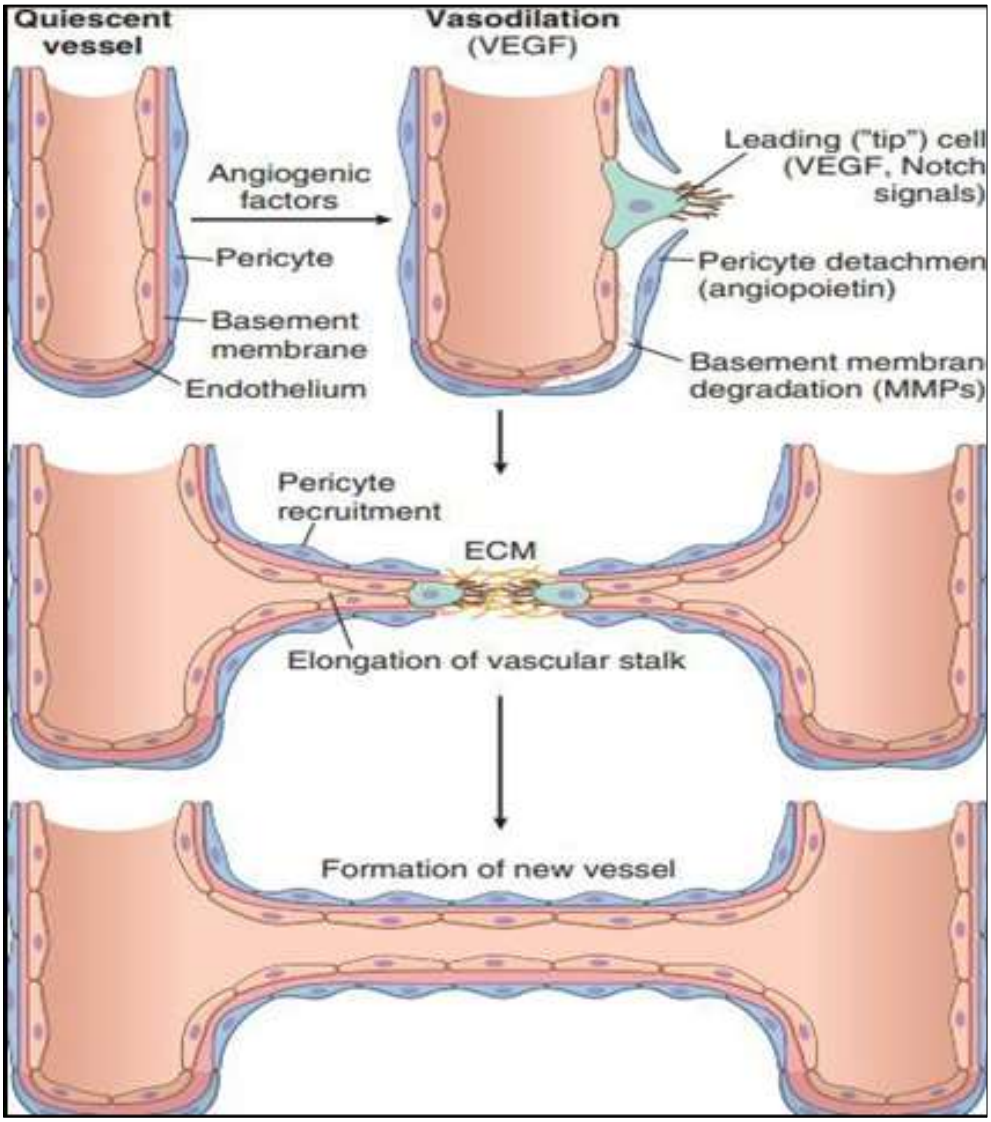

Fig 5: Schematic representation of Steps in Angiogensis (Kumar *et al*., 2014) [8]

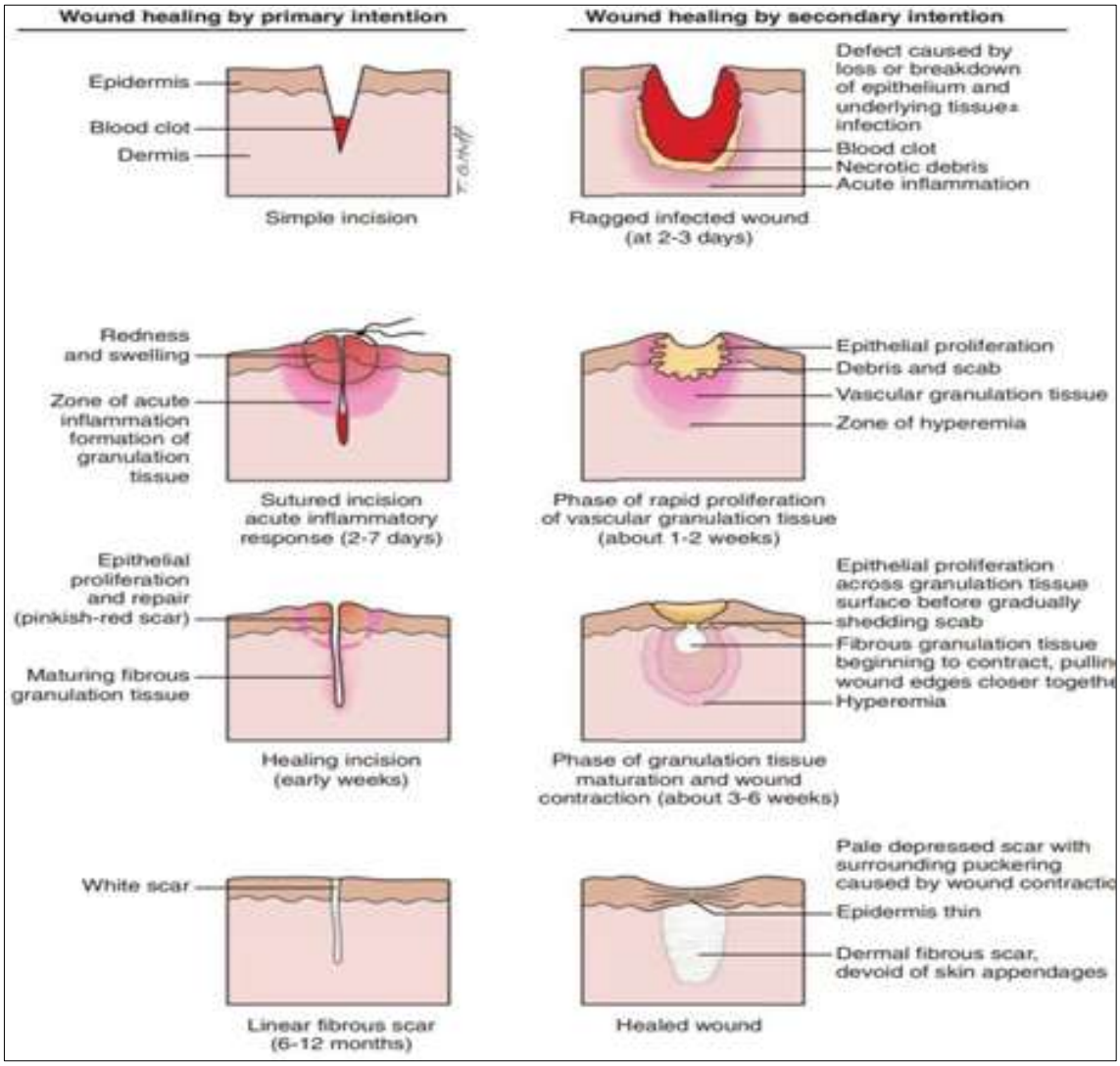

Fig 6: Schematic representation of different phases of wound healing (Zachary *et al*., 2012) [20]

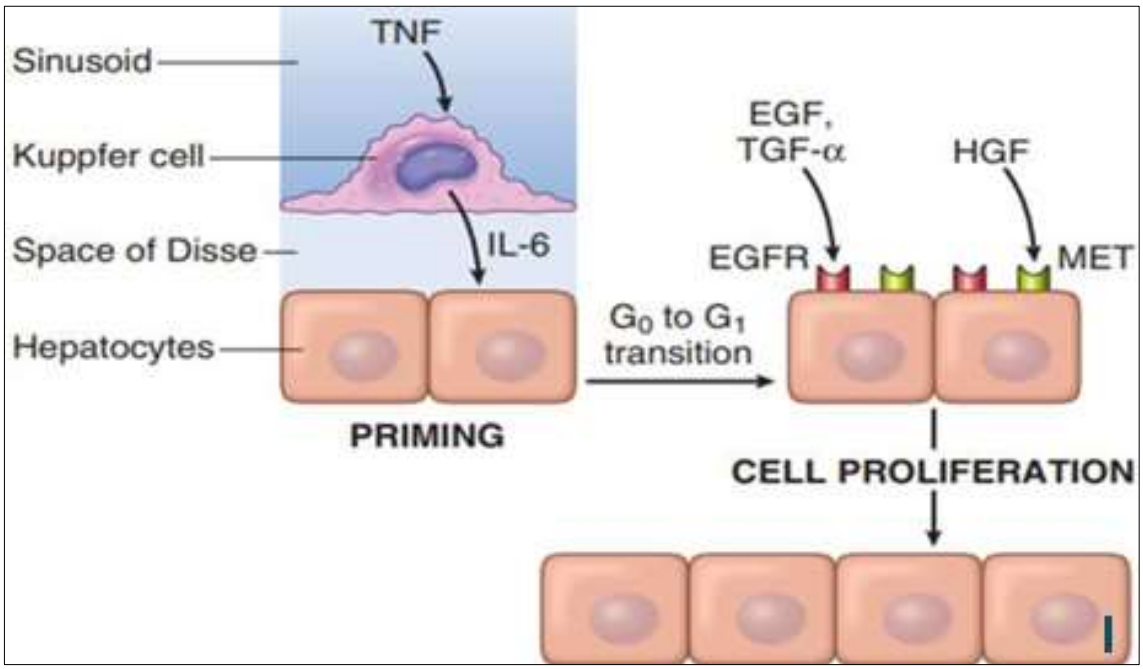

Fig 7: Schematic representation of Hepatocyte regeneration (Kumar *et al*., 2014) [8]

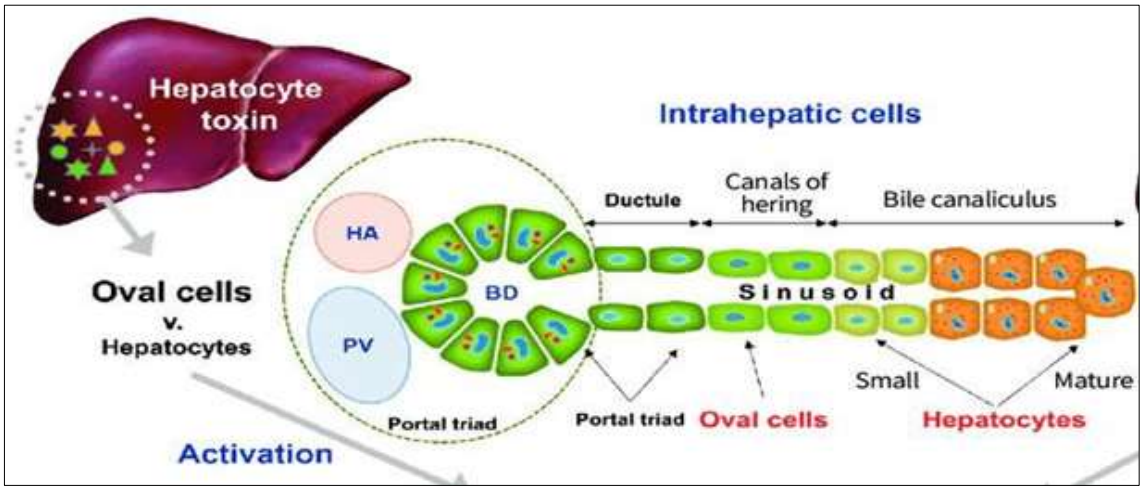

Fig 8: Schematic representation of liver regeneration from progenitor cells (Kim *et al.*, 2019) [7]

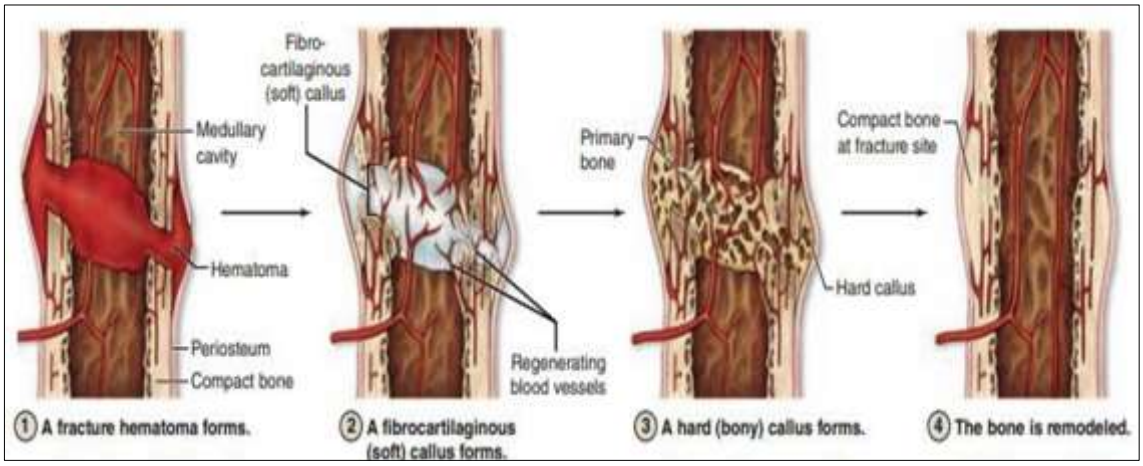

Fig 9: Schematic representation of Fracture healing steps (Mescher, 2018) [11]

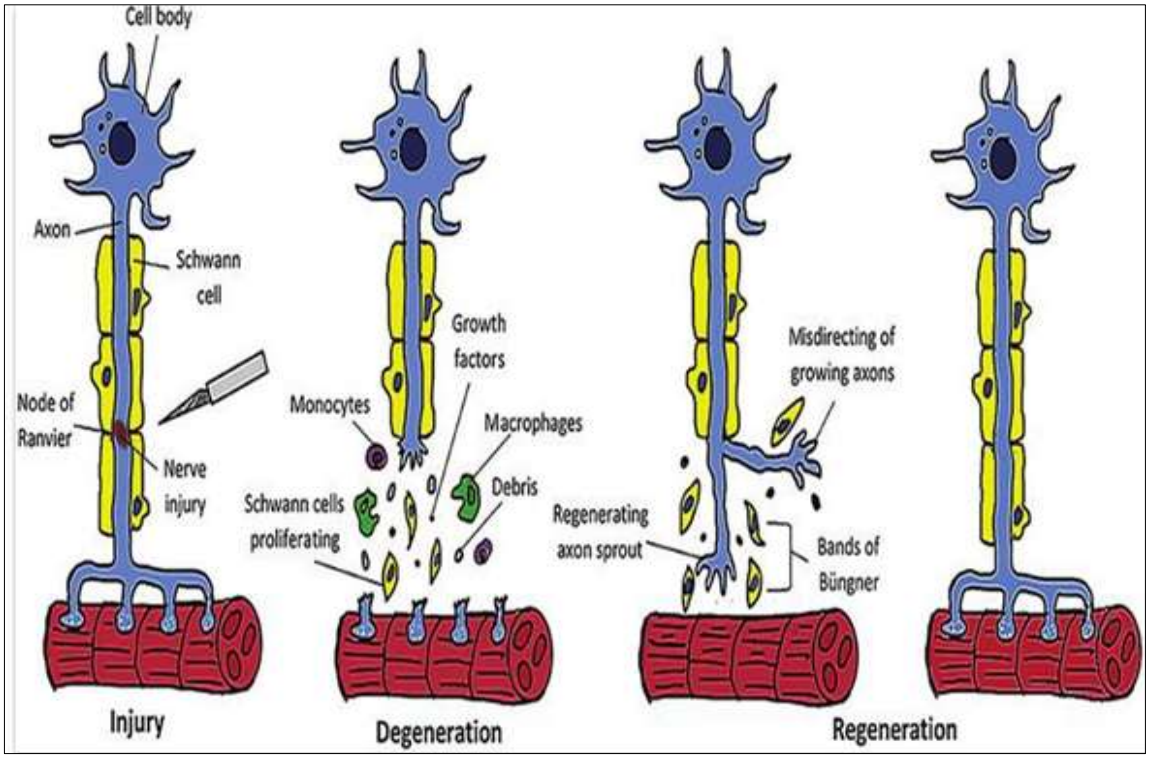

Fig 10: Schematic representation of Peripheral nervous system regeneration (Alvites *et al*., 2017) [2]

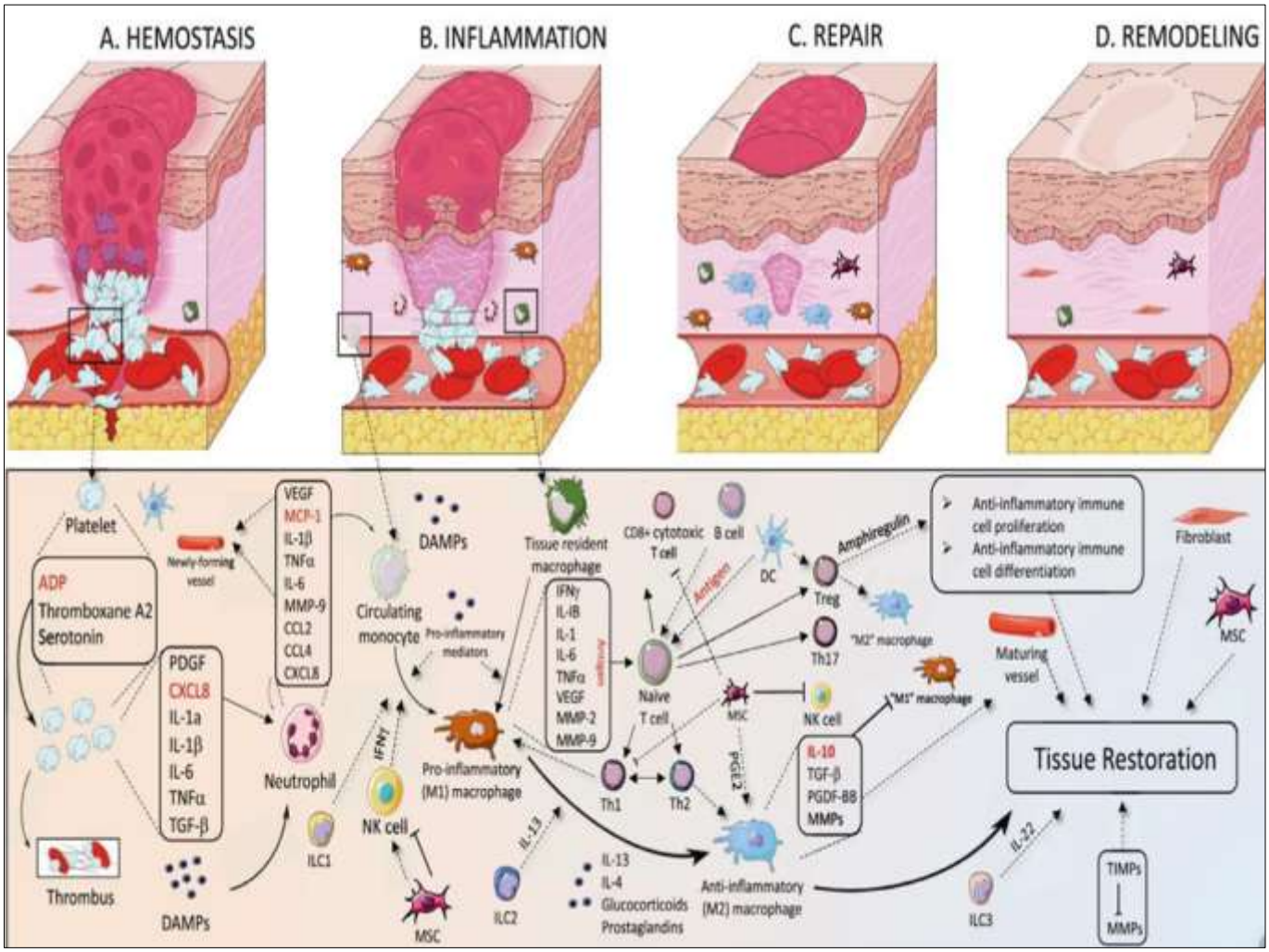

Fig 11: The immune microenvironment in tissue healing (Alaribe *et al*., 2016) [1]

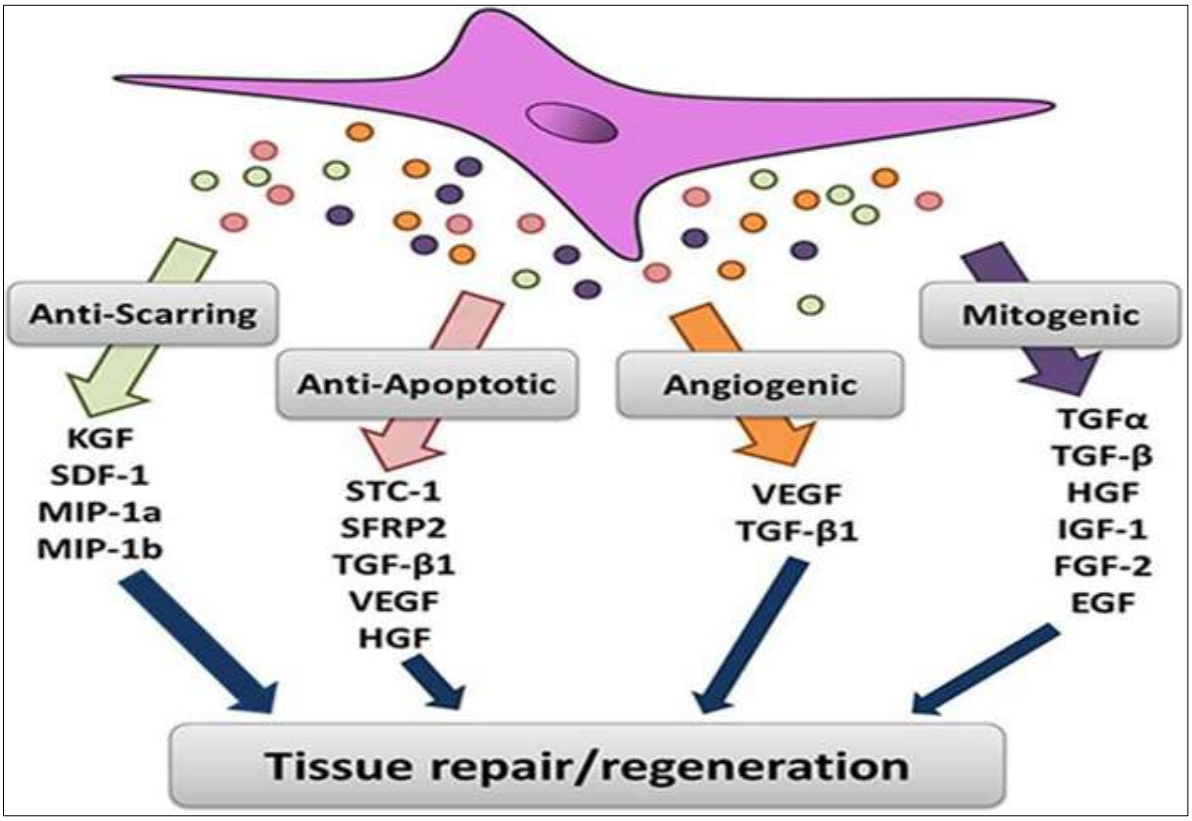

Fig 12: Schematic representation of Paracrine action of mesenchymal stem cell (Ramaswamy *et al*., 2016) [14]

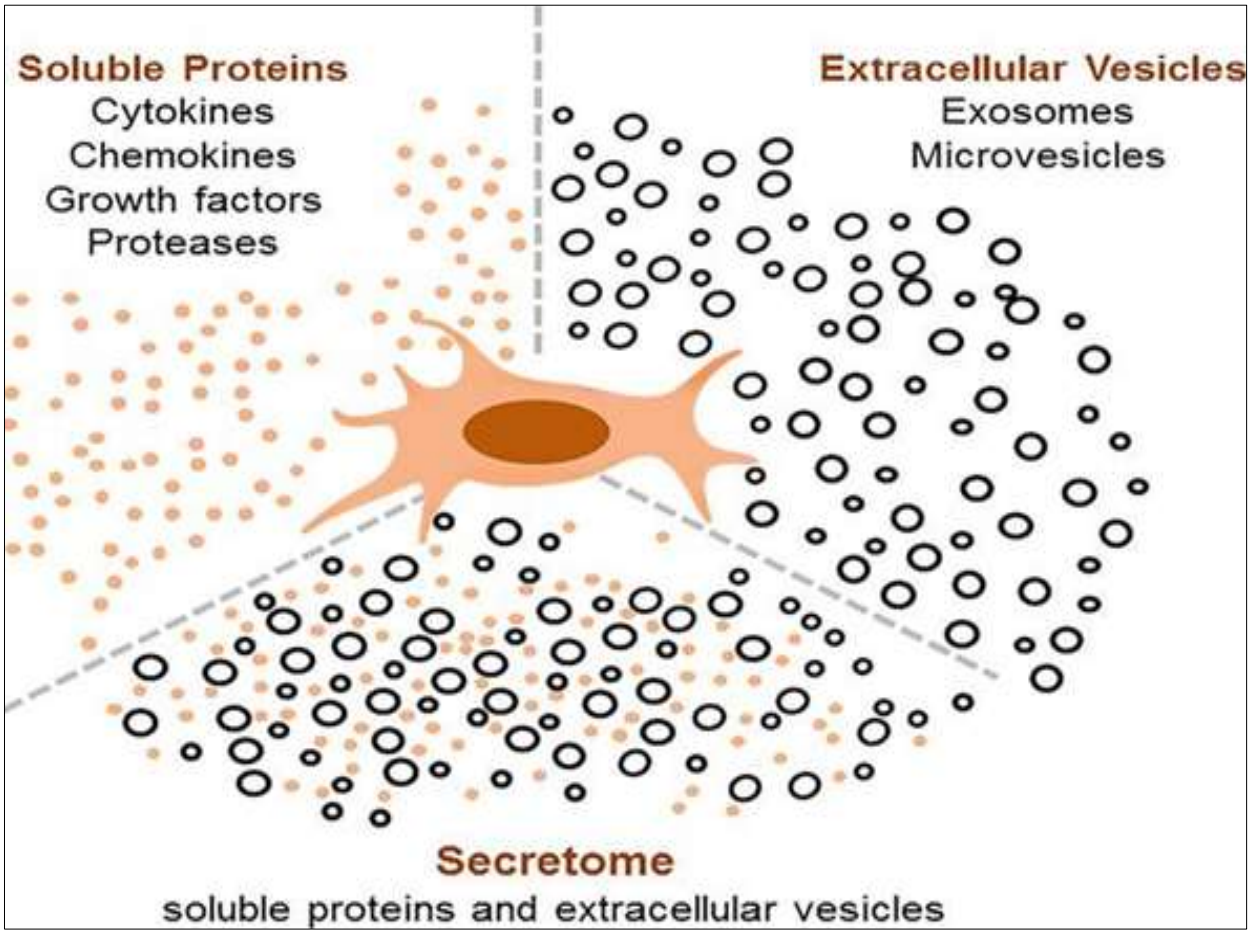

Fig 13: Schematic representation of Secretome used for preparation of conditioned media (Driscoll *et al*., 2019) [3]

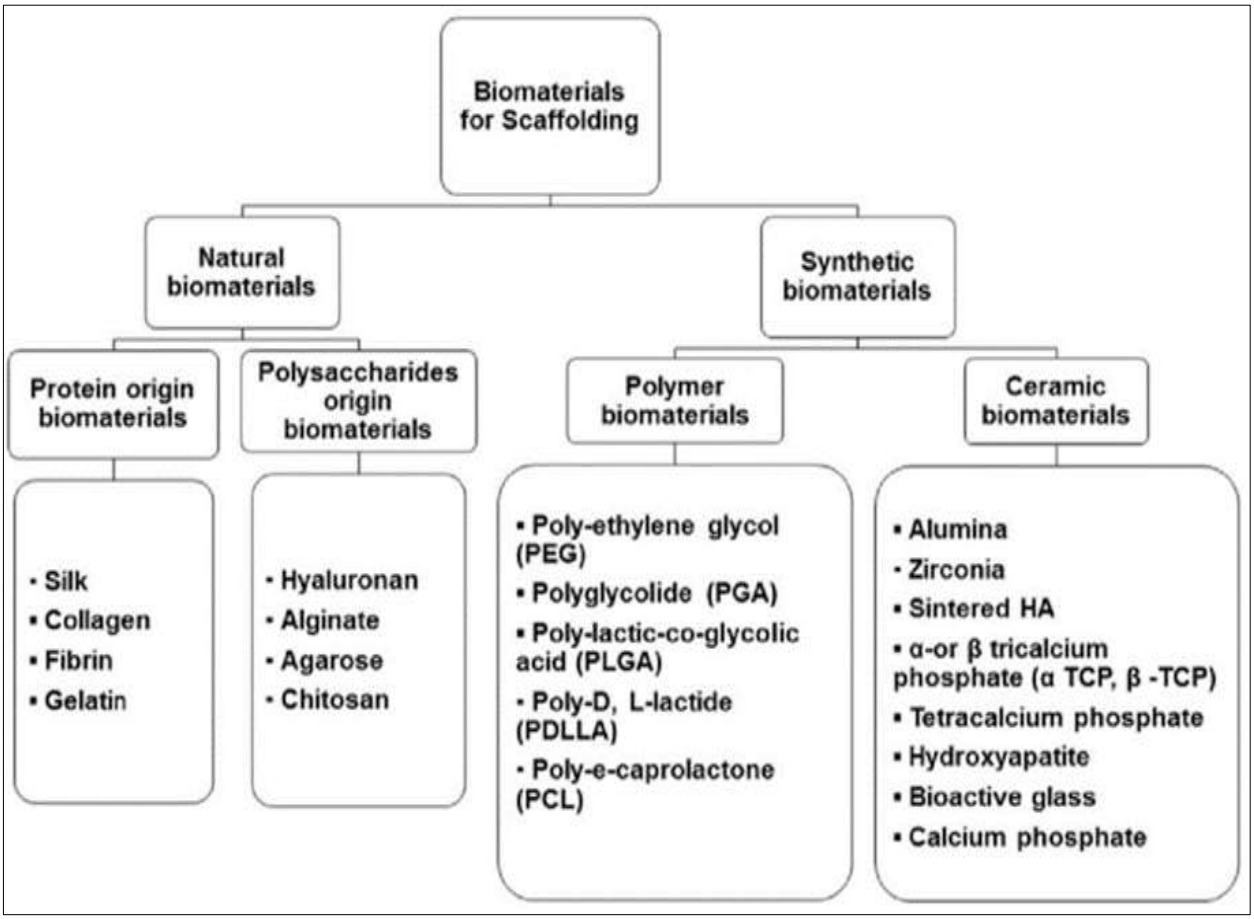

Fig 14: Classification of scaffold (Alaribe *et al.* 2016) [1]

Fig 14a: Buffalo calf presented with umbilical hernia (Umesh, 2023) [18]

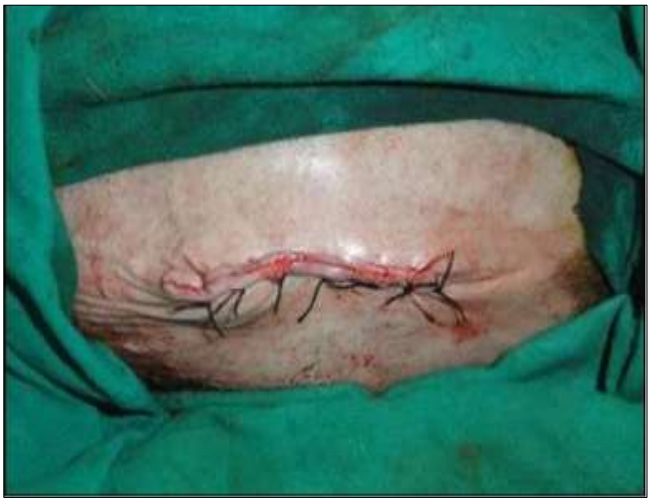

Fig 14c: Repaired hernial defect and skin sutures are applied (Umesh, 2023) [18]

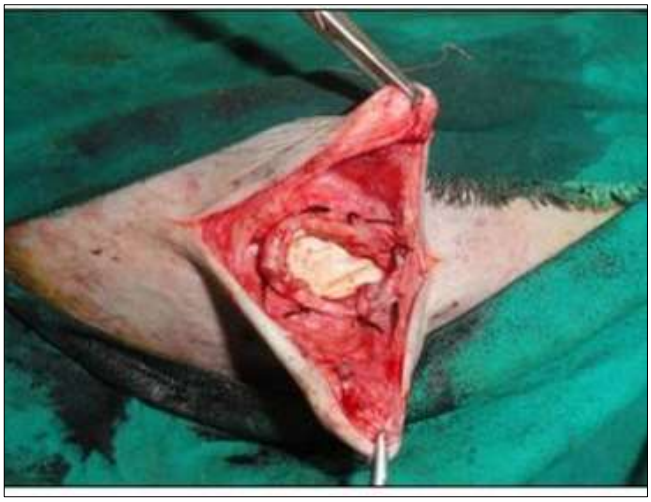

Fig 14b: Repair of umbilical hernia with bubaline rumen extracellular matrix (Umesh, 2023) [18]

Fig 14d: Animal on the 12th day after hernia repair and suture removal (Umesh, 2023) [18]

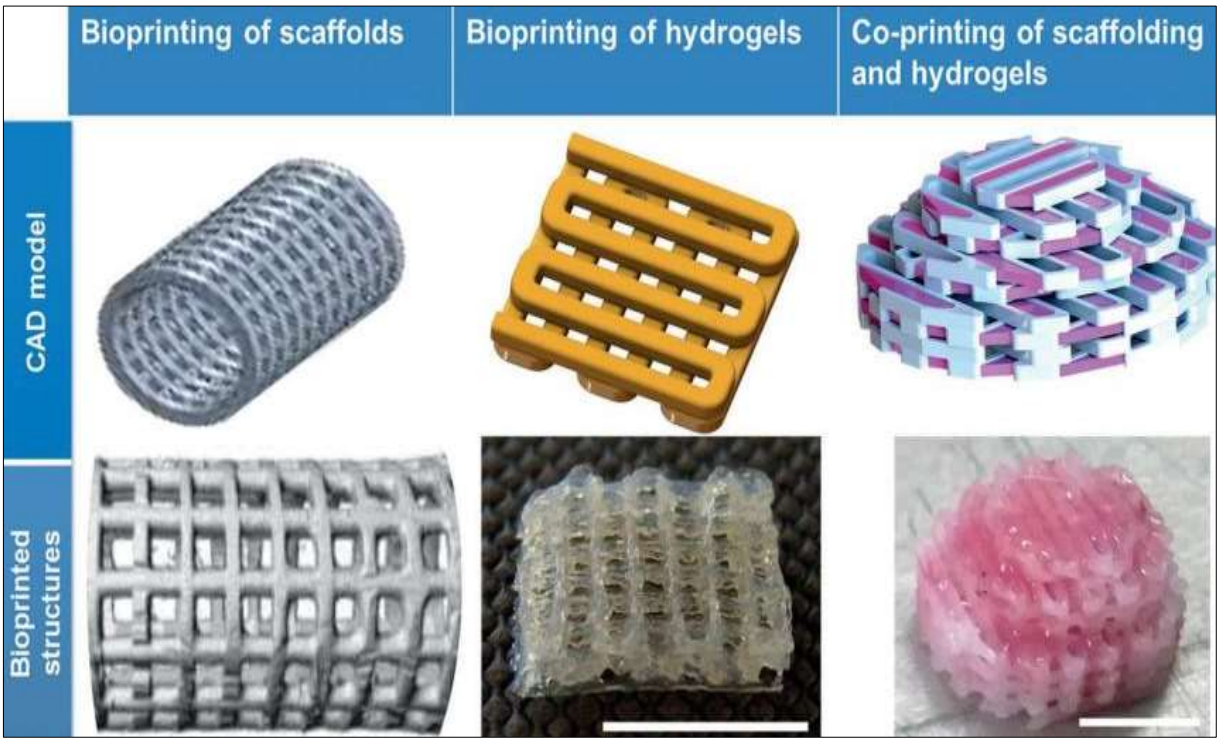

Fig 15: Co-printing of the scaffold and the hydrogel as well as bioprinting of the two materials (Pati *et al*., 2016) [13]

Conclusion

The process of tissue repair and regeneration is used after any injury and aids in determining the patient's prognosis; it is less intrusive and more affordable than transplantation. An new area of study called "regenerative medicine" focuses on the replacement, regeneration, or repair of cells, tissues, or organs in order to improve function.

Tissue engineering is the technique of combining scaffolds, cells, and physiologically active substances to produce functional tissues. It has its roots in the development of biomaterials. Damaged tissues or entire organs can be mended, maintained, or improved by putting together functional constructs.

Extracellular matrix (ECM) scaffolds and stem cells are frequently used in tissue engineering to repair diseased or damaged tissue and build artificial organs by growing epithelium on a variety of ECM substrates.

Scaffold-based systems utilize organic or synthetic materials as a scaffold for seeded cells to aggregate, multiply, and migrate, eventually producing a three-dimensional (3D) structure, such as organoids.

Scaffold-free systems rely on physical factors, such as spheroids to analyze tumor models, or specialized culture plates to promote the self-aggregation of cells. These innovative methods, such as 3D bioprinting, organoids, and spheroids tissue models, are now becoming more significant.

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