

Tissue Repair: Cellular Growth, Fibrosis, and Wound Healing

Dr. Md. Sadequel Islam Talukder

MBBS, M. Phil (Pathology)

3 May 2002

Replacement of injured or dead cells to repair tissue after inflammation is essential to survival of an individual.

Injurious stimuli trigger the activation of genes that are involved in cell replication.

Repair of tissues involves two distinct processes:

- 1) Regeneration and
- 2) Fibroplasia or fibrosis

Regeneration denotes replacement of injured cells by cells of the same types, sometimes with no residual trace of the previous injury.

Fibroplasia denotes replacement by connective tissue, which leaves a permanent scar.

Repair of tissue involves the following mechanisms:

- Cell migration
- Cell proliferation
- Cell differentiation
- Cell-matrix interactions.

Cell-matrix interaction is important for repair. The orderly regeneration of the epithelial tissue of the skin and viscera requires the basement membrane (BM). This specialized extracellular matrix (ECM) functions as an extracellular scaffold for accurate regeneration of pre-existing structures. Maintenance of BM integrity provides for the specificity of the cell type and popularity and influence cell migration and growth.

In adult tissue, the size of a population of cells is determined by the rates of cell proliferation, differentiation, and death by apoptosis. Increased cell number may result from either increased proliferation or decreased cell death.

The impact of differentiation depends on the circumstance under which it occurs. Replication of differentiated cells occurs in certain adult tissues; for example, after partial hepatectomy, liver cell division continues until the signals for such division is abrogated. Apoptosis is induced by a variety of physiologic stimuli and is controlled by a number of genes.

Control of Normal Cell Growth

Cell proliferation can be stimulated by injury, cell death and mechanical deformation of tissues.

Cell replication is controlled largely by chemical factors in the microenvironment, which either stimulate or inhibit cell proliferation.

Cell Cycle and Proliferative potential

The cells of the body are divided into three groups on the basis of their proliferative capacity and their relationship to the cell cycles. The cell growth cycle consists of G₁ (presynthetic), S (DNA synthesis), G₂ (premitotic), and M (mitotic) phases.

Most mature tissue composed of

- Labile cells or continuously dividing cells
- Stable cells or quiescent cells
- Permanent cells or nondividing cells

Labile cells follow the cell cycle from one mitosis to the next and continue to proliferate throughout the life, replacing cells that are continuously being destroyed. Examples are stratified squamous surface epithelium of skin, oral cavity, vagina and cervix; lining mucosa of all the excretory ducts of the glands; the columnar epithelium of the gastrointestinal tract and uterus; the transitional epithelium of the urinary tract and cells of the bone marrow and haematopoietic tissues. Regeneration is derived from a population of stem cells, which have unlimited capacity to proliferate and whose progeny may undergo various streams of differentiation.

Stable cells normally demonstrate a low level of replication, can undergo rapid division in response to stimuli and are thus capable of reconstitute the tissue of origin. They are considered to be in G_0 but can be stimulated into G_1 . Examples are parenchymal cells of virtually all the glandular organs, such as the liver, kidneys, and pancreas; mesenchymal cells such as fibroblasts and smooth muscle; and endothelial cells.

The underlying supporting stroma of the parenchymal cells – particularly the BM – is necessary for organized regeneration, forming a scaffold for the replicating parenchymal cells.

Permanent cells have left the cell cycle and cannot undergo mitotic division in postnatal life. Examples are most nerve cells and cardiac and skeletal muscle cells. Neuron destroyed in central nervous system is permanently lost. They are replaced by supportive elements, glial cells. Skeletal muscle cells have some proliferative capacity. Regeneration appears to occur from transformation of the satellite cells attached to the endomysial sheaths. Regenerative capacity of cardiac muscle is limited, and most large injuries to the heart are followed by connective tissue scarring.

Molecular Events in Cell Growth

Molecular events in cell growth are complex and involve an increasing array of intercellular pathways and molecules. It is important because aberration in such pathways may underlie development of cancer and abnormal cellular response in a variety of diseases.

Growth factors induce cell proliferation by affecting the expression of **protooncogenes**, involved in normal growth control pathways. The expression of these genes is tightly regulated during normal growth and regeneration. Alteration in the structure of such protooncogenes can convert them into **oncogenes**, which contribute to uncontrolled cell growth characteristic of cancer.

There are three general schemes of intercellular signaling:

- Autocrine signaling
- Paracrine signaling
- Endocrine signaling

Autocrine signaling: Cells respond to the signaling substances that they themselves secrete. This type of signaling occurs in compensatory hyperplasia and in tumours. Tumour cells frequently overproduce growth factors that can stimulate their own growth and proliferation.

Paracrine signaling: A cell produce molecules that affect only in a target cell in close proximity. This is common in connective tissue repair of wound healing.

Endocrine signaling: Hormones are synthesized by cells of endocrine glands and act on target cells distant from their site of synthesis being usually carried by blood.

Cell Surface Receptors

Cell growth is initiated by the binding of a signaling agent, most commonly a growth factor, to a specific receptor. Receptor proteins can be located on the cell surface of the target cells or found in either the cytoplasm or the nucleus. A receptor protein has binding specificity for particular ligands, and the resulting receptor ligand complex initiates a specific cellular response.

Three major classes of cell surface receptors are important for cell growth:

- 1) Receptors with intrinsic kinase activity
- 2) Receptors without intrinsic catalytic activity
- 3) G-protein linked receptors.

Receptors with intrinsic kinase activity

These types of receptors have an extracellular domain for ligand binding; a single transmembrane region; and a cytosolic domain. Many growth factors are dimeric proteins, contain two regions for receptor binding, and form stable receptor dimers by simultaneously binding two receptors. Dimerization of the receptor is followed by receptor autophosphorylation, creating binding sites for a series of cytosolic proteins. Such cytosolic proteins include (1) a series of adaptor proteins; (2) components of the phosphoinositide-3-kinase (PI-3-kinase) pathway; (3) phospholipase C- γ in the protein kinase C pathway; and (4) members of the src family of tyrosine kinase. Collectively, these four systems, in turn, generate a cascade of responses that ultimately signals irreversible commitment of the cell to enter S phase of the cell cycle.

Receptors without intrinsic catalytic activity

These types of receptors have an extracellular domain for ligand binding; a single transmembrane region; and a cytosolic domain, which directly associate with and activates one or more cytosolic protein tyrosine kinase, which, in turn, phosphorylate the receptor.

G-protein linked receptors

These receptors contain seven transmembrane loops and are frequently called seven-spanning receptors, is associated with a variety of important functions. For example, receptors for the inflammatory chemokines as well as the hormones epinephrine and glucagon are in this class. Ligand binding activates a signal transducing G protein complex, which in turn, activates an effector system that generates intracellular second messengers.

Signal Transduction System

- Signal transduction is the process by which extracellular signals are detected and converted into intracellular signals, which, in turn, generate specific cellular responses.
- Signal transduction systems are arranged as networks of sequential protein kinase; the most important ones involved in regulation of cell growth are the mitogen-activated protein kinase (MAP kinase), PI-3-kinase, inositol-lipid (IP₃), cyclic adenosine monophosphate (cAMP), the Janus Kinase/Signal transducer and activators of transcription (JAK/STAT) signaling system, and the stress kinase system.

Mitogen-Activated Protein Kinase Pathway

- It is particularly important in signaling by growth factors.
- Ligand binding by a receptor tyrosine kinase results in autophosphorylation of the receptor and binding of adaptor protein, which ultimately lead to activation of the Ras protein. Inactive Ras is in the guanosine diphosphate (GDP) binding form, which is converted by activation to the active GTP form, initiating a cascade of distal kinases, which culminate in change in gene expression.

- Activation of Ras is counteracted by GAP (GTP activating protein), which switches Ras to the inactive GDP form.
- The net result of this pathway is activation of a protein phosphorylation cascade, which amplifies the signal and stimulates quiescent cells to enter the growth cycle.

Phosphoinositide-3-kinase Pathway

Phosphoinositide-3-kinase (PI-3-kinase) generates membrane-associated lipid mediators, which act as second messengers to recruit and activate a series of intracellular kinases. The activity of these kinases eventually leads to cellular responses that are correlated with cellular survival, such as phosphorylation of glycogen synthase kinase 3 and increased glycogen synthesis.

Inositol-Lipid Pathway

The IP₃ signaling system can be coupled to either tyrosine kinase or seven-spanning G protein-linked receptors causing activation of a G protein, which then activates phospholipase C_γ. Phospholipase C_γ cleaves PIP₂ to IP₃ and DAG. The IP₃ then diffuses in the cytoplasm and associate with IP₃-sensitive calcium channels in the membrane of the endoplasmic reticulum, causing release of calcium stores. DAG and calcium also activate protein kinase C, which then phosphorylates a variety of cellular components important in cell growth and metabolism.

Cyclic Adenosine Monophosphate Pathway

Binding of hormones to seven-spanning receptors is coupled through G proteins to activation of adenylate cyclase and generation of the second messenger cAMP. Elevated levels of cAMP activate protein kinase A, which, through a series of intermediate steps, stimulates expression of target genes.

JAK/STAT Pathway

Members of the cytokine receptors superfamily lack intrinsic kinase activity. After ligand binding, the receptor associates with and activates one or more protein kinases present in the cytosol, designated Janus Kinases (JAKs). The JAKs phosphorylate the receptors as well as downstream proteins designated STATs (signal transducer and activators of transcription). In general JAK/STAT system mediates functional as opposed to proliferative responses.

Transcription Factors

Transcription factors have a vital role in controlling cell growth. Transcription factors are phosphorylated by specific proximal kinases and such phosphorylation can change the subcellular localisation of the transcription factor or its affinity for DNA, which alters gene expression.

Cell Cycle and the Regulation of Cell Division

Two types of molecular controls regulate the events leading to cell division: (1) a cascade of protein phosphorylation pathways involving a group of proteins called *cyclins* and (2) a set of *checkpoints* that monitor completion of the molecular events and, if necessary, delay progression to the next phase of the cycle.

Cyclins and Cyclin-dependent Kinases

The entry and progression of cells through the cell cycle are controlled by changes in the levels and activities of *cyclins*. Cyclins perform their functions by forming complexes with a group of constitutively expressed proteins called *cyclin-dependent kinases (CDKs)*. Different combinations of cyclins and CDKs are associated with each of the important transitions in the cell cycle. Cyclin B is synthesized when cell moves into G₂ and it binds to constitutive CDK1, creating the cyclin B/CDK1 complex, whose activity is necessary for cells to enter M phase. The complex is activated by phosphorylation, the active kinase then phosphorylates a variety of proteins involved in mitosis, DNA replication, depolymerization of the nuclear lamina, and mitotic spindle formation. After mitotic division cyclins B are degraded by the *ubiquitin-proteasome pathway*. In this pathway, proteins are first conjugated to the small protein cofactor ubiquitin,

and the modified protein is specifically recognized and degraded within proteasome, a large multisubunit proteolytic complex.

The active CDK complexes are regulated by binding of CDK inhibitors, such as p21 and p27, as well as other kinases and phosphatases. The inhibitors control the cell cycle by balancing the activity of the CDKs.

Checkpoints

Checkpoints represent a second mode of cell cycle regulation and provide a surveillance mechanism for ensuring that certain transitions occur in the correct order and that important events are completed with fidelity. Checkpoints sense problems in DNA replication, DNA repair and chromosome segregation. Activated checkpoints send signals to cell cycle machinery that arrest the cell cycle. By delaying progression through the cell cycle, checkpoints provide more times for repair and diminish the possibility of mutations. Checkpoints systems cause cell cycle arrest either by promoting inhibitory pathways or by inhibiting activating pathways.

Growth Inhibition

The other side of coin in cellular growth control is growth inhibition. The molecular mechanisms of growth inhibition are similar to those of growth stimulation and intertwine along their intercellular routes. A good example of a growth inhibitory signaling system involves the polypeptide growth factor transforming growth factor- β (TGF- β). TGF- β signals through cell surface receptors with serine/threonine kinase activity. The activated kinase phosphorylates its own cytoplasmic domain as well as substrate proteins. TGF- β inhibits cell cycle progression into S phase by affecting the function of both transcription factors and cell cycle control proteins.

Growth Factors

Some of the growth factors act on a variety of cell types, whereas others have effects on relatively specific targets. Growth factors also have effects on cell locomotion, contractility and differentiation.

Some growth factors

- Epidermal growth factors
 - EGF
 - Transforming growth factors
- Platelet derived growth factors (PDGF)
- Fibroblast growth factor (FGB)
- Transforming growth factors β (TGF- β)
- Vascular endothelial growth factors (VEGF)
- Angiopoietins (Ang)
- Insulin like growth factors (IGF)
- Hepatocyte growth factors (HGF)
- Connective tissue growth factors (CTGF)
- Myeloid colony-stimulating growth factors (CSFs)
- Cytokines
 - Interleukins
 - Tumour necrosis factor (TNF)
 - Interferon α , β
- Nerve growth factor (NGF)

EGF/TGF- α :

- Is mitogenic for a variety of epithelial cells and fibroblasts
- Widely distributed in tissue secretions and fluid

PDGF:

- Is stored in platelets and released on activation.
- Also be produced by a variety of cells, including activated macrophages, endothelial cells, smooth muscle cells and tumour cells.
- Causes both migration and proliferation of fibroblasts, smooth muscle cells and monocytes and has other proinflammatory properties.

FGFs:

- Produced by a variety of cells
- Functions:
 - New blood formation (angiogenesis) – has ability to induce all the steps necessary to new blood vessels formation.
 - Wound repair – participate in macrophage, fibroblast, and endothelial cell migration in damaged tissue and migration of epithelium to form new epidermis.
 - Development – play a role in skeletal muscle development and in lung maturation.
 - Haemopoiesis – development of specific lineage of blood cells and development of bone marrow stroma.

VEGF:

- Promotes angiogenesis in cancer, chronic inflammatory states, and healing wounds.

TGF- β :

- Have both growth stimulatory and inhibitory function.
- TGF- β is a growth inhibitor to most epithelial cells.
- TGF- β also stimulates fibroblast chemotaxis and production of collagen and fibronectin by cells.

Cytokines:

- Have growth promoting activities to a variety of cells

Extracellular Matrix and Cell-Matrix Interactions

- Cells grow, move and differentiate in intimate contact with the ECM.
- The ECM is secreted locally and assembles into a network in spaces surrounding the cells.
- The matrix proteins sequestered molecules to provide turgor to soft tissues or minerals to provide rigidity to skeletal tissues. It also provide reservoir for growth factors controlling cell proliferation.

- Provides a substratum for cells to adhere, migrate, and proliferate and can directly influence the forms and function of cells.
- Degradation of ECM accompanies morphogenesis and wound healing as well tumour invasion and metastasis.
- Three groups of macromolecules are associated to form ECM:
 - Fibrous structural protein – collagen and elastin.
 - Adhesive glycoprotein – fibronectin and laminin
 - Gel of proteoglycans and hyalurunan.

These macromolecules assemble into two general organizations:

- Interstitial matrix
- Basal membrane (BM)

Collagen:

- Collagen is the common protein in the animal world.
- Collagens are composed of a triple helix of three polypeptide α chains.
- About 30 α chains form at least 14 distinct collagen types

Major types of collagens:

Type	Characteristics	Distribution
I	Bundles of handed fibres with high tensile strength	Skin (80%), bone (90%), tendons, most other organs.
II	Thin fibrils; structural protein	Cartilage (50%), vitreous humour
III	Thin fibrils; pliable	Blood vessels, uterus, skin (10%)
IV	Amorphous	All basement membrane
V	Amorphous/fine fibrils	2-5% interstitial tissue, blood vessels
VI	Amorphous/fine fibrils	Interstitial tissues
VII	Anchoring filaments	Dermal-epidermal junction
VIII	Probably amorphous	Endothelium-Descemet membrane
IX	Possible role in maturation of cartilage	Cartilage

Elastins, Fibrilins, and Elastic Fibres

- Although tensile strength is provided by members of the collagen family, the ability of tissue to recoil is provided by elastic fibres.
- Central core of elastic fibre contains elastin, which is surrounded by a peripheral microfibrillar network called fibrilin.

Adhesive Glycoproteins and Integrins

- These protein link ECM components to one another and to cells.
- Important such proteins are:
 - Laminin
 - Fibronectin
 - Integrin

Fibronectin:

- Primary role of fibronectin is to attach cells to a variety of matrices.
- Produced by fibroblasts, endothelial cells, and other cells.
- Associated with cell surface, BMs, and pericellular matrices.
- Binds to a number of ECM component including collagen, fibrin and proteoglycans and to cells.

Laminin:

- Laminin is the most abundant glycoprotein in BMs.
- Binds specific receptors on the cell surface with matrix components such as collagen type IV, and heparan sulfate.

Integrins:

- Integrins are the major family of the cell surface receptors that modulate cellular attachment to the ECM.
- It also mediates important cell-cell interactions involved in leukocytes adhesion.
- Integrins are transmembrane glycoproteins.

Matricellular Protein:

- These proteins do not function as structural components of the ECM but interact with matrix proteins; cell surface receptors or other molecules that interact, in turn, with cell surface.

Proteoglycans and Hyaluronan:

- Common proteoglycans are heparan sulfate, chondroitin sulfate and dermatan sulfate.
- They have diverse roles in regulating connective tissue structure and permeability.
- Hyaluronan is found in the ECM of many cells.
- Serves as ligand for core proteins such as cartilage link protein, aggrecan and versican.

Repair by Connective Tissue (Fibrosis):

- Persistent tissue destruction, with damage to both parenchymal cells and stromal framework is a hallmark of chronic inflammation.
- Repair cannot be accomplished solely by regeneration of parenchymal cells. Repairing occurs by replacement of parenchymal cells by connective tissues, which in time produce fibrosis and scarring.

- There are 4 components to this process:
 1. Formation of new blood vessels (angiogenesis)
 2. Migration and proliferation of fibroblast
 3. Deposition of extracellular matrix
 4. Maturation and organization of the fibrous tissue (remodelling)

Granulation tissue:

Repair begins early in inflammation. Sometimes as early as 24 hours after injury, if resolution has not occurred, fibroblasts and vascular endothelial cells begin proliferating to form granulation tissue by 3 to 4 days. It is the hallmark of healing. Granulation tissue is pink, soft and granular in appearance. Histologic features that are characteristic: the formation of new small blood vessels (angiogenesis) and proliferation of fibroblasts. The new blood vessels are leaky allowing passes of proteins and red cells into the extracellular space. Thus, new granulation tissue is often oedematous.

Angiogenesis:

Four steps are needed in the development of new capillary vessels:

- 1) Proteolytic degradation of the basement membrane of the parent vessels to allow formation of a capillary sprout and subsequent cell migration.
- 2) Migration of endothelial cells toward the angiogenic stimulus.
- 3) Proliferation of endothelial cells, just behind the leading front of migrating cells.
- 4) Maturation of endothelial cells, which includes inhibition of growth and remodelling into capillary tubes.
- 5) Recruitment of periendothelial cells including pericytes and vascular smooth muscle cells, to support the endothelial tubes.

All these steps are controlled by interactions among growth factors, vascular cells and the ECM.

Growth Factors and Receptors:

- Although many growth factors exhibit angiogenic activity, most evidence points to a special role for VEGF and the angiopoietins in vasculogenesis.
- These factors are secreted by many mesenchymal and stromal cells, but their receptors are largely restricted to endothelium.
- VEGF expression is stimulated by certain cytokines and growth factors (e.g., TGF- β , PDGF, TGF- α) and tissue hypoxia.

Extracellular Matrix Proteins as Regulators of Angiogenesis:

- The key component of angiogenesis is the motility and directed migration of endothelial cells.
- These processes are controlled by several classes of proteins, including integrins, matricellular proteins and proteases.

Fibrosis (Fibroplasia)

- Fibroplasia occurs within the granulation tissue framework of new blood vessels and loose ECM that initially form at the repair sites.
- Two processes are involved in fibrosis:

- Emigration and proliferation of fibroblasts
- Deposition of ECM by these cells.

Fibroblasts Proliferation

- Granulation tissue contains numerous newly formed blood vessels.
- VEGF promotes angiogenesis but also is responsible for a marked increase in vascular permeability, leading to increased deposition of plasma protein in the ECM and provides a provisional stroma for fibroblast ingrowth.
- Migration of fibroblasts to the site of injury and their subsequent proliferation are triggered by multiple growth factors (TGF- β , PDGF, EGF, and FGF) and fibrogenic cytokine (IL-1 and TNF- α).
- Macrophages are important constituent of granulation tissue, responsible for clearing extracellular debris, fibrin, and other foreign material. These also elaborate TGF- β , PDGF and therefore promote fibroblast migration and proliferation.
- If the appropriate chemotactic stimuli are present, mast cells, eosinophils and lymphocytes may be increased in number. Each of these can contribute directly or indirectly to fibroblast migration and proliferation.
- TGF- β appears to be the most important growth factor involved in inflammatory fibrosis, because of the multitude of effects that favour fibrous tissue deposition. TGF- β causes fibroblast migration and proliferation, increased synthesis of collagen and fibronectin, decreased degradation of ECM by metalloproteinases. TGF- β is also chemotactic for macrophages.

Extracellular Matrix Deposition

- When repair progresses, the number of proliferating endothelial cells and fibroblasts decreases.
- Fibroblasts progressively become more synthetic and deposit increase amount of ECM.
- Fibrillar collagens form a major portion of the connective tissue in repair sites and are important for the development of strength in healing wound.
- Many of the same growth factors regulate fibroblast proliferation also stimulate ECM synthesis.
- Net collagen accumulation depends not only on synthesis but also on collagen degradation.
- Ultimately granulation tissue scaffolding is converted into a scar composed of spindle shaped fibroblasts, dense collagen, fragments of elastic tissue, and other ECM components.
- When scar matures, vascular regression continues, eventually transforming the richly vascularized granulation tissue into a pale, avascular scar.

Tissue Remodeling

- Remodeling of the connective tissue framework is an important feature of both chronic inflammation and wound repair.
- The net result of ECM synthesis versus degradation results in remodeling.
- Degradation of collagen and other ECM proteins is achieved by a family of matrix metalloproteinase, which are dependent on zinc ions for their activity.
- Metalloproteinase consists of:
 - Interstitial collagenase which cleaves the fibrillar collagen I, II, III

- Gelatinase (or type IV collagenase), which degrade amorphous collagen and fibronectin.
- Stromelysin, which act on a variety of ECM components including proteoglycans, laminin, fibronectin, and amorphous collagens.
- Metalloproteinases are produced by fibroblasts, macrophages, neutrophils, synovial cells and sometimes epithelial cells.
- Secretion of metalloproteinase is induced by certain stimuli, including growth factors (PDGF, FGF), cytokines (IL-1, TNF- α), phagocytosis, and physical stress.
- Metalloproteinase is inhibited by TGF- β and steroids.

Wound Healing

Processes of Wound Healing

- Induction of an acute inflammatory process by the initial injury.
- Regeneration of parenchymal cells.
- Migration and proliferation of both parenchymal and connective tissue cells.
- Synthesis of connective tissue and parenchymal components.
- Collagenization and acquisition of wound strength.

Mechanism Involved in Wound Healing

- The mediators of acute inflammation.
- The role of growth factors.
- Cell-ECM interaction in cell migration, proliferation and differentiation.
- The mechanism of angiogenesis and fibrosis

General Principles of Wound Healing

There are two types of wound healing

- Healing by first intension or primary union
- Healing by second intension

Healing by First Intension

- Healing occurs in a clean, uninfected surgical incision approximated by surgical suture.
- The incision causes death of a limited number of epithelial cells and connective tissue cells as well as disruption of epithelial BM continuity. The narrow incisional space immediately fills with clotted blood containing fibrin and blood cells. Dehydration of surface of clot forms the well-known scab that covers the wound.
- Within 24 hour, neutrophils appear at the margins of the incision, moving toward the fibrin clot. Epidermis at its cut edges thickens as a result of mitotic activity of basal cells and within 24 to 48 hours, spurs of epithelial cells from the edge both migrate and grow along the cut margins of the dermis, depositing BM components as they move. They fuse in the midline beneath the surface scab, then producing a continuous but thin epithelial layer.
- By day 3, the neutrophils have been largely replaced by macrophages. Granulation tissue progressively invades the incision space. Collagen fibers are present in margins of the incision, but at first these are vertically oriented and do not bridge the incision. Epithelial cell proliferation continues, thickening the epidermal-covering layer.
- By day 5, the incisional space is filled with granulation tissue. Neovascularization is maximal. Collagen fibres are more abundant and begin to bridge the incision. The epidermis recovers its normal thickness. The differentiation of surface cells yields mature epidermal architecture with surface keratinization.
- During the second week, there is continued accumulation of collagen and proliferation of fibroblasts. Leucocytes infiltrate, oedema, and increased vascularity have largely disappeared.
- By the end of the first month, the scar comprises a cellular connective tissue devoid of inflammatory infiltrate, covered by intact epidermis. The dermal appendages that have been

destroyed in the incision line permanently lost. Tensile strength of the wound increases, but it may take months for the wounded area to obtain its maximal strength.

Healing by Second Intension (Wound with Separated Edges)

- Healing occurs when there is more extensive loss of cells and tissue, such as, in infarction, inflammatory ulceration, abscess formation and surface wounds that create large defects.
- Common denominator in this entire situation is a large tissue defect that must be filled.
- Regeneration of parenchymal cells cannot completely reconstitute the original architecture.
- Abundant granulation tissue grows in from the margin to complete repair.
- Secondary healing differs from primary healing in the following respects:
 1. Inevitably, large tissue defects initially have more fibrin and more necrotic debris and exudates that must be removed. Inflammatory reaction is more intense.
 2. Much larger amount of granulation tissue is formed.
 3. Occurrence of wound contraction in large surface wound. Contraction may ascribe due to presence of myofibroblasts.

Whether a wound heals by primary or secondary intension is determined by the nature of the wound, rather than by the healing process itself.

Wound Strength

- When wound sutures are removed, usually at the end of the first week, wound strength is approximately 10% of the strength of unwounded skin but it increases rapidly over the next 4 weeks.
- This rate of increase then slows at approximately the third month after the original incision and then reaches a plateau at about 70% to 80% of the tensile strength of unwounded skin, which may persist for life.
- The recovery of tensile strength results from increased collagen synthesis, exceeding collagen degradation during the first 2 months and from structural modification of collagen fibres, when collagen synthesis ceases at later times.

Summary of Wound Healing

- Early phase of inflammation, followed by stage of fibroplasia, followed by remodeling and scarring involves in wound healing.
- Different mechanisms occurring at different times triggers the release of chemicals signals that modulate the orderly migration, proliferation and differentiation of the cells, and synthesis and degradation of ECM proteins. These proteins, in turn, directly affect cellular events and modulate all responses to soluble growth factors.

Systemic Factors That Influence Wound Healing:

- **Nutrition**
 - Nutrition has profound effects on wound healing

- Protein deficiency and vitamin C deficiency inhibit collagen synthesis.
- **Metabolic status**
 - Can change wound healing
 - Diabetes mellitus is associated with delayed wound healing
- **Circulatory status**
 - Can regulate wound healing
 - Inadequate blood supply usually caused by arteriosclerosis or venous abnormalities that retard venous drainage also impair healing.
- **Hormones**
 - Glucocorticoids have anti-inflammatory effects that influence various components of inflammation and fibroplasia.
 - These agents also inhibit collagen synthesis.

Local Factors That Influence Wound Healing

- **Inflammation**
 - Is the single most common cause of delayed wound healing.
- **Mechanical factors**
 - Early motion of wound can delay wound healing
- **Foreign bodies**
 - Unnecessary sutures or fragments of steel, glass or even bone, constitute impediments of wound healing.
- **Size, location and type of wound**
 - Wound in richly vascularized areas such as face heal faster than in those in poorly vascularized ones, such as the foot.
 - Small injury produced by intentionally heals faster than larger ones caused by blunt trauma.

Pathological Aspects of Wound Healing

(Complications in Wound Healing)

Complications in wound healing can arise from abnormalities in any of the basic repair process. These abnormalities can be grouped into three general categories:

- 1) Deficient scar formation
 - 2) Excessive formation of the repair components
 - 3) Formation of contractures
- Inadequate formation of granulation tissue or assembly of scar can lead to two types of complication: **wound dehiscence and ulceration.**

- Excessive formation of components of the repair process can also complicate wound healing. The accumulation of excessive amount of collagen may give rise to a raised tumourous scar known as **keloid** or hypertrophic scar.
- Excessive formation of granulation tissue, known as **exuberant granulation tissue (proud flesh)** may protrude above the level of the surrounding skin and blocks re-epithelialization. Incisional scar or traumatic injuries may follow exuberant proliferation of fibroblasts and other connective tissue elements that may recur after excision.
- Contraction in the size of a wound is an important part in the normal healing process. An exaggeration of this process is called contracture. Contracture results in deformities of the wound and the surrounding tissue. In contrast to orderly wound healing the disease is associated with persistence of initial stimuli for fibroplasia or the development of immune or autoimmune reaction. In such reactions, lymphocytes-monocytes interactions sustain the synthesis and secretion of growth factors and fibrogenic cytokines, proteolytic enzymes, and biologically active molecules.

-----0000-----