# CHAPTER II

## EVENTS FROM INJURY TO REPAIR.

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INJURY TO REPAIR:

A variety of insults: mechanical, thermal, chemical, microbial and immunological cause disruption in anatomical and functional continuity. Living organisms respond to injury by marshalling series of events that culminate in wound repair. Many of these events seem to have a temporal and a remote purpose.

In most wounds, an immediate need post-wounding, is to arrest blood and lymph loss. This is achieved by a prompt clot formation. Earlier it was believed that role of fibrin (apart from hemostasis) clot is to serve as scaffold to direct and guide the migrating fibroblasts. In recent years however Ross (7) and Kington et al, (8) have clearly established an important and contributing role of platelets and fibrin in wound healing. Once the hemostasis has been achieved inflammatory responses both vascular and cellular soon follow. This subserves many functions debridement and forestalling / controlling infection. While these functions of inflammation are well recognised, the inflammatory response has also a critical role in healing of wound. Wound macrophages direct and control fibroblast migration, proliferation and functions. Angiogenesis is also promoted by macrophages. Leibovich and Ross (9) have undisputably demonstrated the pivotal role of macrophage in healing process.
CURRENT CONCEPTS OF EVENTS FROM INJURY TO REPAIR.
Wound repair involves various phases - hemostasis, granulation, collagenation, wound contraction, epithelization and scar maturation. There are number of factors, chemical and cellular that direct, regulate and terminate these events. The interaction between various events of body's response to injury is briefly reviewed in the following pages. Fig. 2-1 diagrammatically summarises these events.

**HEMOSTASIS:**

Hemostasis is the forerunner of wound healing. This involves both cellular and chemically mediated responses.

Platelets react with thrombin and collagen at the site of injury to form primary hemostatic plug within the ruptured vessel. The primary clot so formed helps in containing blood loss. This is soon followed by chemical cascade reaction that culminates in deposition of fibrin. Fibrin deposition effectively seals the damaged blood and lymph vessels. Within a short time wound coagulum is formed and consists of neutrophils, monocytes, lymphocytes etc.

The role of platelet and blood coagulation in tissue repair is being now appreciated. As will be described later, (Chapter-3) platelets, fibrin and products of fibrinolysis play an important role in reparative process (8, 10). It is well known that
in thrombocytopenic patients, the healing is notoriously poor (7, 8) other coagulopathies are also associated with poor healing (11, 12).

Hemostasis is followed by inflammation in healing of wound. Cell migration is dependent upon fibrin in the early stage of injury.

INFLAMMATION:

Metchnikoff (13) was first to recognize chronological sequence of events from injury to healing. Inflammation is perhaps the most important event in wound repair. Inflammatory reactions eliminate or restrict the injurious agents and also forestall/fight microbial invasion. Both vascular and cellular responses have been assigned to serve this primary role of attacking offending agent/s that threaten to continue their destructive activity in wound.

Vasodilatation and increased capillary permeability bring more of blood, with hosts of specific and non-specific agents to the wound site to dilute or neutralize the offending agent. The migration of neutrophils, lymphocytes and monocytes into the wound area are like soldiers equipped with ability to attack microbes and other injurious agents.

The cellular phase of inflammation as Sanberg (14) referred, holds the key that kinders wound healing. It is the macrophage of the wound that plays pivotal role. Elegant work of Leibovich & Ross (9) has convincingly shown that neutrophils and lymphocyte fight the infection and while macrophages not only fight the obnoxious agent but also call-forth and direct the reparative process. Macrophage liberates host of factors (Table 2-1) which herald inflammatory reactions and healing process (15).
Table 2-1: SECRETORY PRODUCTS OF MONO-NUCLEAR PHAGOCYTE

1. POLYPEPTIDES
   - Inter leukin 1α & 1β
   - Tumor necrosis factor-(Cachetin) (TNF)
   - Interferon -
   - PDGF, FGF, TGF,
   - Insulin like activity
   - Thymosin
   - Erythropoietin
   - β-endorphin
   - Plasma cytome growth product
   - Neutrophil activating factor.

2. COAGULATION FACTORS
   - Intrinsic path: IX, X, V.
   - Extrinsic - VII prothrombin.
   - Surface activities - Tissue factor prothrombinase
   - Prothrombolytic activity
   - Plasminogen activator
   - Antithrombolytic activities
   - Plasmin inhibitors

3. COMPLEMENT (C) COMPONENTS:
   - Classical path - C_1, C_4, C_2, C_3, C_5
   - Alternative path - Factor B, Factor D.
   - Active fragments - C_{3a}, C_{3b}, C_{5a}, B_{b}

4. OTHER ENZYMES: Neutral proteases
   - Plasminogen activator
   - Elastase
   - Collagenases
   - Phospholipase - A_2
   - Glucosaminidase.

5. INHIBITORS OF ENZYME & CYTOKINES:
   - Protease inhibitors
   - α_2 - Maeroglobin
   - Collagenase inhibitors.

6. PROTEINS OF EXTRACELLULAR MATRIX:
   - Fibronectin, Thrombospondin, Chondroitin sulphate proteoglycan.

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### Table 2-2: CHEMOATTRACTANTS IN WOUND REPAIR (17)

<table>
<thead>
<tr>
<th>CHEMOATTRACTANTS</th>
<th>TARGET</th>
<th>RECRUIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₅ᵃ Elastin peptide</td>
<td>Inflammatory cells</td>
<td>Phagocytes</td>
</tr>
<tr>
<td>Fragment peptides</td>
<td>Neutrophils</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Monocytes</td>
<td></td>
</tr>
<tr>
<td>PDGF, Fibronectin</td>
<td>Connective tissue cells</td>
<td>Matrix producing cells</td>
</tr>
<tr>
<td>Lymphokines, Monokines</td>
<td>Fibroblasts, Smooth muscle cells</td>
<td></td>
</tr>
<tr>
<td>Complement peptides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibronectin, Laminin</td>
<td>Endothelial cells</td>
<td>Vascular system</td>
</tr>
<tr>
<td>Monokines</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Vascular and cellular phase of inflammation are brought about by number of agents referred to as mediators of inflammation - histamine, 5-HT bradykinin, leukotriene-B\textsubscript{4} etc. Though most of these substances are known to take part in vascular phase of inflammation, some agents like 5-HT may also play an important role in fibroplasia (16).

Cell migration paves the way for inflammation. Different cells are recruited to wound site. Substances that are generated by complement activation, fibrinolysis, kinin activation, collagen products, chemicals released from various infected tissues and products of bacterial growth participate in cell migration (17). These substances are called 'chemoattractants' (Table 2-2). As is evident, some chemoattractants promote migration of specific type of cells. This specific relationship is explained on the basis that the attracted cell has receptors for specific chemoattractants. The cell migration triggered by chemoattractants could be haptotactic, chemokinetic and chemotaxetic. Of the various chemoattractants, those which promote migration of cells that directly participate in healing (fibroblasts endothelial cells etc) include platelet derived growth factor (PDGF), fibronectin, lymphokines, complement peptides, monokines and laminin.

Wound debridement is another important function subserved by inflammatory cells, besides ushering in repair process. Macrophage and granulocytes are the major wound cells that take part in debridement. No single cellular mechanism underlines wound debridement. Without debridement, wound would go un-mended. It removes tissue that is heavily contaminated by dirt and bacteria. Secondly, debridement cleanse the wound by removing devitalised tissues and increases the ability of the wound to resist infection. In uncomplicated wound, debris is removed usually by 3rd to 5th day and by that time fibroblasts and capillaries invade
the entire wound area. Thus, inflammation plays role in the healing in different ways. Hence it has been emphasized 'no inflammation - no repair' (18).

Conventionally, healing of skin wounds is described to have various phases that follows hemostasis and inflammation - fibroplasia, collagen synthesis, wound contraction, scar remodulation and epithelization. All these phases occur in all types of skin wound. However, one or the other phase/s may be predominant depending upon the type of wound. For example, in open wounds contraction assumes more importance and in resutured incision wound granulation phase and collagenation.

It is important to know that all phases of wound healing occur independently and pathology may affect one or other phases without disturbing the other. Therefore it is reasonable to suspect a given drug may promote / inhibit the healing phases selectively. (refer therapeutics and wound repair).

FIBROPLASIA:

This is the last lap of response to tissue-loss and bridges the anatomical discontinuity (possibly functional). In most tissues healing is by fibroplasia whereas in liver, endothelium and epithelium healing is by regeneration where parenchymal cells have proliferative potential.

The role of fibroblast in wound healing is of paramount importance; it would not be exaggeration to say that healing process is epitomized by life-story of fibroblast. Wound fibroblast are derived from mesenchymal cells, particularly the adventitia of blood vessels in the neighbourhood of the wound. Fibroblast migration is guided by the fibrin - fibronectin - collagen - scaffold (19). Fibroblasts are invariably seen in the wound by 3rd day. Platelets and other scores of factors
stimulate migration, proliferation and functions of fibroblast. By 4th or 5th day collagen is synthesized and laid in matrix. Depending on the site and size of wound fibroblast proliferation approximately lasts for 2 to 4 weeks (20).

The fibroblasts of the granulation tissue serve two main functions (1) formation of collagen rich extracellular matrix and (2) wound contraction.

**ANGIOGENESIS:**

Neovascularization occurs throughout the life span of an organism following repair process or under certain pathological conditions. Angiogenesis involves migration and proliferation of endothelial cells followed by the formation of capillary buds which later canalize. New vessel formation starts from 3rd or 4th day of injury and by 7th day wound shows maximum neovascularization. Later, supporting structures, basal membrane develop from surrounding mesenchyme. Newly formed vessel is fragile and lacks basement membrane. This is mainly responsible for profuse bleeding on slight injury a characteristic feature of newly formed capillaries. There is constant remodelling of the vasculature which involves obliteration of many of the initial capillaries (21). As wound healing slows and scar maturation occurs capillaries gradually regress and the red vascular rich wound tissue transforms into a white relatively avascular, cell-poor scar (22).

The establishment of characteristic vascular patterns are attributable to rate of flow, volume and pessure of blood (23). Hypoxia is the principal stimulus of endothelial proliferation and capillary formation. Transplantation technique studies have revealed that with many exceptions, most transplants of normal adult and embryonic tissue lack an appreciable angiogenesis (24). These facts support the concept that angiogenic factors are present in tissues. Many tissues secrete
angiogenic factors. Epidermis has specific, heat labile diffusible but non-dializable 'Epidermal angiogenic factor' (25). Epidermal growth factor (EGF), Fibroblast growth factor (FGF), prostaglandin E, and copper ions are known to induce neovascularization and promotes the release of angiogenic factors (26). Heparin supports endothelial proliferation (27). Fractionization and characterization of angiogenic factors are in progress and antiangiogenic factors are also being described (22). Exogenous administration of angiogenic factors may enhance or adversely affect vessel formation (28,29).

**COLLAGEN METABOLISM:**

The dynamic extracellular matrix of normal and injured or neoplastic tissues is composed of different classes of macromolecules: fibre - proteins collagen elastin, structural glycoproteins fibronectin, laminin, entactin, and proteoglycans (30). Studies over the past decade have established that the extracellular matrix provides and may control, a rapidly changing milieu in which the epithelium is organized. It is within the setting of extracellular matrix, that the process of wound healing occurs. Furthermore, evidences have been accumulating that suggest interaction between the components of extracellular matrix and the epithelial cells, may be, similar to those that take place during embryogenesis and early stages of neoplastic invasion (31).

In all tissues, the collagen molecules are essentially the same. However, there are subtle variations which determine biological function. Collagen is largely a fibrous protein, approximately constituting 1/3 of the body's total protein. It is a long stiff rod shaped protein and consists of triple helix of three polypeptide chains called \( \alpha \) - chains, wrapped rope like around each other. At present more than ten genetically distinct collagen types have been recognised (Table 2-3).
<table>
<thead>
<tr>
<th>TYPE</th>
<th>TISSUE DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>skin, bone, Tendon.</td>
</tr>
<tr>
<td>II</td>
<td>tumour cell cultures. Skin, liver, cartilage</td>
</tr>
<tr>
<td>III</td>
<td>foetal skin, blood vessels, Intestine.</td>
</tr>
<tr>
<td>IV</td>
<td>basement membrane.</td>
</tr>
<tr>
<td>V</td>
<td>ubiquitous.</td>
</tr>
<tr>
<td>VI (intimal)</td>
<td>aortic intima placenta</td>
</tr>
<tr>
<td>VII (long chain)</td>
<td>anion anchoring fibrils.</td>
</tr>
<tr>
<td>VIII (endothelial)</td>
<td>endothelial cell cultures.</td>
</tr>
<tr>
<td>IX</td>
<td>HMW-LMW cartilage.</td>
</tr>
<tr>
<td>X</td>
<td>short chain cartilage.</td>
</tr>
<tr>
<td>XI</td>
<td>1 alpha : 2 alpha cartilage.</td>
</tr>
</tbody>
</table>

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In connective tissue, collagen functions in many ways: (i) provides structural support to tissues, (ii) generates tensile strength that is necessary to hold tissues together as functional units, (iii) regulates growth, maintenance, remodeling and rigidity of bone. (iv) interacts with platelet and promotes aggregation. (v) plays key role in cell differentiation during embryonic development. (vi) purports unidirectional strength of tendons. (vii) underlines the flexibility of the skin and contributes to elasticity of large arteries.

**COLLAGEN SYNTHESIS:**

Biosynthesis of collagen is basically similar to that of other-proteins. However, it is a complex process, since multiple post-ribosomal enzymatic modifications occur during collagen synthesis.

Collagen is a complex protein containing unique amino acids hydroxyproline and hydroxylysine and glycine at every third residue. The mol.wt. of collagen is approximately 270,000 daltons. The three alpha-chains making up the collagen molecule are approximately equal in length, about 1000 amino acids to a single peptide chain.

The sequence of biosynthesis of collagen is given in fig. 2-2.

Hydroxyproline and hydroxylysine characteristic of all collagens are absent when pro- chains are initially synthesized, specific proline and lysine residues are hydroxylated by prolyl and Lysyl hydroxylases respectively. Both the enzymes require four co-factors (a) molecular oxygen, (b) ferrous iron (Fe²⁺), (c) -ketoglutarate and (d) ascorbic acid.
**Fig. 2-2 : STAGES OF PROCOLLAGEN SYNTHESIS**

A. translocation of procollagen m-RNA - Pro-α-chain synthesis

B. hydroxylation and glycosylation.
   Rough endoplasmic reticulum
   (i) Hydroxylation of pro-α- chains.
   (ii) Synthesis of peptidyl-4-hydroxyproline: catalysed by prolyl-4-hydroxylase
       (rate limiting step in collagen production)
   (iii) Synthesis of peptidyl-3-hydroxylproline: catalysed by prolyl-3-OHxylase
   (iv) Synthesis of peptidyl hydroxylysine: lysyl oxidase catalysis.

C. assembly and secretion
   pre-α-Chain
   disulphide linkage → endoplasmic reticulum.
   procollagen polypeptide
   in

D. secretion
   membrane of endoplasmic reticulum
   → Cisternal space
   → Golgi apparatus
   → exocytosis

E. extracellular modification
   tropo collagen
   → excision of registration peptide
   → procollagen peptidase
   Conversion of tropocollagen to functional proteins: (insoluble collagen)
   → assembly of fibril by 1/2 stagger arrangement
   → cross linking: fibrillogenesis: bundle formation.

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All vertebrate collagens contain glucose and galactose linked through the hydroxyl group of hydroxylysine residues. Therefore, glycosylation is subsequent to hydroxylation. The two hexoses are found as galactosyl hydroxylysine or glucosyl-galactosyl hydroxylysine. Glycosylation is catalysed by galactosyl and glucosyl transferases. Soon after the pro-α-chains are released from the ribosomes into the cisternae of the endoplasmic reticulum interchain disulphide bonds are formed and the triple-helical conformation rapidly assumed by the pro-α-chains. (Fig. 2-2). Once the assembly of procollagen molecules is complete they are secreted via Golgi apparatus into the extracellular space. At this stage terminal peptides are cleaved by specific peptidases to produce extracellular tropocollagen which is insoluble. Further modification of insoluble collagen also takes place in extracellular space i.e. the formation of the precursors of the stabilizing cross-links.

**COLLAGEN CROSS LINKING** : (33)

Initially, the tropocollagen molecules aggregate, by a self assembly process, in a specific stagger arrangement determined by the charge distribution along the collagen molecules. This alignment brings lysine and hydroxylysine residues in adjacent molecules into a position allowing interaction, which produces the cross links. Cross linking is an unique mechanism of the fibrous protein collagen based on aldehyde formation from lysine or hydroxylysine side chains.

Two pathways of cross-linking can be defined in the fibrillar collagens (a) based on allysine, the lysine derived aldehyde. (b) on hydroxyallysine—hydroxylysine derived aldehyde (Fig. 2-3).

Lysyloxidase, a copper metalloenzyme converts the amine side chains of specific lysine and hydroxylysine residues into aldehydes. Lysyloxidase requires pyridoxal phosphate as a co-factor. This enzymatic activity is highest against
Fig 2-3: SCHEME OUTLINING TWO ROUTES OF CROSS-LINKING IN COLLAGEN (33)

(a) LYSINE

\[ \text{lysyl oxidase} \]

lysine, norleucine

\[ \text{hydroxylysine} \]

\[ \text{hydroxylysino} \]

\[ \text{norleucine} \]

\[ \text{hydroxylysine} \]

\[ \text{lysine-5 ketonorleucine} \]

\[ \text{hydroxylysine-5 ketonorleucine} \]

\[ \text{hyroxypyridinium cross-links} \]

\[ \text{mature products still unknown} \]

(b) HYDROXYLYSINE

\[ \text{hydroxyallysine} \]

\[ \text{hydroxylysine} \]

\[ \text{histidine} \]

\[ \text{hydroxy mesodesmosine} \]

\[ \Delta \text{ For dehydro: Signifies the natural aldamine forms for the various components.} \]
nascent fibrils of native collagen, the site of action of this enzyme appears to be the growing fibril.

Polyfunctional cross-links provide fibre-stability. Both inter-and intra-molecular cross-linking can occur. The type of cross-linking present depends on the tissue, probably, the biological function of the tissue. Further, as yet unidentified, reducible and non-reducible cross-links have been described. Cross-linking is an extremely important event responsible for the mechanical properties of collagen, particularly tensile strength of the wound. It also determines solubility characteristics of collagen.

COLLAGENOLYSIS WOUND REMODELLING AND SCAR MATURATION:

Healed scar show maximum collagenolytic activity, there is no sharp demarcation between the end of fibroplasia and the beginning of scar maturation. Collagenases cleave the main chain of collagen at physiological pH and temperature. Not all tissue collagenases are identical. Collagenase act at specific site breaking glycine-isoleucine bond. Fibroblast, macrophage, bacteria are the sources of wound collagenase. Collagenase activity may be increased by colchicine, cytochalasin B, epidermal growth factor, heparin, prostaglandins and neutral protease, platelet factor-4 and cationic proteins from cartilage and aorta control the catalytic activity of collagenase (34).

The mechanisms that regulate collagen deposition and collagenolysis determine the nature of scar. Scar remodelling is the final and long continuing process. Once the scar matures, it becomes more dense and contains less fluid.
During remodelling the original size of the scar is reduced, this is known as contracture wound contracture occurs within months after injury, depends on several factors - viz. age, tension, pressure, oxygen supply (35) and collagen mass etc. (35).

The strength of scar increases steadily over long period of time and scar gradually becomes less elastic. The scar collagen has thinner fibrils and lacks the well organized architecture seen in normal tissue. Scanning electron microscopy has revealed different morphology of collagen fibrils in healing wound (36).

Mechanical stress promotes collagen synthesis and deposition often resulting in hypertrophic scar (37). Keloid and hypertrophic or weak scar is the result of imbalance between collagen deposition and degradation, manifested by over abundant connective tissue formation. It is said that there is an immune basis triggering reaction which occur with inflammation and hypertrophic scar. Keloid is loaded with immunoglobulin - g. In hypertrophic scar prolylhydroxylase levels are more than those found in normal scar. There is also evidence of high concentration of collagenase inhibitors like α-1, antitrypsin, α-2 macroglobulin in keloid and hypertrophic scar (37). Approaches to the control of hypertrophic scar formation include—collagen synthesis, cross-linking and lysis of fibre protein. Currently interest is focused on epithelial mesenchymal cell interaction. It has been referred that this interaction regulates fibroblast and collagenase production in the healing of mammalian wounds (38).
PROTOEOGLYCANS:

All connective tissues contain a varying amount of components that lie between cells and fibers and referred to as ground substance. This amorphous matrix contain high concentration of specific class of negatively charged polysaccharides composed of repeating disaccharide units known as 'proteoglycans' (27, 39). Different tissues have a considerable heterogeneity in their proteoglycans components (Table 2-4). In fact, the manner in which proteoglycans are organised in the matrix is largely unknown. Except hyaluronic acid all other glycosaminoglycans are covalently linked to protein to form proteoglycans. There is increasing evidence that hyaluronic acid has a special function in tissues through which cells are migrating during progress of wound repair (40). It is reported (41) that increased local production of hyaluronic acid which attracts water and thereby swells the matrix, may be a general strategy used to facilitate cell migration during morphogenesis and repair. Glycosaminoglycans are important in stabilizing fibres and this is true with tendon and skin. The presence of particular types of glycosaminoglycan may determine ultimate fibre size and possibly their arrangement in the tissue. Nevertheless, changes in hexosamine, uronic acid the basic moieties of proteoglycan appear to be unrelated to wound tensile strength (42). The functional importance of collagen and proteoglycans interaction is obscure. However, it has been shown that tissue which develops small diameter collagen fibril is rich in hyaluronic acid whereas those with large diameter collagen fibril contains high concentration of dermatan sulphate (43). There is evidence that proteoglycans affect the ultimate physical characteristics of collagen fibril in vivo (44).

Numerous changes continue to occur long after the wound is covered by epidermis; wound colour fades red to pink to white, fibroblast proliferation slows.
Table 2-4: MATRIX GLYCOSAMINOGLYCANS

<table>
<thead>
<tr>
<th>GLYCOSAMINOGLYCANS</th>
<th>MOL.WT.</th>
<th>LINK TO PROTEIN</th>
<th>DISTRIBUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyaluronic acid</td>
<td>4000 to 8x10^6</td>
<td>—</td>
<td>skin, vitreous body cartilage, synovial fluid.</td>
</tr>
<tr>
<td>Chondroitin 4-sulphate</td>
<td>5000 to 50,000</td>
<td>+</td>
<td>cartilage, cornea</td>
</tr>
<tr>
<td>Chondroitin-6sulphate</td>
<td>5000 to 50,000</td>
<td>+</td>
<td>bone, skin, arteries.</td>
</tr>
<tr>
<td>Dermatan sulphate</td>
<td>15,000 to 40,000</td>
<td>+</td>
<td>cornea, bone, skin arteries.</td>
</tr>
<tr>
<td>Heparan sulphate</td>
<td>5,000 to 12,000</td>
<td>+</td>
<td>skin, blood vessels</td>
</tr>
<tr>
<td>Heparin.</td>
<td>6,000 to 25,000</td>
<td>+</td>
<td>lung, arteries, cell. surface.</td>
</tr>
<tr>
<td>Keratin sulphate</td>
<td>4,000 to 19,000</td>
<td>+</td>
<td>lung, liver, skin mast cell</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>cartilage, cornea. intervertebral disc.</td>
</tr>
</tbody>
</table>
down, scar becomes relatively acellular, collagenases continue to degrade collagen, fibrils organised, bundles become broad, tight and oriented in the direction of wound stress. Currently, it has been said that multiple hormonal control of normal collagen turnover is involved with growth and remodelling of scar. (See below):

**FIBRONECTIN IN WOUND HEALING:**

The role of fibronectin is prodigious in wound repair. Throughout the healing period fibronectin is present particularly in association with the fibrin clot and developing collagen fibres. Fibronectin is a glycoprotein. There are two types of fibronectin: (a) cell surface fibronectin and (b) plasma fibronectin. These two protein molecules are very similar but not identical (45). Fibronectin in plasma may play an important part in the removal of collagenous and other debris from blood after injury. Plasma fibronectin was formerly known as cold insoluble globulin-opsonic protein (45).

Cell surface fibronectin is distributed widely; fibroblasts, astroglial cells, cultured epithelial cells, endothelial cells, primitive mesenchyme, extracellular fibrils in connective tissue, basement membrane, platelets, amniotic and cerebrospinal fluids.

Biological activities (45, 46) of fibronectins include:

1. cell-cell aggregation.
2. reversion of transformed phenotype:
   (a) cell-alignment over lapping.
   (b) fibroblastic morphology.
   (c) cell-surface morphology.
   (d) micro filament bundle-organisation.
   (e) increased cell motility.
3. reticulo endothelial system - clearance of colloids.
4. specific binding to macromolecules.
   (i) collagen.
   (ii) fibrin and fibrinogen.
   (iii) Heparin.

   By an action of factor XIII fibronectin is cross-linked with fibrin and collagen (47). Fibronectin mediates binding of macrophages and fibroblasts to fibrin or collagen fibres.

   Fibroblasts were the first cells recognised as ones that synthesize and secrete fibronectin. More recently fibronectin has been shown to be synthesized by a number of cell types including myofibroblasts, schwann cells, endothelial cells, epithelial cells, and macrophages (46). Fibronectin is readily cleaved by a variety of enzymes including thrombin, plasmin, trypsin, plasma transglutamase cathepsin-D and leukocyte elastase (46).

   Fibronectin actively takes part in growth regulation. Removal of all detectable fibronectin from cell surface does not activate growth from cell surface. Fibronectin has multiple physiological actions: extra cellular matrix formation and reticulo-endothelial organization in tissue repair (48).

**WOUND CONTRACTION:**

Wound contraction is an independent process by which the size of the full thickness open wound is reduced and is characterized by the centripetal movement of the whole thickness of surrounding skin. In lower animals contraction of wound is a major process. In human beings it is seldom complete except in
small wounds and it may result in significant deformity, loss of function, the extent of which depends upon the size and the location of wound (49).

Wound contraction involves entire dermis cells including epithelial cells. Contraction proceeds at a fairly uniform rate at about 0.6 to 0.75 mm/day (50). Maximum contraction occurs between 7 to 14th day. If the wound is not completely closed by 12th to 15th day contraction usually ceases. Generation of isotonic force is necessary for wound closure. The size of the wound does not affect contraction but the shape does. Circular wound contracts slowly than rectangular one.

The mechanism of wound contraction is highly disputable and debatable. Many theories have been proposed however no consensus yet. Contraction is a cell-mediated phenomenon. Wound margins contain the mechanism for wound contraction; repeated daily excision of wound margins completely inhibits contraction (49). At the end of 14th day contraction no longer occurs (49). Early wound closure appear to be mediated by contractile force produced by a circumferentially arranged band of fusiform-shaped epidermal cells at wound margin (51). Gabbiani et al (52) described that wound contraction is mediated by functionally and structurally differentiated wound bed fibroblast called 'myofibroblast', and the actions of the myofibroblasts are consistent with the 'picture frame and pull' concepts of wound closure.

Recently, Ehrlich (53), while not convinced with the theory of myofibroblasts as major cellular component for wound contraction reported that fibroblasts generate force of contraction and collagen controls it. Matrix resorption is an integral part of wound contraction. It is referred that wound matrix rich in type III collagen contract more readily than one made from type-I (53).
It is also known that there are three types of fibroblasts at the stage of scar contracture - namely - mobile fibroblasts, myofibroblast and traction fibroblast or spindle fibroblast. Biochemical analysis have revealed that substantial amount of actin is present in wound and granulation tissue at the contractile stage of scar tissue development (54). Cytochalasin inhibits contraction by disrupting actin filaments. As with any scientific data which are ephemeral, explanations of the mechanism of wound contraction are inconclusive and have added more chaos (54).

Wound contraction can be both beneficial or detrimental. In man wound contraction can lead to distortion, disfigurement and impairment of function. Fortunately, wound contraction is not a major problem except in extensive burns. The rapidity of the healing of open wound depends upon contraction to considerable extent. Many factors affect wound contraction: viz - wounds bathed in exudate do not contract, vitamin C deficiency has no effect on wound contraction (49). Colchicine, inhibits wound contraction like glucocorticoids. Adenosine monophosphate with N-acetyl-D-glucosamine antagonize dinitrophenol action on wound contraction and completely restores the process. X-ray irradiation arrest wound contraction (750 roentgens). Wound splinting retards contraction (50). Much remains obscure to pave the way for therapeutic modalities to contain unwanted wound contraction.

EPITHELIZATION:

Epithelium is a tissue composed of closely aggregated cells that are in apposition over a large part of their surfaces. These cells are specialized for the function of absorption, secretion, transport and protection and sensory receptors.
Re-epithelization is the most important component of wound repair.

A wound by definition remains as wound until it is covered by mature surface epithelium. Epithelization, like an example of tissue regeneration, depends on several factors: size, location, shape, impairment of blood supply and pathological modification of wound. Wound which affect only epidermis heals by simple re-epithelization.

Basically, the important stages of re-epithelization are:

(i) cell mobilization.
(ii) migration.
(iii) proliferation.
(iv) differentiation.

In case of skin wounds epithelial cells originate from epithelial cell of hair follicle and sebaceous glands. Within 12 hours of the injury to the epithelium, changes in epidermal cell morphology occur. The process of epidermal cell migration is known as 'epiboly.' These cells appear to move by rolling or sliding over one another as described electron microscopic studies (55). Although confirmative evidences are lacking, it has been said that migrating epidermal cells possesses fibrinolytic and collagenolytic activity (56). Moist wound environment allows more rapid epidermal migration than air exposed wound (22). After the epidermis has covered the wound, maturation of epithelium takes place and stratum corneum is formed. It is also noted that suture track become epithelialized and these epithelialized tracks become absorbed after the removal of stuture.
The aqueous extract of homogenized epidermis contain substances with specific growth inhibitory effect on keratocytes. These provisional - inhibitory factors are not completely purified, and are referred to as 'epidermal chalones'. Subtypes of epidermal chalones have been described without adequate scientific proof and seem to involve in the control of repithelization (55, 57).

Peptide growth factors like EGF & TGF - (alpha) accelerate regeneration of epidermis (58). Topical application of selected growth factors may be useful in accelerating healing of partial thickness injury. The type of wound dressing can also affect re-epithelization. Epithelization is more rapid with hydrophobic rather hydrophilic dressings.

GROWTH FACTORS IN WOUND HEALING:

Growth factors can be defined as those hormones or hormone like substances that regulate the growth of cells either in the intact animal or in tissue cultures. Several growth factors have been described and a number of them have been purified (Table 2-5). In order to grow, the cells must receive two signals that are separate and distinct: 1) Signal to double its size (2) A signal to replicate DNA. It is clear from the studies of Ross et. al. & Pledger et. al (59, 60) that the first signal comes from PDGF that 'primes' the cell and the second comes from a factor in platelet poor plasma.

PDGF is released from alpha-granules of platelets only during blood clotting (61). A basic glycoprotein bearing disulphide linkages reduction of which abolishes mitogenic activity. The role of PDGF in wound healing is as follows:

(a) principal mitogen for fibroblast in the presence of either TGF - alpha or EGF.
(b) mediator of inflammation & repair.
Table 2-5: PEPTIDE GROWTH FACTORS REGULATORS OF TISSUE REPAIR

(a) **GROWTH FACTORS CHARACTERIZED (DETAIL / PARTIAL)**

(i) Platelet derived growth factor (PDGF)

(ii) Transforming growth factor. Alpha & Beta. (TGF - TGF\(\alpha\) TGF -\(\beta\))

(iii) Epidermal growth factor. (EGF)

(iv) Fibroblast growth factor. (FGF)

(v) Macrophage derived growth factor (MDGF)

(vi) Tumor necrosis factor.

(vii) Growth inhibitory factors.

(viii) Proteases.

(ix) Insulin like growth factors - I & II.

(x) Interleukin-2.

(xi) Nerve growth factor.

(b) **GROWTH REGULATING AGENTS**:

(i) Growth hormone.

(ii) Insulin.

(iii) Placental lactogen.

(iv) Plasmin activator.

(v) Thrombin.

(vi) Vasopressin.

(vii) Transferrin.

(viii) Relaxin.

(ix) Prolactin

(x) Chalones (\(\xi\))
(c) potent chemotactic protein for inflammatory cells & fibroblast.
(d) activates neutrophils initiating superoxide synthesis and release, neutrophil granule release and neutrophil aggregation.
(e) stimulate fibroblast to synthesize collagenase - matrix remodelling.

PDGF, TGF-α and TGF-β are the principal autocrine and paracrine growth factors that take part in inflammation and repair. In vitro, studies indicate that TGF-β is an essential regulator of cell replication and differentiation, hence regarded as 'pan-regulin' for tissue repair (62, 63).

The initial observation which lead to the recognition of epidermal growth factor (EGF) was that daily subcutaneous injections of extracts of the mouse submaxillary gland into newborn mice resulted in precocious opening of eyelids and eruption of incisors. Now, receptors of EGF have been described, EGF binding capacity can be affected by glucocorticoids (64).

Many studies have now shown that several different proteases can stimulate proliferation of cultured animal cells. Currently the biological significance of protease - stimulated cell-division remains obscure. Furthermore, following injury thrombin produced at the site of wound not only takes part in clot formation but also signals the cell division necessary for wound healing (65).

The concept of 'chalone - inhibitory growth regulator's remains circumstantial and falls short of direct scientific proof (57). Therefore any description of chalone is provisional and operational.
THERAPEUTICS AND WOUND REPAIR:

With obvious exceptions, it is rather surprising that data available in the therapeutic armamentarium about the implications of drug administration on wound healing is not comprehensive. Drugs modulate tissue repair, patients on steroids, anticancer drugs and diabetics suffer from poor healing. Although the implications of systemic and local administration of drugs on wound healing is well realized, much remains to be described. It is not clear, whether drugs employed for different purposes in wounded patients affect healing. "I dress wound. God heals it" - is no longer tenable, since advanced wound care employ various medicated dressings and wide variety of chemical substances to accelerate wound repair. Yet, many wound problems need deserved attention and judicious medical aid. What factors cause the development of weak scar and wound dehiscence? What is the pharmacological strategy to control poor and over healing? To answer these wound therapy requires to be more rationalized; so that measures to avoid unwanted fibrosis, contracture, risk of wound failure and fistulae would be defined.

Antimicrobials, antiinflammatory agents, local anesthetics, hemostatics, antiseptics, astringents and ointment bases are frequently employed for wound management. Reports are pouring in, which suggest that wound medication and concomitant systemic drug use interfere with wound repair (66). Perioperative use of drugs must be considered on the basis of their effects on wound healing. Infection delays wound healing and chemotherapeutic agent preferred should not disturb repair. Laboratory & clinical investigations have demonstrated that nutritional deficiency (67), trace elements (16, 66, 68), adrenal steroids (16), insulin (16), non-steroidal antiinflammatory drugs (69), histamine liberators (70)
anticancer drugs (71), heparin (72), hypnotic & sedatives (16) and plant extracts (73) affect wound healing. Until more is known about the therapeutics of repair, practitioners are left with few choice of drugs for wound problems.

It is desirable that drugs which influence the reparative process are ideally designated as prohealer or antihealer or scar modulator (Table 2-6).

Pharmacological control of reparative process depends on understanding of the importance of fibroblast life history at the wound site. Further more, it is important, to bisect the biological significance of various peptide growth factors that take part in wound repair. The knowledge about the factors that affect healing is also essential. Local factors - blood supply, temperature, surgical trauma, surgical materials, nerve supply, oxygen tension, infection influence wound healing. Similarly, uremia, jaundice, ische'mic heart disease, cancer, diabetes mellitus, cytotoxic drug therapy, NSAIDS are known to alter tissue repair, besides, age of the patient.

Current therapeutic modalities to contain keloid formation are unsuccessful. In fact, betamino propfihitrile, glucocorticoids and tocopherol have been investigated from this angle but far from reaching goal.

It is also true that drugs produce differential effects on various wound parameters. This makes the aim of wound therapy more difficult. For example, heparin reduces gain in breaking strength (72) and promote epithelization and produce beneficial effects on thermal wound (Chapter 3 & 5).

Ideally, wound dressings should enhance healing, reduce pain, protect the wound from trauma, toxins and absorb exudate. It should not induce irritation and allergy. Ideal wound dressings has not been manufactured. It is established that occlusive dressings promote epithelization by 30-45 %, increase collagen synthesis and reduce pain - an universal phenomenon (22). Therefore, absorbable dressing is to be employed in exudative phase and occlusive type for later stages of wound healing.
Table 2-6: **DRUGS AND TISSUE REPAIR**

(a) **PRO-HEALERS (?)**:
- Insulin, vitamin-A, B₁, B₆, & C.
- Trace elements eg: Zinc.
- Gentian violet, placental extract.
- Antifibrinolytics, Phenytion.

(b) **ANTI-HEALERS (?)**:
- Glucocorticoids, betaaminoproprionitrile.
- Heparin, Cytochalasin, NSAIDS, colchicine.
- Antineoplastic drugs, immunosuppressants.
- Semicarbazide, vitamin-E, progesterone.
- Potassium permanganate.

(c) **OTHERS (?)**:
- Trocinate, oxygen, topical agents - antiseptics, ointment bases.
Thorough hemostasis should be obtained to prevent haematomas since, haemotoma paves the way for infection readily. Hemostasis induced by electro surgery, suturing or protein coagulant technique produce tissue necrosis, if extensive prolong healing (22). Advanced research has accomplished functional relationship between blood coagulation, inflammation, tissue repair and cancer (74). Thus, there is need for a new perspective pharmacologic reappraisal for wound medication.

Can wound healing be controlled? what mechanism trigger and call-off the healing process? Drugs do modify hemostasis, fibrinolysis, inflammation collagen metabolism and pathological state of wound. Does this provide required therapeutic needs? future research would kindle the light to design more promising avenues for wound care.
CHAPTER - II

B: BLOOD COAGULATION, FIBRINOLYSIS AND WOUND HEALING

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   (c) Contact - Activation system 39

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***************
It is important to draw attention to the obvious fact that in healing of different tissue wounds the sequence and timing of events is astoundingly similar. All start with blood coagulation followed by inflammation. The protective role of hemopoietic and hemostatic system in variety of environmental perturbation is well known (75). The hemostatic system has a complex function involving both rapid effective response to injury and mechanism to limit the clot formation. Haemostasis is being regulated by multifactorial balance between procoagulant and anticoagulant mechanisms. Blood coagulation is basically a proteolytic process. Clotting bridges the gap between the injury and inflammation, stops the blood loss and restores some of the mechanical and physical integrity of the damaged tissue.

Blood coagulation is a process of extreme importance to response to injury and repair. Platelet may be regarded as the band master in the orchestra of tissue repair. The plasma contact activation system bears intimate relationship with inflammation, classical complement cascade and fibrinolysis (76, 77). There have been number of reports linking inflammation, cell proliferation, immune response and cancer with coagulation (74). There is formidable list of chemotactic factors associated with coagulation, kinin formation and fibrinolysis (78). Thus blood coagulation assumes great significance in tissue repair. Manipulation of blood coagulation by pharmacological means may have bearings on wound repair.
In 1953 the process of blood coagulation was described as 'FOUR FACTOR THEORY'.

THE 'FOUR - FACTOR THEORY' OF BLOOD CLOTTING :

Cellular damage

\[ \text{Prothrombin} + \text{Ca}^{2+} \]

Thrombo plasmin

\[ \text{Thrombin} \]

\[ \text{Fibrinogen} \]

\[ \text{Fibrin} \]

Later the idea of an extrinsic (tissue activated) system each culminating in prothrombin activation has become firmly established. Now, blood coagulation is a well recognized cascade process referred to as cybernetic system. (Fig. 2-4). Each basic reaction is accelerated by accessory factors. Basically there are three main chemical reactions that occur in coagulation of blood in the given order and each one is dependent upon the previous one: (a) formation of autothrombin-\(\mathbf{c}\) (Factor \(X^\alpha\)), (b) formation of thrombin, (c) formation of fibrin. The formation of fibrin is due to thrombin, formation of thrombin is due to autothrombin - \(\mathbf{c}\) \(^1\) and formation of autothrombin-\(\mathbf{c}\) is due to enzymatic degradation process. The materials required for the autocatalytic coagulation cascade are primarily found in plasma, platelets and fixed tissues. Some of the controlling components of blood
FIG. 2-4.

REATIONS INVOLVED IN NORMAL COAGULATION OF BLOOD

BLOOD COAGULATION MECHANISMS

**INTRINSIC PATHWAY**

XII $\rightarrow$ XII$^a$

XI $\rightarrow$ XI$^a$

**EXTRINSIC PATHWAY**

Tissue Damage

Tissue Thromboplastin

Platelet Aggregation

IX $\rightarrow$ IX$^a$ + VII

V + Phospholipid (Platelet Factor-3)

Platelets

**FIBRINOlytic ENZYME SYSTEM**

Prothrombin

II $\rightarrow$ Thrombin

II$^a$

Fibrinogen

I $\rightarrow$ Fibrin

XIII$^a$

Stabilized Fibrin

Fibrin Degradation Products

Plasminogen

Plasmin
coagulation consists of positive and negative feedback, retarded chain reactions, multiple enzyme involvements and integration with organ function.

There are 13 coagulation factors (Table 2-7) that take part in fibrin clot formation which is dependent upon the interplay between procoagulant and inhibitory factors. Clotting factors are conveniently grouped into:

(a) **VITAMIN K DEPENDENT FACTORS**: Synthesized in liver
   (i) X
   (ii) IX
   (iii) VII
   (iv) II

(b) **FACTOR V AND VIII**

(c) **FIBRINOGEN AND XIII.**

(d) **CONTACT ACTIVATION FACTORS** (See below)

The actual contribution of extrinsic and intrinsic pathways to the generation of factor Xa will depend upon the physiological activation sites and on the available concentrations of factor VIIa and IXa. The extrinsic activation of factor X is by tissue factor - factor VIIa complex. This alone does not provide adequate haemostasis. The classical intrinsic system of clotting is imitated by the contact activation reactions that result in factor IXa formation. (There are several links between these systems: (1) activation of VII by \( \alpha \)-factor XIIa or \( \beta \)-factor XIIa, (b) activation of factor VII by factor IXa & Xa and (c) IX activation by tissue factor - factor VIIa.)
<table>
<thead>
<tr>
<th>FACTOR</th>
<th>COMMON SYNONYM</th>
<th>ROLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Fibronogen</td>
<td>Terminal substrate of the coagulation system. Polymerizes into fibrin fibers upon proteolysis by thrombin.</td>
</tr>
<tr>
<td>III (?)</td>
<td>Tissue factor ! '?</td>
<td>- - - -</td>
</tr>
<tr>
<td>IV (?)</td>
<td>Ca²⁺ !!</td>
<td>- - - -</td>
</tr>
<tr>
<td>V</td>
<td>Proaccelerin</td>
<td>Non enzymatic procofactor for Xα in the prothrombinase complex. Labile factor.</td>
</tr>
</tbody>
</table>
Stable factor. &
<p>|        | Antihaemophilic factor - A | Non enzymatic pro-co-factor for IXα in the factor X activation complex. |</p>
<table>
<thead>
<tr>
<th>IX</th>
<th>Antihaemophilic factor - B (Christmas factor)</th>
<th>Vitamin-K dependent. Zymogen of factor IXα which activates factor X.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>Stuart - power factor</td>
<td>Vitamin-K dependent. Zymogen of factor Xα. The protease of prothrombin complex.</td>
</tr>
<tr>
<td>XI</td>
<td>Plasma thromoplastin</td>
<td>Zymogen of factor XIα which converts IX to IXα.</td>
</tr>
<tr>
<td>XII</td>
<td>Hageman factor</td>
<td>Zymogen factor XIIα which activates Factors IX &amp; prekallikrein.</td>
</tr>
<tr>
<td>XIII</td>
<td>Fibrin stabilizing factor</td>
<td>Zymogen of a transglutamase which covalently cross-links fibrin monomere with each other.</td>
</tr>
<tr>
<td>Prekallikrein</td>
<td>Fletcher factor</td>
<td>Zymogen of Kallikrein which activates Factor XII and cleaves high mol. wt. kinogen to liberate brady kinin.</td>
</tr>
<tr>
<td>High mol. wt.</td>
<td>Flaujeac</td>
<td>Non enzymatic contact activation co-factor for Factor XIIα and kallikrein.</td>
</tr>
<tr>
<td>Kinnogen</td>
<td>Fitzgerald</td>
<td></td>
</tr>
<tr>
<td>(HMWK)</td>
<td>Williams factor</td>
<td></td>
</tr>
</tbody>
</table>
FIG. 2-5. CONTACT ACTIVATION SYSTEM

PK

HMWK

K

PK

HMWK

HMWKα

BK

HMWK

HMWKα

XI

XI

- ACTIVATING SURFACE
- ZYMOGEN
- ENZYME CO-FACTOR

HMWK: HIGH MOLECULAR WEIGHT KININogen
PK: PREKALLIKREIN
BK: BRADYKININ
CONTACT ACTIVATION SYSTEM : (Fig. 2-5) The contact system of blood coagulation comprises those blood elements which are sensitive to the effect of contact with specific surfaces. The activation of this system is probably initiated by the binding of factor XII to a negatively charged surface where auto activation occurs. Factor XII, prekallikrein (PK), high molecular weight Kininogen (HMWK) and factor XI have been shown to be major proteins involved in contact activation system of blood coagulation (Fig. 2-5). In addition during the past two decades considerable evidences have accrued that these four proteins are required to initiate, amplify and propagate surface mediated body defense reaction by activating c1. (75). More emphasis should be given in this regard so that the role of contact activation system in inflammatory conditions will be better explored. Hageman factor activates several other biological systems.

The biological activity of Hagemen factor other than blood coagulation include :

(i) Activates the fibrinolytic system - by an action on plasminogen (75).
(ii) Release of kinin.
(iii) May have role in activation of acute inflammatory response (77).

However, the biological significance of these actions of Hageman factor remains elusive.

FIBRINOLYSIS :

Vertebrate fibrin has a transient existence. Fibrinolysis is essential for survival. The knowledge of presence of fibrin dissolving enzyme and fibrinolytic system stems from several sources of information. Primarily, the clot lysis is activated to limit clot formation. Plasminogen synthesized in liver, present in plasma and in the inter-cellular space, on activation liberate plasmin
FIG. 2-6. OUTLINE OF FIBRINOLYTIC SYSTEM

Contact Activation

Kallikrein

Proactivator

Intrinsic Activator

Tissue Plasminogen Activator (TPA)

Plasminogen Activator Inhibitor

PA1

Unknown Cellular Source

Pro Urokinase

Urokinase

C1 Inactivator

Plasminogen

Plasmin

Streptokinase

Plasminogen Activator

Thrombin

Fibrin (soluble)

Fibrin (insoluble)

FDP (long relatively)

Activation

(-) Inactivation

L2 AP: Antiplasmin

HRG: Histidine Rich Glycoprotein

PAF: Platelet Activating Factor

EACA: E-Amino Caproic Acid

B' A' C': Activation Steps
which splits fibrin. Like coagulation cascade, known components of fibrinolytic system are plenty (Fig. 2-6).

The most potent naturally occurring stimulator of plasminogen activator appears to be fibrin itself. Many factors are involved in the process of clot dissolution (Fig. 2-6). The process of blood coagulation and fibrinolysis are interlinked. Hageman factor activates both coagulation and fibrinolysis. Plasmin derived from plasminogen hydrolyses number of clotting factors, factor V and VIII are particularly susceptible. Released fibrin degradation products influence coagulation, may act as anticoagulants especially fragment X & Y (79).

Fibrin normally disappears from the wound site when it has performed its function (79) as a temporary hemostatic barrier and as a supporting frame work for the developing capillary beds and the migrating fibroblast. Further, fibrinolytic system is a part of a mechanism regulating fibrin deposition in tissue repair (80).

Fibrinolytic components function in a variety of other biological processes including macrophage activation, neovascularization and neoplasia (80). The stress of injury and surgical operation result in an initial increase in the spontaneous fibrinolytic activity of blood followed by a period of reduced activity in the post injury or post operative period (81). Platelet secretes plasminogen activator inhibiting factor, this may protect the blood clot against premature lysis (80). EGF induces plasminogen activator, Protein-C is one of the physiological stimulator of fibrinolysis.

The role of plasminogen activator/plasminogen-plasmin system is not restricted to fibrinolysis but extends to proteolytic events during variety of
physiological and pathological processes. In particular, this system seems to be involved in terminal differentiation, growth regulation, tissue remodeling and tumour invasion (82, 83). Thus, fibrinolytic system seems to be important for reparative process.

It is apparent that several physiological events operate at the site of injury-hemostasis, clot-lysis and inflammation which are activated by thrombin. Any deviation of these processes may alter wound repair.

**PLATELET AND WOUND REPAIR:**

The platelet or thrombocyte is an anucleate disc shaped cell that has been known to play role in primary hemostasis, blood coagulation and thrombosis. Platelets are produced in the bone marrow from precursor cell megakaryocyte by fragmentation. The released thrombocyte contain no rough endoplasmic reticulum or Golgi complex. The mature platelet contains at least two types of granules - dense and α-granules. Wide variety of substances activate platelets to release granules. Commonly, thrombin adenosine di-phosphate (ADP) and collagen induce platelet release action. Activated platelets in vivo play a complex and critical role in hemostasis and wound repair (7). Platelets also appear to play an yet unclear role in the protection of the endothelium (84).

It has been suggested that platelets play an important role in initiating the early fibroproliferative response that occurs in healing wounds (84). Platelet derived growth factor a principal mitogen, initiates DNA synthesis and cell proliferation in culture; is responsible for thrombocyte role in wound repair (84).

The α-granules of platelets contains platelet factor-4, beta thromboglobulin, fibrinogen along with PDGF, whereas dense granules contain calcium, serotonin, ADP & ATP, (Table 9-7).
<table>
<thead>
<tr>
<th>Granules</th>
<th>Factors</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Granule</td>
<td>Platelet derived growth factor</td>
<td>mitogenic for fibroblasts</td>
</tr>
<tr>
<td></td>
<td>platelet factor - 4</td>
<td>antagonise heparin</td>
</tr>
<tr>
<td></td>
<td>- Thromboglobulin.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thrombospondin. Fibrinogen.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fibronectin. Albumin. Histidine -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rich glycoprotein. Transform growth</td>
<td></td>
</tr>
<tr>
<td></td>
<td>factor.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>vonWillebrand factor.</td>
<td></td>
</tr>
<tr>
<td>DENSE GRANULE</td>
<td>adenosine diphosphate.</td>
<td>platelet aggregation</td>
</tr>
<tr>
<td></td>
<td>calcium.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>serotonin</td>
<td>platelet aggregation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>fibroblast growth &amp; metabolism.</td>
</tr>
<tr>
<td>Others</td>
<td>hydrolytic enzymes</td>
<td>activate Hageman factor and prekallkrein</td>
</tr>
<tr>
<td></td>
<td>Platelet factor - 3.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Thromboxane - A₂</td>
<td>platelet aggregation.</td>
</tr>
</tbody>
</table>
The functional significance of platelet factor-4 is uncertain and known to antagonize the effects of heparin.

When a blood vessel is injured, the smooth muscle wall rapidly contracts - this is due to the release of serotonin from platelets. Normal spontaneous haemostasis depends mainly on the formation of a haemostatic plug which fills the gap. At first this plug consists of platelets. The formation of hemostatic plug involves first adhesion of platelets to other tissues followed very rapidly by the aggregation of platelets to each other. Within a few minutes the platelets become packed much more closely. Platelet adhesion to injured endothelium is markedly increased by several substances like, collagen fibres, catecholamines, ADP and thrombin. Ionized calcium, fibronogen, thrombin and adrenaline also potentiate platelet aggregation.

Decreased platelet number is associated with incomplete conversion of prothrombin. Clearly, platelets provide materials which greatly accelerate the coagulation (74). Platelet factor-3 provide optimal conditions for the activation of clotting factors (75).

Though the signals that initiate wound healing response are largely uncharacterized, it is reasonable to suspect the role of platelet response to wounding since thrombocytes participate in the first stage of wound healing - blood coagulation. This is partially fulfilled by the investigation of Knighton et al. which demonstrated the capacity of thrombin activated platelets stimulate angiogenesis, collagen synthesis (8). It is now known that PDGF is responsible for this phenomenon. Therefore, it is understandable that patients with thrombocytopenia with or without clotting disorders show poor wound healing (7, 8).
Platelets arrest bleeding, protects endothelium, release chemoattractants, secrete mitogen, induce fibroplasia and angiogenesis. Thus, thrombocyte can be assumed to have pivotal role in tissue repair. Many drugs affect platelet function, what are the implications of these agents on wound repair? Research in this area is just beginning. Acetyl salicylic acid, retard wound healing (85, 69) whether aspirin anti inflammatory effects or antiplatelet action or both are responsible for wound healing suppressant effects is not clearly understood. Indomethacin is known to have controversial actions on wound repair (16, 69). Information in these regards are sparse. Advanced pharmacological research alone can provide, convincing explanation about how platelet function modulators affect the process of repair. This would define the importance of thrombocytes in wound healing more convincingly.

**FIBRIN DEPOSITION : FIBRINOLYSIS & WOUND REPAIR :**

Unless, bleeding is arrested wound repair cannot proceed; Fibrin deposition provide a biostable union between tissue planes and initiates and paves the way for repair. The composition of fibrin glue is important in its recolonization of fibroblasts. Fibrin besides functioning as biological glue is bioactive as well. Fibrin degradation products participate in chemotaxis and known to induce neovascularization (86). Implantation of homologous plasma clot stimulate physiological repair process and the fibrinolytic system is a part of mechanism regulating fibrin deposition in tissue repair (87). Increased plasmin level suppress both prolyl hydroxylase activity and collagen synthesis (86). Factor XII helps to regulate the size of the clot, stimulates fibrinolysis, activates neutrophil, induces chemotaxis and collagenase (88). Fibroblast move very slowly into the clot formed in the absence of Factor - XIII (46). Thrombin is a potent chemoattractant for monocytes (89). Fibrin degradation products whose mol.wt. 50,000 at
high concentration inhibit collagen synthesis other fragments may increase synthesis (86). Fibrin containing gel induces angiogenesis (90). Thus, research in the past two decades has clearly demonstrated that blood coagulation and related processes have intricate link with tissue repair.

Grossly, platelets, blood clot together with macrophage constitute wound repair stimulation triad. Therefore, hemostasis and clotlysis have profound impact on wound repair. In fact, this is gaining importance in the concerned research field. Fibrin formation may be necessary for cell proliferation (88). If so, reinforcement of blood coagulation and inhibition of clot formation by pharmacological agents is likely to disturb the process of repair. Probe into this potential area would no doubt, help us to understand the intricacies of hemostasis linked body's response to injury.