Chapter 1

Introduction

1.1. a. Wound

A wound is the discontinuation of tissue anywhere within the body or on its surfaces by a wide number of causes and it can either be microscopic or macroscopic. The skin is composed of collagen, elastin and amorphous ground substances like proteins, polysaccharides, glycoprotein, globular proteins, salt and water. It can be reemphasized that wound healing is not an isolated single phenomenon, but a complex series of biological events, the end result of which is preservation of life.

Accordingly the process of wound healing and epithelialization occurs whenever the whole body substrate is adequate for survival after injury or wound. Hence, a wound can be defined as the “Disruption of anatomical or functional continuity of living tissue produced by physical, chemical or microbial insult to the tissue and wound healing refers to the restoration of continuity of living tissue.” The different agents which play an important role in wound healing are, circulations of blood, displacement of inter and intracellular water in the tissue space or disruption of cell membranes.

Literature on wound healing states that the bulk of collagen formation and gain in strength of healing skin occurs during second week and reaches maximum at the end of second and third week. Extensive investigations have confirmed that there is involvement of several “humoral factors” in wound healing. These are gel like materials (like water, electrolyte, enzymes, proteins, polysaccharides) which occupy the spaces between bundles of connective tissue fibres. Cellular mechanism of wound healing involves different processes that have bearing on the understanding of injury, inflammation, regeneration, repair / healing. Conversely wounds have been used as models for the investigation of cellular biochemistry and physiology.
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The process of cutaneous healing follows a general scheme of the following sequential order: Platelet aggregation; blood clotting; formation of fibrin; In an inflammatory phase cellular injury; alterations in the ground substance; endothelial and capillary proliferation; fibroblastic proliferation; collagen production; epithelial proliferation and lastly surface covering. Healing is said to be complete when disrupted edges are firmly joined by collagen, dead spaces are obliterated or the surface covered by epithelium, leading to partial or complete restoration of function.

1.1. b) Classification of wounds

Based on the nature of the healing process, wounds can be classified as acute or chronic wounds. Acute wounds comprise of tissue injuries that heal completely, with minimal scarring within the expected time frame lasting usually for 8–12 weeks. The primary cause of acute wounds includes mechanical injuries in the form of abrasions and tears which are caused by frictional contact between the skin and hard surfaces. Mechanical injuries also include penetrating wounds caused by sharp objects and bullets and surgical wounds caused by surgical incisions (ex. removal of tumours). Another category of acute wounds include burns and chemical injuries, which arise from a variety of sources such as radiation, electricity, corrosive chemicals and thermal sources. The temperature of the source and the exposure time influence the degree of a thermal burn. Extensive burns will normally require specialist care because of the complications involved. Chronic wounds on the other hand, due to persistent injury that interferes with the healing process and may need several weeks for complete healing. Such wounds may also fail to heal due to repeated tissue insults or underlying pathological condition such as diabetes and malignancies, persistent infections, poor primary treatment and other patient related
factors. These result in a disruption of the orderly sequence of events occurring during the wound healing process. Chronic wounds include decubitis ulcers (bedsores or pressure sores) and leg ulcers (venous, ischaemic or of traumatic origin). Wounds are also classified based on the number of skin layers and area of skin affected. Injury that affects the epidermal skin surface alone is referred to as a superficial wound. Partial thickness wounds are due to injury involving both the epidermis and the deeper dermal layers, including the blood vessels, sweat glands and hair follicles. Deeper wounds occur when the underlying subcutaneous fat or deeper tissues are damaged in addition to the epidermis and dermal layers.
1.2. a. Wound healing

From the above facts, all wound healing essentially comprises two discrete phenomenon, viz. Inflammation and Repair.

i) Inflammation:

Inflammation is a cellular response to tissue injury, where further rapid accumulation of polymorph nuclear leucocytes takes place. During inflammatory phase various endogenous substances released are: histamine; serotonin; neurotransmitters; prostaglandins; leukotrienes; bradykinins; platelet activating factor (PAF) and superoxide radicals. Altogether inflammation is the characteristic response of mammalian tissue to injury. The Roman Celsus described that the four cardinal signs of inflammation are rubor (redness); calor (heat); dolor (pain) and tumor (swelling).\textsuperscript{11}

After inflammatory response to injury, the proliferation of cells is responsible for the repair of wound. It includes the capillary dilation and increased permeability resulting in fluid exudation into wound area from blood and lymph. The constituents of this exudate play a role in ‘chemo taxis’ to attract the cellular elements at the site of wound.

Stability of the lysosomes which contain hydrolytic enzymes plays an important role in the inflammatory phase that accompanies wound healing. Depending upon the type of cell there are thirty distinct enzymes present in the lysosomes. Lymphoid tissue, especially spleen is one of the richest sources of hydrolytic enzymes of lysosome origin.
Hence, cellular infiltration appears to play an important role in the process of wound healing.

ii) Wound repair:

Voluminous literature on wound healing indicates that the process is divided into five distinct interrelated and often concurrently processing events viz,

- Cellular phase (granulation)
- Wound contraction phase (narrowing of wound area)
- Collagen deposition (collagenation)
- Epithelial covering (epithelization)
- Scar remodeling (cicatrisation)

**Cellular phase:**

Immediately after the injury the cellular phase (granulation) begins. It activates a cascade of chemo attractants and mitogens that recruit cells like neutrophils, macrophages, fibroblasts and endothelial cells e.g. PDGF (Platelet derived growth factor).

Most of the cells respond to chemical signals in their environment by alteration in metabolism, movement and growth differentiation.\(^{12}\)

The undifferentiated mesenchymal cells at the wound margin cause platelet aggregation and blood clotting. Further, formation of fibrin as inflammatory response to bacteria, foreign bodies and cellular injury also causes alterations in the ground substance. These responses cause proliferation of endothelial cells, epithelial cells, fibroblast and leads to further collagen production.
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The vascularised connective tissue, bearing a leukocyte infiltrate known as 'Granulation tissue' mainly contributes for obliteration of dead space of the wound.

After fifth day, granulation tissue invades the entire wound area and during this period fibroblasts starts synthesizing muco-polysaccharides, collagen, probably glycoprotein and elastin.

Some of the fibroblasts modify into myofibroblasts that develop contractile characteristics and respond like smooth muscle to pharmacological agents.\textsuperscript{13-15}

Certain cytotoxic drugs like cyclophosphamide and 5-fluorouracil inhibit this granulation phase (cellular phase).

Wound contraction phase

The term contraction means diminision in size of an open wound. Generally this is a result of the centripetal movement of the whole thickness of the surrounding skin or deformity resulting from contraction in area where the skin overlies and is attached to the fascia of the muscle or to the tendon finally leading to resorption and remodeling of scar.\textsuperscript{16}

Rapid contraction of wound occurs after a lag phase from the first to the fourth day. By the fourteenth day the wound size is reduced by anything ranging from 40% - 80%. However, it has been observed that contraction may start within one to two days after injury.

The contraction of the open wounds is found to be independent of new tissue formation and epithelization.
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Magnitude of contraction varies with shape, size, site of the wound, oxygen supply and species. The excision wound ultimately heals by a combined process of wound contraction and epithelization.\textsuperscript{17}

Gabbiani \textit{et al}\textsuperscript{13} proposed that wound contraction is due to a specialized cell called the myofibroblast, the prominent characteristic of this cell is its content of micro filaments.

In lower animals contraction seldom results in deformity or loss of function. But in man it may result in a ‘contracture’ and cause deformity or functional deficit depending upon the site and size of the wound.

Different theories are advanced to explain the mechanism of wound contraction. They are:

1. \textit{Push theory}: The wound margins may be pushed in by extension of surrounding skin.

2. \textit{The growth and push theory}: According to this the wound margins may grow and push themselves inward by pushing the surrounding skin.

3. \textit{The sphincter theory}: It proposes that contractile material at the wound edge may act as a sphincter.

4. \textit{The picture frame theory}: According to this theory, active cells within the margin of the wound may migrate inward, pulling on the material within the margins of the defect.

5. \textit{Pull theory}: This pleads that the material within the defect may exert tension and pulls the margins of the defect inward.
Amongst all the proposed theories ‘The Pull Theory’ is more readily accepted and precise.\textsuperscript{18}

Certain drugs which are have anti-fibroblastin activity can decrease wound contraction viz., antiproliferative agents, cellular poisons like cyanides, dinitrophenol.\textsuperscript{19-20}

The modified fibroblasts (myofibroblasts) are linked together by desmosomes into the surrounding stroma and forms basal lamina. On contraction they actively pull the edges inward. This almost resembles the smooth muscle cells pharmacologically which is confirmed by chemical and immunologic means.\textsuperscript{21-22}

Collagen phase:

Each collagen molecule is composed of 3 polypeptide or $\alpha$-chains arranged in a triple helix in which most of the $\alpha$-chains are identical. Characteristic feature of this phase is the ‘collagen formation’ which begins from fifth day of wounding and continues till complete healing.\textsuperscript{23}

The first step in collagen synthesis is the formation of a precursor polypeptide which is then modified by the hydroxylation of certain proline and lysine residues and further glycosylation of some hydroxylysine residues. This causes the removal of non-helical extensions and converts the procollagen molecule to collagen.

Fibroblast and smooth muscle like cells which appear on the third and fourth day after injury are attracted to collagen, collagen chains, collagenous peptides, fibronectin and fibronectin fragments.
Fibrous proteins like reticular and elastin, support collagen aggregation during collagen maturation. They are found to be relatively less important in the process of healing.

Collagen derived from different species and different tissue in the same species differs in nature, but their basic characteristics that differentiate them from other proteins remain same.

Collagen maturation comprises of cross linking between tropocollagen and other collagen molecule leading to the formation of tougher and less soluble form of collagen which is resistant to degradation by tissue collagenases.

Tropocollagen, the collagen synthesized and extruded from fibroblast into the extra-cellular space undergoes maturation and gets organised there.\textsuperscript{24-26}

Tropocollagen is soluble in weak salt solutions and the three peptide chains within the collagen molecules are held together by weak electrostatic forces.

Ultimately collagen content is balanced between its production and degradation. The collagen synthesis is highest during the healing process.

Drugs like colchicine may favour accumulation of collagen without favouring its maturation and thereby might decrease the wound strength.

Collagen contains large molecules of glycine, proline, hydroxyproline all of which form a unique helical structure.

Collagen comprises 30% of the body’s protein and its continual production must be balanced by degradation and absorption of collagen.

By the third week the wound develops greatest mass of collagen which is often described as ‘Healing Ride’ and the collagen content remains higher in wound
than that in the surrounding skin for a long period.\textsuperscript{27, 28} The wound strength at this time corresponds to its collagen content.

Three polypeptides of $\alpha$-chains arranged in the triple helix constitute a collagen molecule. Each $\alpha$-chain has helical sequence of some 333 repetitive Glycine-12-triplets.

The different types of collagen are:-

- **Type-I Collagen:**

  It is commonly found in adult dermis, fascia, bone etc. Type-I collagen is heteropolymer with two of its $\alpha$-chains termed $\alpha$-1 chain identical. But the third is termed $\alpha$-2 chain similar but not identical.

- **Type-II Collagen:**

  This is found solely in cartilages and has three identical $\alpha$-1 chains that have slight differences in the amino acid sequence as compared to $\alpha$-1 chains of type-I collagen.

- **Type-III Collagen:**

  Type III Collagen is generally present in the skin but most abundantly in infancy and wound. As the wound matures type-III collagen is replaced by type-I and has three identical chains, somewhat different from those in type-I and type II collagen.

- **Type-IV Collagen:**

  This type of collagen has been identified in certain basement membrane and has three identical chains, somewhat different from those mentioned above. It is
distributed pericellularly and is found in type-I collagen. This also has two identical 
α-1 chains and α-2 chain that are different from those in type-I.

Drugs like hydrocortisone and anti-proliferatives like cyclophosphamide, 5-
flourouracil are known to interfere with healing by affecting collagen synthesis.

Epithelization:

Epithelization means complete covering of wound surface by regenerating 
epithelial cells.

Wound healing suggests that aging is accompanied by decreased inflammatory 
and proliferative responses, delayed angiogenesis, delayed remodeling and slower 
reepithelization^{20}.

*First stage:*

This phase starts within few hrs. after injury, the cell starts migrating from 
margins over the wound bed and there appears to be a tumbling or ‘Leap trogging’ 
followed by formation of basement membrane beneath.

*Second stage:*

In this stage proliferation occurs in marginal epidermal cells (within 1mm 
from epidermis) which later migrate into the wound area.
Third stage:

Finally, the newly formed migrated cells start functioning on keratinization in epidermis. After differentiation epidermis loses its capacity to divide.

Normally in excision wounds, these stages are found simultaneously.

Epithelization contributes to wound strength before collagen production. In a ‘steady state’ of epithelial cells, cell production is balanced by cell loss. Utilizing H-thymidine, the basal cells gain the ability to synthesize DNA and to divide in a random manner.

Cell movement is induced by a breach of continuity of cell to cell contact with a loss of contact inhibition and a change in the humoral environment. The movement is more rapid in moist than in dry wounds and in wounds that are isothermic.

The epithelium over scar is thinner and is less firmly attached to the underlying scar tissue than normal, because of short and sparse ‘Rete-Pegs’, which in normal skin strengthen this attachment.

‘Aber Crombies’ contact inhibition mechanism suppressing cellular mitosis is also an example for negative feedback. Many pharmacological agents viz. Corticosteroids are reported to interfere with epithelization and it is noted that hyperoxia accelerates epithelization without affecting wound contraction.

Scar remodeling (Maturation):

In higher animals a scar replaces the damaged tissue. Remodeling of scar refers to a process by which the scar changes its shape, bulk, and gradually collagen fibers are oriented along the tension lines.
Granulation, contraction, collagenisation and epithelization restore the tissue continuity by forming a scar. The scar so formed undergoes certain changes which constitute scar ‘maturation’ or ‘remodeling’.

Inter molecular cross-linking of collagen fibers provides the best mechanical structure and contributes for increased wound strength in late phase.

The wound may gradually enlarge forming a hypertropic scar or massive ‘keloids’ due to altered collagen metabolism. Thus keloids would appear to be simply a disease of uncontrolled collagen synthesis, with normal collagen lysis. The factors interfering in collagen metabolism obviously interfere with scar maturation, the ultimate process of healing.

With advancing age, the scar fibres and their bundles become more closely packed to favour inter molecular cross-linking.

This organized collagen contributes for increased wound strength in late phase. During the first three weeks, the wound strength correlates with scar collagen content. Normally, the scar decreases in the bulk gradually, normal pigmentation returns and scar become insignificant.

The factors that are reported to have suppressant effect on healing process are corticosteroids, anti-inflammatory agents, chronic stress, ACTH, hypoxia, diseases like diabetes, malignancy, anaemia, uremia and jaundice.

The factors known to have pro-healing effect are: vitamin-A, Vitamin-C, Vitamin-E, trace elements like zinc, iron, copper and also sleep, nutritional diet, high oxygen tension, warm environment, insulin, microcrystalline collagen, honey etc.
Certain cytotoxic drugs like cyclophosphamide, 5-fluoruracil, colchicines, certain foreign bodies, aging, hypothermia, trauma, local irradiation etc. also suppress the healing process.\textsuperscript{30,31}

However, it is not certain whether or not these agents accelerate normal wound healing.

1.2. b. Monitoring of wound repair:

Wound healing is a complex process, the monitoring of which involves the following features:

i) Physical (contraction and epithelization)

ii) Mechanical (tensile strength or wound breaking strength) attributes

iii) Biochemical (collagen & mucopolysaccharides)

iv) Histological (microscopic features)

Repair and healing is monitored by any of the methods that measure the above mentioned attributes. The techniques of wounding may modify the attributes and particularly these attributes are better delineated in a particular wound model.

So monitoring of one particular wound model does not truly assess the healing, hence different wound models have been developed.

A method was introduced for estimation of breaking strength to assess the healing which became popular after introduction and pioneered the studies on rate or gain in breaking strength of tissues. Contraction and epithelization were the only parameters employed.\textsuperscript{32}
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The role of collagen in wound healing plays an important role and hence the hydroxy proline estimation and also other studies involved are weights of granuloma for histological features and tensile strength.

i. Physical attributes:

Contraction, epithelization and scar remodeling are monitored by measuring total wound area, open wound area, and scar area e.g. bulk, shape and colour etc.

Excision wound is ideal to study these attribute which gives the rate of healing, wound contraction and epithelization.\(^{33}\)

The wound area is marked by tattooing or by putting scattered stitches around the margin during operation.

One of the methods used is the wound area is traced on a paper, weighed and compared with that of reference paper to directly measure the area.

Complete epithelization can be assessed by noting the time for complete covering of the raw surface of the wound. ‘Thorostat’ is a sophisticated technique with electron opaque marker and is reported for identification of migrating epithelial cells.\(^{34}\)

Scar remodeling can be assessed by mere observation and assessing the physical changes in collagen.

Granuloma studies are done by quantifying granuloma itself by determining overnight dry weight.

This study was modified by Cotton pellet granuloma technique of D’Arey as described by Turner.\(^{35}\)
ii. Mechanical attributes:

Skin exhibits tension and extensibility. The tension of skin is related to the content and direction of the elastic fibres of the dermis, which diminishes with age.

The extensibility of the skin is the amount to which it stretches before it breaks. It is made up of two parameters, elastic stretch and non-elastic stretch or plastic flow. So tensile strength is measured in terms of load applied in unit of cross sectional area as expressed in Lbs/sq. inch or kg/sqcm or kg/sq mm.

The wound breaking strength in different areas of the body can vary but the tensile strength remains steady due to tissue thickness. Breaking strength of incised resutured wound can be measured by noting the load applied per unit area to disrupt the wound. Both breaking strength and tensile strength can be monitored by Tensiometer or Constant water flow technique.36

iii. Biochemical attributes:

Biochemical activities in a wound attain a higher rate than in the surrounding tissue. Increased activity may extend as far as 7.5mm on either side of an incision wound.

For estimating the collagen content, hydroxyproline an amino acid in collagen is measured by colorimetric methods, spectrophotometric method or chromatographic methods.37

iv. Histological attributes:

Histological studies are done by examination of granulation tissue. A sponge biopsy technique is employed to study the repair process and this method is modified by Ehrlich et al.15 by subjecting polyvinyl sponge disc granuloma to microscopic examination.
1.3. Wound dressing materials (WDM)

Wound dressing materials are the dressing materials that are used to heal or promote the wound. Wound dressing based on natural materials such as alginic material is well known, in literature as well as from commercial point of view, in wound management.\textsuperscript{38-41} Chemically treated calcium alginate being a natural haemostat, alginate based dressings are indicated for bleeding wounds. Another interesting parameter is the gel forming property of alginate, which helps in removing the dressing without much trauma and reduces the pain experienced by the patient during dressing changes.\textsuperscript{42} It provides a moist environment that leads to rapid granulation and reepithelialization. In a controlled clinical trial, significant numbers of patients dressed with calcium alginate were completely healed at 10\textsuperscript{th} day, compared with the member of the paraffin gauze group. Calcium alginate dressings provide a significant improvement in healing split skin graft donor sites.\textsuperscript{43} In another study with burn patients, calcium alginate significantly reduced the pain severity and was favored by the nursing personnel because of its ease of care. The combined use of calcium sodium alginate and a bio-occlusive membrane dressing in the management of split-thickness skin graft donor sites eliminated the pain and the problem of seroma formation and leakage, seen routinely with the use of bio-occlusive dressing alone.\textsuperscript{44} Different marketed products of alginate based WDM are listed below.
Table 1: Marketed alginate based wound dressing materials with company names.

<table>
<thead>
<tr>
<th>Product</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>AlgiDERM</td>
<td>Bard</td>
</tr>
<tr>
<td>AlgiSite</td>
<td>Smith &amp; Nephew, Inc.</td>
</tr>
<tr>
<td>Algosteril</td>
<td>Johnson &amp; Johnson</td>
</tr>
<tr>
<td>CarraSorb H</td>
<td>Carrington</td>
</tr>
<tr>
<td>CuraSorb</td>
<td>Kendall</td>
</tr>
<tr>
<td>CuraSorb Zinc</td>
<td>Sherwood-Davis &amp; Geck</td>
</tr>
<tr>
<td>Dermaceae</td>
<td>B. Braun</td>
</tr>
<tr>
<td>FyBron</td>
<td>Gentell</td>
</tr>
<tr>
<td>Gentell</td>
<td>Hyperion Medical, Inc.</td>
</tr>
<tr>
<td>Hyperion Advanced</td>
<td>ConvaTec</td>
</tr>
<tr>
<td>Alginate Dressing</td>
<td>DeRoyal</td>
</tr>
</tbody>
</table>

Another naturally occurring biopolymer used in the production of WDM is Chitosan. Chitosan is derived from chitin, a major component of crustacean outer skeletons. This material is known in the wound management field for its haemostatic properties. Further, it also possesses other biological activities and affect macrophage function that helps in faster wound healing. It also has an aptitude to stimulate cell proliferation and histoarchitectural tissue organization. The biological properties including bacteriostatic and fungistatic properties are particularly useful for wound
treatment. Like alginate material, several references on chitosan in wound treatment are available. Flexible, thin, transparent, novel chitosan–alginate polyelectrolyte complex (PEC) membranes caused an accelerated healing of incision wounds in a rat model compared with conventional gauze dressing. Closure rate and appearance of PEC membrane treated wounds were comparable with Opsite1-treated wounds. Application of the photo-cross-linkable chitosan hydrogel on full-thickness skin incisions made on the backs of mice, significantly induced wound contraction and accelerated wound closure and healing compared with the untreated controls. Healing at split skin graft donor sites was studied by dressing half with chitosan and the other half with a conventional dressing. It showed that chitosan facilitated rapid wound re-epithelialization and the regeneration of nerves within a vascular dermis. Early returns to normal skin color at chitosan-treated areas were demonstrated. Treatment with chitin and chitosan demonstrated a substantial decrease in treatment time with minimum scar formation on various animals. Biochemistry and histology of chitosan in wound healing has been reviewed by Muzzarelli et al. and Feofilova et al. Drug incorporated bilayer chitosan wound dressing showed excellent oxygen permeability, controlled water vapor transmission rate, and water-uptake capability. It exhibited excellent antibacterial activity by in vitro culture for 1 week. Chitosan has been studied widely as a wound dressing material, however, a wound-dressing product based on chitosan is yet to be commercialized.
1.4. Classification of WDM

Wound dressing materials are basically classified on the basis of their origin i.e., Natural and synthetic as below:

i) Passive products

ii) Interactive products

iii) Bioactive products

Traditional dressings like gauze and tulle dressings that account for the largest market segment are passive products. Interactive products comprise of polymeric films and forms, which are mostly transparent, permeable to water vapor and oxygen but impermeable to bacteria. These films are recommended for low exuding wounds. Bioactive dressings are either designed to deliver bioactive compounds or these dressing materials have endogenous activity (proteoglycans, collagen, non-collagenous proteins, alginates or chitosan).

Food Drug Administration (FDA) of USA reclassified the dressing categories as,

i) Non-resorbable gauze/sponge dressing

ii) Hydrophilic wound dressing

iii) Oclusive wound dressing

iv) Hydrogel wound and burn dressing

v) Interactive wound and burn dressings
1.5. Wound healing animal models.

Different wound models in every species ranging from amphibian to alligator, from tadpoles to trout and from mongoose to man have been employed to study these complex processes of wound healing. The commonly used animals for the purpose are rats, rabbits and guinea pigs. In another study, some observations were made in animal models which may have to be judiciously transferred to human beings. Open wounds of 4 sq. cms. were made on the backs of adult male New Zealand white rabbits (1.8 to 2.5kg body weight) and the effect of vinblastin and colchicine (tropically applied) on wound healing were studied.

Morton and Molane,34 employed circular wounds of 2.5cm diameter made on depilated dorsal thoracic region in rats. The measurements were taken by either putting sutures on the margin of wound or after tattooing or after marking the margin with an indelible ink.

It is possible to differentiate the process of contraction and epithelization in these studies by planimetric methods made by series of photographs taken at different time intervals.

Results are expressed as percentage closure of the original wound size at a given time measurement. Another characteristic is that its ‘Half life’ can be calculated, which is time in days required to reduce its area by one half and is known as wound half closure time.

Church and Warren62 employed a 2 x 2 full thickness excision wound in the web of the fruit-bat and concluded it is to be superior to all the methods.
However, it has not gained popularity. In a study a new technique by controlled application of heat and cold is employed so that the excision wound may be produced.

The rate and extension of contraction, epithelization time, scar features are most commonly employed parameters. Other parameters include collagen estimation, histological studies and hydroxyproline estimation in granulation tissues of open wounds.

In incision wound models, the rate of gain in breaking strength is an important aspect estimated by "Tensiometer or Constant water flow technique".\textsuperscript{63}

Estimation of breaking strength is another parameter to measure wound strength. Full thickness skin wounds of suitable length (6 cm\textsuperscript{2}) on the back, parallel to and 1cm lateral to vertebral column have been described for the purpose of studying breaking strength in experimental animals.\textsuperscript{64}

The sutures using preferably black thread or Michels clips were used to close these wounds. Sutures are removed a day or two prior to the measurement of breaking strength.

Quantitative estimation of granuloma formed on foreign bodies implanted in dead wound has been shown to be a reliable parameter in the study of wound healing. Granulation tissue is also used to estimate tensile strength, collagen content and for histological studies.

The granulation tissue may be grown on foreign bodies like polyvinyl sponges implanted in dead space wounds. Subcutaneous implantation of any inert foreign body like plastic of suitable dimensions seems to induce granuloma formation.
I.6. Plants selected for the study

**ISAPGOL**

![Isapgol plant](image.png)

Figure 1: Photograph of Isapgol plant.
ISAPGOL

Botanical name : Plantago ovata Forsk.

Family : Plantaginaceae.\textsuperscript{65}

Part used : Dried seed husk

Geographical source:

Widely grown in India, West Asia, Pakistan, Persia, Mexico and Mediterranean regions. It is grown as a cash crop in Gujarat, Punjab and Uttar Pradesh.

Chemical constituents:

It contains Polysaccharides on hydrolysis it gives xylose, arabinose, galactouronic acid and rhamnose. SHI contains \textasciitilde{}15\% of non-polysaccharide material\textsuperscript{66} and the remaining 85\% appears to consist of a single polysaccharide comprising D-xylose (\textasciitilde{}62\%), L-arabinose (\textasciitilde{}20\%), L-rhamnose (\textasciitilde{}9\%) and D- galactouronic acid (\textasciitilde{}9\%)\textsuperscript{5}. The sugars present and their approximate proportions were first determined by Laidlaw and Percival.\textsuperscript{67, 68} Out of two polysaccharide fractions separated from the husk mucilage, one (eq.wt.700; uronic acid 20\%) is soluble in cold water while another (eq.wt.4000; uronic acid 3\%) is soluble in hot water.\textsuperscript{67} The SHI is hygroscopic in nature and absorbs large quantity of water to become mucilaginous.

Therapeutic uses

The dried seed husk of Plantago ovata, is indigestible and is a source of soluble dietary fiber. It is used to relieve constipation, irritable bowel syndrome and diarrhea. Also used as a regular dietary supplement to improve and maintain regular
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GI transit. The inert bulk of the husk helps provide a constant volume of solid material irrespective of other aspects of the diet or any disease condition of the gut.\textsuperscript{65} Few recent research reveals that they are promising in lowering cholesterol and controlling diabetes.\textsuperscript{69-71} The husk may also be combined with other ingredients. For example, Blackstrap molasses is sometimes used with for its high mineral and vitamin content, as well as being an excellent carrier. A typical dose is one to three teaspoons per glass of water. The seeds can be used for the same purpose at a lower cost. The standard dose is 3.5gm dissolved in 250 ml of water.\textsuperscript{72-73}
TAMARIND

Botanical name :  *Tamarindus indica* Linn.

Family :  Leguminosae.\textsuperscript{74,75}

Part used :  Dried seed kernels

Geographical source of plant:

The plant is widely cultivated in tropical Africa, Asia, Mexico, Germany and Japan. It is cultivated all over India, especially in Andhra Pradesh Tamil Nadu and Karnataka. In the United States, it is a large-scale crop introduced for commercial use, second in net production quantity to India.
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Chemical constituents:

Its sticky pulp is rich source of non-starch polysaccharides (NSP) or dietary fiber such as gums, hemicelluloses, mucilage, pectin and tannins. It is rich in tartaric acid which gives a sour taste to food but is also a very powerful antioxidant. The fruit contains many volatile phytochemicals such as limonene, geraniol, safrole, cinnamic acid, methyl salicylate, pyrazine and alkylthiazoles. Together these compounds account for the medicinal properties.\textsuperscript{76}

The tamarind seed polysaccharide (TSP) is obtained from the tamarind seeds and contains about 65 percent of total dry weight of seeds kernel. It is a neutral hydrophilic, mucoadhesive polymer structurally similar to xyloglucan. Chemically it contains D-mannose and D-galactose units.\textsuperscript{77-79}

Therapeutic uses

It contains many health benefiting essential volatile chemical compounds, minerals, vitamins and dietary fibres. Its pulp has been used in many traditional medicines as laxative, digestive and as a remedy for biliousness and bile disorders. Traditionally the bark is used in treatment of paralysis, urinary discharge and gonorrhea. Seeds are useful in vaginal discharge and in treatment of ulcer.\textsuperscript{80,81} In northern Nigeria, fresh stem bark and fresh leaves are used as decoction mixed with potash for the treatment of stomach disorders, general body pain, jaundice and yellow fever, as blood tonic and skin cleanser. In Indonesia, Malaysia, Philippines and Japanese traditional medicine, asem leaves are used as herbal infusion for malarial fever, the fruit juice as an antiseptic, and for scurvy and even cough cure. Throughout Southeast Asia, the fruit is used as a poultice and applied to foreheads of fever sufferers.\textsuperscript{82} It is used in Ayurvedic medicine for gastric and/or digestion problems and as a cardioprotective.
PIGEONPEA

Botanical name : *Cajanus cajan* Spreng.

Family : Papilionaceae

Part used : Dried seeds

Geographical source:

It is cultivated throughout the world in both tropical and sub-tropical regions mainly in India and South Africa. The three major pigeonpea producing regions of the world are India, East Africa and Central America. It is highly drought resistant, so can be grown in areas with less than 650 mm annual rainfall.⁸³

Figure 3: Photograph of Pigeonpea plant.
Macroscopic characters:

An erect shrub, 1.5 to 3 meters in height with silky branches. Leaflets are oblong, indistinctly dotted, flowers are loosely arranged, forming a terminal panicle. Calyx and corolla are 6 mm in height and are yellow in color. Seeds are yellow to reddish brown in color with varying size.

Chemical constituents:

It contains between 57.3 g/100g carbohydrate, 19.2 g/100g protein, 1.5 g/100g fat and 8.1g/100g fiber. It contains protein, amino acids like methionine, lysine and tryptophan.

Uses:

Traditionally the seeds are used as anthelmintic, in treatment of piles and biliousness. Leaves are used in inflammation and wounds. The juice of the flower and leaves are used as antidysenteric and laxative.
1.7. Aims and objectives

(i) Isolation and characterization of polymers of plant origin from three different plants sources viz., Plantago ovata Forsk. Tamarindus indica Linn. and Cajanus cajan Spreng.

(ii) Among three different polymers, isolated seed husk of isapgol (SHI) is highly cross-linked and used without any chemical modification, where as tamarind seed polysaccharide (TSP) and pigeonpea polymer (PPP) are cross-linked with epichlorhydrin so as to make them more suitable as wound dressing materials.

(iii) Two different formulations are prepared i.e., gel and film. Gel formulations are characterized for various by rheological properties like shear stress, torque etc.

(iv) Film formulations are evaluated for film strength, transparency, water vapor transmission, skin irritation test (sensitivity) etc.

(v) Chemical characterization, analysis, standardization of newly isolated natural polymer and chemically modified polymers are undertaken for FTIR, NMR spectral studies.

(vi) Antibacterial activity and In-vivo evaluation of new product on wound closure in excision wound model is undertaken.