Chapter 1

Introduction

1.1 WOUND

A wound is defined as an imperfection in the skin, caused due to physical or thermal damage or as a result of an underlying medical or physiological condition. According to the Wound Healing Society (www.woundheal.org), a wound is a transformation in the normal anatomic structure and function. It may be reversible or irreversible outcome of an injury in which the part affected is cut, torn or punctured which may be due to trauma, surgery or health disorders. Wounds may be classified as those with tissue loss and without tissue loss. The common example of wounds with tissue loss include burn wound (second and third degree burn wounds), diabetic foot ulcer etc., and those without tissue loss include laceration and first degree burn wound. A superficial wound is one which affects the outermost layer of the skin surface (ie epidermis), while a partial thickness wound affects both the epidermis and the deeper dermal layers, including the blood vessels, sweat glands and hair follicles (Naradzay FX and alson R 2005). A full thickness wound affects the underlying subcutaneous fat or deeper tissues in addition to the epidermis and dermal layers.

The further classification of wounds are they may be acute or chronic by nature (Ferreira et al 2006) based on the time required to heal. Acute wounds are usually injuries that cause a break in the skin or can happen suddenly, last for short duration of time, heal completely and may heal on its own with minimal scarring. Acute wounds are further classified into two types namely traumatic wound and surgical wound. A traumatic wound is a minor cut that causes severe tissue injury when a force is more than the strength of the skin or the underlying supporting tissue. A surgical wound is a cut and sutured or lay open to heal by a surgeon. The
acute wounds are caused due to mechanical injuries like abrasions and tears which have the frictional contact between the skin and hard surfaces. The other injuries include penetrating wounds caused by knives and fire arm shots, surgical incisions etc. The variety of sources which arise from burn and chemical injuries are radiation, electricity, corrosive chemicals and thermal sources. The degree of thermal burn is influenced by the source temperature and the time of exposure.

The other type of wound is the chronic wound which are caused due to injury in the tissue. They do not heal in an orderly set of stages with in a time frame. chronic wounds do not heal beyond 12 weeks and tend to occur often (Harding et al 2002). These types of wounds fail to heal due to repeated tissue infections or physiological conditions such as diabetes and malignancies, constant infections, poor primary treatment. Chronic wounds also include ulcers (bedsores or pressure sores) and leg ulcers (venous). Both acute and chronic wounds that are difficult to heal are referred as complex wounds with unique characteristics as shown in Fig.1.1. The properties of complex wounds are:

(a) Extensive loss of the integument which comprises skin, hair, and associated glands.
(b) Infection which results in tissue loss.
(c) Tissue death or signs of circulation impairment.
(d) Presence of pathology.
Fig. 1.1. Optical images of acute and chronic wounds

1.2 WOUND HEALING

Wound healing is the body's regular biological process and occurs as four stages: hemostasis, inflammation, proliferation and time frame (Rothe and Falnga 1989, Shakespeare 2001). For a wound to heal completely all the four stages should occur (Schultz 1999) in an orderly manner and within a stipulated time.
1.2.1 Hemostasis

This is the first stage which starts as soon as a wound occurs, with vascular constriction and fibrin clot formation. Pro-inflammatory cytokines and growth factors such as transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) are released from the clot and surrounding wound tissue in this phase.

1.2.2 Inflammation

This stage is promoted when the bleeding in the wound is controlled and the cells start to move into the wound. During this phase infiltration of neutrophils, macrophages and lymphocytes occur in a sequence. The purpose of the neutrophils is, it aids in the clearance of approaching microbes and any waste in the wound. Cytokines are released which promote inflammatory response and creates additional leukocytes. Additionally they clear the apoptic cells. Macrophages promote the changeover to the proliferative phase of healing by clearing the apoptotic cells to stimulate keratinocytes, fibroblasts, and angiogenesis, to form new tissues shown in Fig.1.2 (a)

![Inflammation phase of a wound healing process.](image)

Fig.1.2. (a) Inflammation phase of a wound healing process.
1.2.3 Proliferation

In this phase within the wound, the migration of epithelial cells and fibroblasts takes place to substitute damaged and lost tissue. In the dermis, fibroblasts and endothelial cell are the known cell types that initializes capillary growth, formation of collagen and granulation tissue formation at the place of injury. The fibroblasts also helps in the production of collagen, glycosaminoglycan and proteoglycans which contribute to the extracellular matrix (ECM) and are shown in Fig.1.2(b).

![Fig.1.2. (b) Proliferation phase of a wound healing process.](image)

1.2.4 Remodeling

The final stage of the wound healing process which takes long duration of time. The characteristic feature of this phase is the vascular density of the wound attains the regular pattern due to the newly formed capillaries. In this stage the extracellular matrix is remodeled to an architecture that resembles the normal tissue shown in Fig.1.2(c).
1.3 CLASSIFICATION OF WOUND DRESSINGS

Numerous ways are used in the classification of dressings based on their role in wound like the debridement, antibacterial, occlusive, absorbent, and adherence (Purna and babu 2000). The materials used to produce the dressings are collagen, hydrocolloid, fibrin, chitosan, alginate (Queen et al 2004). The physical form of dressings include ointment, film, foam, gel etc (Falabella 2006). The other classification of dressings are primary, secondary and island dressing. (Van Rijswijk 2006). Dressings are further classified into traditional, modern, advanced dressings, skin replacement products and wound healing devices.

1.3.1 Traditional Dressings

Wound dressings have been used for several years and dates back to the crude application of plant herbs, animal fat and honey for the treatment of wound. Many traditional plants in Africa exhibit antibacterial activity for treatment of wounds (Kudi et al 1999). The leaves of Guiera senegalensis exhibit anti-bacterial and anti-radical effects for treating wounds and inflammatory swellings. The application of these crude material for the treatment of wound pose potential problems like infection due to the presence of microorganism and chemicals. The quality of wound management materials have shown tremendous improvements due to good sterilization and aseptic practice applied to medicine and surgery.
Traditional dressing can be classified into absorbing and non-absorbing dressing. The common examples of such dressing include Gauze, lint, cotton and tulle. These dressings fulfill a not many properties of an ideal dressing and are restricted in their use as primary dressings and sometimes as secondary dressings.

1.3.2 Interactive dressings

Interactive dressings consists of foams and polymeric films. These possess properties like transparency, permeability to water vapor and oxygen but impermeable to bacteria. These films are recommended for low exuding wounds. Interactive dressings use the body's atmosphere to heal normally.

1.3.3 Hydrogel based dressings

Hydrogel is an arrangement of polymer chains that are not soluble in water, sometimes originate as a colloidal gel in which the dispersion medium is water. Hydrogels contain sufficient water and exists as natural or synthetic polymers. They are flexible and are analogous to natural tissue owing to their water content. Currently hydrogels are used as scaffolds for drug delivery, wound dressing and tissue engineering applications. The advantages of hydrogel dressings are retention of moist atmosphere, high swelling capacity, creating cooling sensation and wounds are healed without a scar.

1.3.4 Bioactive dressings

Bioactive dressings in the recent years has contributed significantly towards wound healing process and are usually referred as biomaterials. They may be derived from natural or artificial sources.(Barlett 1981) The polymers such as collagen (Doillon and Silver 1986), chitin and chitosan (Ishihara et al 2002) alignates and elastin are some of the naturally occurring biomaterials. The advantage of preferring these types of dressings are that they are much superior than the conventional and synthetic dressing materials like gauge and hydrogel dressings.(Pruitt and Levine 1984)
1.4 BIOMATERIALS FOR WOUND DRESSING

In the last 30 years the role of biomaterials has significantly improved in the development of novel treatments. These find a wider application in treating acute and chronic wounds. Biomaterials can be natural or synthetic in origin. These may be defined as inorganic, organic materials or combination of both, which are biocompatible and can be implanted in the human body to replace or repair failing tissue. They may be distinguished from other materials in that they possess a combination of chemical, mechanical, physical and biological properties which render them safe, effective and reliable within a physiological environment. The other advantage of these materials is that they do not require any surgery for their removal. In recent years, medical scientists have attempted to use these materials in fabricating substitutes for damaged, injured or deceased portions of the body. Presently, both synthetic and natural polymers find increasing applications in the medical field. There are certain characteristics that are consistently important for a biomaterial.

- The material should be non-irritant, non-toxic and should not cause any inflammatory reaction to the neighboring tissues.
- It should not be antigenic.
- It should not be carcinogenic.
- It should be sterilizable.
- It should be adequately available for widespread utilization.

The concept of temporary biological dressing, introduced in 1930s has been developed to the point where there use has been extended from burns to all types of granulating wounds. Although the introduction of topical antibiotics has allowed greater percentage of burns to proceed to healing without grafting, the need for wound closure by autografts remains. The constant progress in technology has now made it possible to produce numerous synthetic, bio-synthetic and biological substitutes capable of replacing skin, for limited periods of time. The skin substitutes
now available can be divided into three categories, depending on their origin and physiochemical composition.

* Biological skin substitutes
* Synthetic skin substitutes
* Biosynthetic skin substitutes.

Among the biological covering materials the following are of particular importance

- Homologous skin
- Pig skin
- Human amniotic membrane
- Collagen derivatives
- Cultured allograft

Some of the biomaterials that occur naturally are collagen, fibrin, alignates, chitin, chitosan, elastin and gelatin. These biomaterials have wide spread application.

1.5 COLLAGEN

Collagen the natural constituent of the connective tissue, a mammalian protein is available on earth in large quantities. 20–30% of total body proteins consists of collagen (Harkness 1961). The emergence of collagen has been reported in the early stage of evolution in primitive animals like coral, jellyfish and sea anemones (Bergeon 1967). Bovine skin, tendon, bone, eye (Bilgen et al 1999, Nagai et al 2004, Sadowska et al 2003) porcine skin and rat tail are the common sources from which it can be extracted. The other sources include all living animals like alligators (Wood et al 2008), kangaroos (Johnson et al 1999) etc. It can also be extracted from marine life forms like sponges (Exposito et al 2002), fish (Sugiura et al 2009) which contribute excellent source of collagen. There is change in the collagen properties from animal to animal, therefore it is worth investigating the
sources which contribute the extraction of collagen (Lin and Liu 2006). One half of the total body collagen is present in the skin and 70% of the material other than water present in dermis of skin and tendon is collagen.

1.5.1 The Collagen Molecule

Collagen the essentially studied biomolecule of the extracellular matrix, contributes 25% of the dry weight of mammals and in humans. Twenty nine different types of collagen are identified based on their characterisation and polymeric structure. The primary structure of all these identified exhibit a triple helix shown in Fig.1.3.

![Triple helical structure of collagen.](image)

Collagen I, II, III, V, and XI are found to exhibit continuous triple helix structure with three α chains. They exhibit fibrillar structures and possess homologous sequences that are large and independent of species identified (Timpl 1984). The three basic amino acid that contribute to the triple helical structure of collagen are glycine, proline and hydroxyproline. Based on their molecular sequence three α chains are present in collagen. Three parallel polypeptide strands forms the
right hand triple helix and left-handed polyproline 11-type (PPII) helical conformation coil around one another. The triple helix structure of collagen is contributed by three basic amino acid namely glycine, proline and 4-hydroxyproline. The 3 α chains present is confirmed by its molecular sequence. A single α chain of the collagen helix consists of thousands of amino acids and every third residue is the presence of glycine that results in a repeat sequence Xaa-Yaa-Gly repeat unit, where Xaa and Yaa can be any amino acid. The confirmation of glycine at the third position is necessary to substantiate the tight packing of the three α chains in the tropocollagen molecule. Proline(Pro) occupies the Xaa, 4-hydroxyproline (Hyp) occupies the Yaa position creating Pro-Hyp-Gly as a common repeat triplet in collagen (Ramshaw et al 1998). Twenty five different α chains are present in 29 different types of collagen. Though the 3 chains are identical, triple helices heterotrimeric by nature are more common than the homotrimeric forms.

Many types of collagen are characterized still a few are applied for producing biomaterials based on collagen. Tropocollagen assembles in to 10-300 nm sized fibrils and then the fibrils agglomerate to form collagen fibres which range between 0.5 to 3.0 min diameter. In type IV collagen, the triple helical conformations regions are stopped by short non-helical peptides and large non-helical domains. Fibril linked collagens (type IX, XI, XII, and XIV) possess small chains, type VI is a microfibrilla collagen and type VII is anchoring-fibril collagen (Samuel et al 1998). Of all the types discussed above the most abundant type of collagen in the skin is the Type I collagen which is presently considered as the "gold-standard" in tissue-engineering applications. TypeI collagen has a carbohydrate content below 1% and is a glycoprotein. The sugar components present are single galactose unit or a disaccharide of galactose and glucose O-glycosidically attached via hydroxyl sine residues. Its close resemblance with human tissue has been studied in depth to produce biomaterials applied for medical applications for many long years (Khor 1997).

1.5.2 Collagen - The biomaterial

Collagen, the major constituent of the connective tissue protein in vertebrates. It is used in various forms for sutures, wound dressing agents,
haemostatic agents, surgical tampons, vascular prosthesis etc. Pure collagen has very little immunological activity and is used as an excellent substrate for cell attachment and cell growth. The favorable properties of collagen as a biomaterial have been summarized below:

- Pure collagen has very little immunological activity, this little activity is lost by removing the non-helical telopeptides portion of collagen by enzymatic treatment.
- Both collagen as well as cross linked collagen are physiologically resorbable.
- Collagen has excellent haemostatic property.
- Collagen functions as an ion exchanger and binder of electrolytes, metabolites and pharmaceuticals.
- The positive effect on wound healing is due to the stimulation of fibrinogenesis.
- Collagen is an outstanding substrate for cell attachment and cell in growth.
- The solid collagen material can be easily sterilized by gamma irradiation or by the use of ethylene oxide
- Non-antigenic.
- Biodegradable and bioreabsorbable.
- Non-toxic and biocompatible.
- Well suited with bioactive components
- Collagenous materials can be prepared in various forms i.e., (solution, gel, powder, fleece, film, fabric, tape, sponge, tube etc.)
- Well matched with synthetic polymers
Disadvantages

- Pure type I collagen is very costly.
- Variability of isolated collagen (e.g. crosslink density, fiber size, trace impurities, etc.)
- Hydrophilicity which leads to swelling and more rapid release.
- The degradation rate of enzyme is variable compared to the hydrolytic rating.
- The handling properties are difficult.
- Prone to side effects like bovine spongeform encephalopathy

(Sources: Friess, 1998, Fujioka et al, 1998.)

1.5.3 Preparation of different forms of collagen and its applications in medicine

Collagen can be prepared in various forms like film, shield, sponge, gel, powder, fleece, pellet, fabric, tape, tube etc.

1.5.3.1 Film/sheet/disc major

The chief application of collagen film is as barrier membrane. Films with a thickness of 0.01-0.5 mm made of biodegradable materials are developed by air-drying a casted collagen preparation. Collagen membranes are loaded with drugs by hydrogen bonding, covalent bonding or simple entrapment. These are sterilized and upon hydroxylation undergo bending to maintain the required strength. Collagen film/sheet/disc is used treating infection caused in tissue, typically corneal tissue infection, cancer in liver, and as a carrier for drugs such as tetracycline (Minabe et al 2010). To promote bone formation collagen film and matrix are used as carriers for gene delivery. To monitor the bone development, a composite of collagen and recombinant human bone morphogenetic protein 2 (rhBMP-2) was used and it was observed that the composite rhBMP-2/collagen resulted in the formation of the bone while when collagen alone was used it resulted in no bone formation. (Murata et al
1999). The matrix of collagen loaded with bone morphogenetic protein (BMP), when placed in near contact with osteogenic cells obtained direct osteoinduction without no cartilage formation (Park et al 2000). Biodegradable collagen films or matrices are used as scaffolds for transfected fibroblasts survival. when collagen is combined with other polymers like atelocollagen matrix and added on the surface of the polyurethane films, these exhibit better attachment and proliferation of fibroblasts and gives good support for growth of new cells (Park et al 2000). The matrix films, composed of different combinations of collagen and elastin, are used in tissue calcification and serve as a device for cardiovascular drugs.

1.5.3.2 Collagen shields

The collagen shield was intended for bandage contact lenses by dissolving in the cornea. The ease with which these formulations are applied to the ocular surface and the self administration property gives collagen-based drug delivery systems a distinctive advantage. The shield's mechanical properties is used to defend the corneal epithelium healing from blinking movement of the eyelids. Drug delivery by collagen shields depends on loading followed by discharge of medication by the shield (Leaders et al 1973). The collagen matrix acts as a storage space where the drugs are held in the interstices of the collagen matrix. As tears roll through the shield, it dissolves the shield and forms a layer of biologically adopted collagen solution. This solution lubricates the eye surface minimizing the rubbing action of the lids on the cornea and rises the contact time between the drug and the cornea to, facilitate epithelial healing (Podos et al 1972).

1.5.3.3 Collagen sponges

Pure collagen secluded from bovine skin, puffed-up at pH 3.0 are used for making the sponges. It exists in the physical form of sponge layer by stabilization. Sponges are combined with other materials like elastin fibronectin or glycosaminoglycans (Geesin et al 1996) to obtain excellent elastic activity and fluid-building capacity. The starting material is cross-linked with glutaraldehyde and subsequently copolymerized with other polymers like
polyhydroxyethylmethacrylate (PHEMA). The water absorbing properties of the PHEMA chains enables the membranes to be wet and rises their tensile strength thus affecting the management of wounds and burns that are infected. Collagen sponges are used in the treating severe burns and is a ideal dressing material for different types of wounds, such as pressure sores, donor sites, leg ulcers and decubitus ulcers. The advantages of using the sponge is its ability to absorb wound exudates, maintain a low moist environment around the wound. The other advantages include preventing from mechanical harm and protecting against bacterial infection. The sponge improves the dermal and epidermal healing when coated with a growth factor. (Marks et al 1991).

1.5.3.4 Gel, hydrogel, liposomes collagen

Collagen gels can be injected easily. These gels have a tendency to flow and can be injected easily in systems. The forms in which these collagen injectable gels are available are as (a) suspensions of collagen fibers and (b) non-fibrillar, viscous solutions in aqueous media. Several formulations are patented for ophthalmic applications. They are initially in the liquid form and turn to gel after application on the eye. The gel remains intact in the cul-de-sac of the eye for a longer duration as liquid formulations and acts as an antibiotic (Nair et al 2010). Gel made of a telocollagen is produced by removal of the telopeptide moieties using pepsin, has been used as chondrocytes carriers to restore cartilage defects (uchio et al 2000). There are reports of grafted type I atelocollagen being a favorable matrix for migration of the cell. (Nakagawa and Tagawa 2000). An effort of mixing collagen and PHEMA into hydrogels has been carried out to develop a drug delivery system for anticancer drugs.

1.5.3.5 Pellet/tablet

Collagen can be processed into Minipellets. They can be injected by a syringe needle into the subcutaneous space. The subcutaneous injection administered once in the form of a mini pellet retains interleukin-2 for a long time and reduces the maximal concentration in the serum. This pellet-type carriers are as
local delivery of minocycline and lysozyme. Collagen based pellet is also used as a carrier for gene delivery carrier and is extensively studied. The means of a direct bone formation by collagen complex has been ultra structurally investigated (Nakagawa and Tagawa 2000). This study proved that direct bone formation is induced by BMPs without cartilage formation when atelocollagen type I collagen pellet is used as a carrier.

1.6  COLLAGEN AND ITS ROLE IN WOUND HEALING

Collagen fibers, fleeces and sponges are used for a long time in medicine as haemostatic agents. The collagen sponges are useful owing to their wet strength allowing them to be suturing materials for soft tissue, giving them a way for promoting new tissue growth. The implants based on collagen are used in delivery of cultured keratinocytes and are an excellent replacement for wounds due to burns on the skin wounds (Leipziger et al 1985, McPherson et al 1986). The implanted collagen sponges, amorphous connective tissue do not filter the implanted collagen and contains GAG, fibronectin, new collagen and various cells primarily fibroblasts and macrophages. When these cells attach themselves to the extracellular matrix like the implanted collagen sponge, there is an increase in the production of new collagen (Postlethwaite et al, 1978). Collagen sponge undergoes degradation into peptide fragments and amino acids. This generally takes 3-6 weeks by the collagenases and is relied on the degree of cross linking. This implant is replaced by the native collagen type I produced by fibroblasts.

1.7  COLLAGEN AS WOUND DRESSING MATERIAL

Natural collagen is used in the treatment of surgical and abdominal repair process (Van der Laan et al 1991). collagen based wound dressing can be easily restructured easily owing to their availability in abundance, simple membrane structure and uniformity. All these properties aid in the improvement of a new surgical adhesives prepared from porcine collagen and polyglutamic acid. These adhesives essentially stops air from leaking out of damaged lungs during the extended process of recovery. The absorption of such collagen-based adhesives can
be synchronized by altering the collagen content of the system. Collagen based wound dressings are in use for many years for the treatment of burns and ulcers (Doillon et al 1986, Peters 1980, Yannas et al 1982). A few common and commercially available skin, dermal substitutes, and dressings like Alloderm™ (human dermis), Amniograph™ (amniotic membrane), Integra® (acellular collagen GAG scaffold), and Oasis ™ (porcine skin), are used for medical applications. Collagen when mixed with alginate facilitates the inflammatory phase in the wound healing and provides the required mechanical strength, an important feature of the collagen fibrils. Collagen dressings can be synthesized into a semi-occlusive polymer film (Zitelli 1987). The advantage of using these films are, they are less affected by bacteria and provides permeability to air and vapour. They reduce contraction and scarring, increase the rate of tissue growth. Biobrane® is commercially booming example of dressing used in wound care. It is made of a silicone membrane knitted with a nylon membrane which is built-in with porcine collagen peptides. This composite promotes granulation and acts as an attachment for full-thickness wounds (Lal et al 2000).

1.8 OTHER APPLICATIONS OF COLLAGEN

Several researches have reported the use of collagen in powder form and displays tremendous attachment to wound, better hemostatic properties, tissue fluid binding and formation of a vascular granulation bed. (Chvapil et al 1973). Films developed from hydrolyzed collagen have been used as a tissue sealants for replacement of the suture, since it satisfies the characteristics property of the biomaterial like biodegradability, non-toxicity and does not stop the healing process. (Chvapil et al 1973). Collagen acts as a natural hemostat and its application to dental therapy by clot formation and stabilization (Wikesjo et al 1992). Collagen serves as an excellent biologic scaffold for endothelial and progenitor cells from the periodontal ligament (Prosthlewaite 1978). Upon oral application, homogenized reconstituted collagen mixed with cell culture media has been used for endodontic repair (Bashutski and Wang 2009). Membranes based on collagen have been extensively used in periodontal and implant therapy, acts as barriers to avoid the migration of epithelial cells and allow wound cells to grow with regenerative
potential (Wang 1998). Several investigators have studied type I collagen as a membrane barrier for use in guided tissue regeneration (GTR) procedures. Collagen is absorbable, does not require any surgical procedure for removal and exhibit unique properties (Pitaru et al 1998).

1.9 OTHER NATURAL BIOMATERIALS

1.9.1 Fibrin

Fibrin is a biopolymer responsible in the natural blood clotting process. It is a polypeptide derived from pre protein fibrinogen and consists of plasma-protein fibrinogen and thrombin. The formation of fibrin occurs as a final step in blood coagulation and produces a clot that helps in the process of wound healing. Soluble fibrin monomers (Blomback 1978) are produced when thrombin cleaves small peptide from the fibrinogen after activation with calcium ions. Insoluble polymerized fibrin clot is formed by covalently cross linking these monomers through the action of factor xiii in shown in Fig .1.4.

![Fig.1.4. Formation of Fibrin Clot](image)

Numerous factors influence the formation of fibrin clot formation such as the thrombin to fibrinogen ratio, the ionic strength and divalent cation concentration of the medium, or the presence of very specific types of plasma proteins (e.g., serum albumin) regularization rate of fibrin polymerization, structure and composition
(Helgerson 2004). Fibrin monomers are generated by the catalytic enzyme thrombin and are obtained in both human and bovine forms. The human form is preferred (Webster and West 2002). Human plasma fibrinogen is used in fibrin glue (Weisel 2004). Thrombin is animal form of fibrinogen. The marine sources like salmon contributes fibrinogen.(Manseth et al 2004)

1.9.1.1 Fibrin as a Biomaterial

Fibrin exhibits favorable properties of a biomaterial like excellent biocompatibility, biodegradability, injectability and the presence of several extracellular matrix proteins. It is the earliest biopolymer used as a biomaterial (Nair and Laurencin 2007). This bioresorbable adhesive coating is helpful for soft tissues and organs such as cardiovascular and thoracic tissues. Fibrin is used as a graft sealant, hemostatic and antibacterial agent .It is an efficient scaffold material for engineering of tissue. Fibrinogen stimulates the movement of epidermal cells and keratinocytes (Donaldson et al 1989, Krasna et al 2005). Fibronectin, a glycoprotein in fibrin glue, improves cellular migration during wound healing (Horch et al 1998, Clark et al 1982). Clinical application of a single cell suspension of in vitro expanded autologous human keratinocytes in fibrin sealant and delivers directly into the defected skin .The cell-glue system adheres and spreads over the defect resulting in generation of new tissues within few weeks (Kopp et al 2004). Additionally, cultivation of keratinocytes onto a fibrin layer in vitro maintains isolation (Pellegrini et al 1999):

An alternative treatment for chronic wounds and severe burns includes isolation of a small portion of the patient's skin, growth of the resultant single keratinocytes in vitro onto a supportive 3T3 feeder layer and transfer of the developed epidermal sheet directly to the wound. However it is an expensive and time consuming process and careful enzymatic detachment and handling of the cell sheet is critical. Meana et al 1998, reported a fibrin gel either with or without human fibroblasts as a base for a dermal equivalent. This technique involved the cultivation of low cell numbers on the gel system and successfully developed new epithelium within 10-14 days. The cell layer was removed yearly from the culture flask without
enzymatic treatment and easily transplanted into the defected skin. Gorodetsky et al 1999, developed a novel technology to transport cells from fibrin derived micro beads instead of conventional fibrin gel system. Horch et al 2001 found that a keratinocyte/fibrin matrix suspension, after injection to skin lesion sites, resulted in the rejuvenation of epidermal tissue, which helps in the wound-healing process. Apart from wound healing of the skin, fibrin-based biomaterials are used in the repair process of urinary system. Urothelial cell/fibrin matrix suspensions have been transplanted to lesion sites and help to regenerate multilayered urothelium in urethral reconstruction procedures (Wechselberger 1998, Bach 2001). Fibrinogen can be used as a chaperone that binds to unfolded polypeptides and prevents them from binding to one another (Tang et al 2009). Adding fibrin glue to calcium phosphate granules has been reported to provide a viable scaffold for bone tissue engineering when thrombin is used to polymerize the fibrin to the calcium phosphate (Nihouannen et al 2006). Photochemically cross-linked native, unmodified fibrinogen has been reported to form a seal five times stronger than commercial fibrin glue and cures within 20 seconds, providing a rapid and high-strength tissue sealant (Elvin et al 2009).

1.9.2 Elastin

Human elastin is found predominantly within blood vessel walls and lung tissue. The ligamentum nuchae (the well-developed ligament that connects the base of the skull to and along the spinous processes of the vertebrae) of ungulates (hoofed herbivores) is almost pure elastin, with a small amount of collagen (Fung 1993). Other elastic proteins similar to elastin, are resilin, retrieved from the flexible joints of arthropods (spiders, crustaceans, centipedes, insects), and abductin, harvested from the hinges of scallops (Fung 1993). Due to the difficulty of purifying elastin, this protein has not been widely used as a biomaterial, nor have its mechanical properties been studied extensively (Debelle and Tamburro 1999, Daamen et al 2001). Elastin, unlike collagen, cannot be fixed with aldehydes (Fung 1993). Elastin is an insoluble, tightly cross-linked polymer (Mithieux et al 2004) composed of a number of tropoelastin molecules covalently bonded together. Tropoelastin is a
product intracellularly and cross-linked extracellularly (Fung 1993). The cross-links occur at hydrophilic regions, while hydrophobic regions separate the cross-links. Despite being mostly hydrophobic, elastin is highly hydrated (Debelle and Tamburro 1999). The secondary structure of elastin is a β-turn which is dynamic. The aforementioned cross-links continually change conformation, resulting in the high-entropy, and non stressed state of elastin. The amino acid composition of elastin includes glycine, alanine, valine, and proline, in order of respective proportions from greatest to least (Debelle and Tamburro 1999).

1.9.2.1 Application of elastin in biomedical research

Pure elastin scaffolds have been a popular tool for vascular tissue repair and replacement. New extracellular matrix (ECM) proteins, such as collagen bundles, are able to attach and proliferate within these scaffolds, allowing for better integration of the new tissue into the host tissue (Simionescu et al 2006). Copper has been found to be a useful promoter of elastin production by smooth muscle cells. This approach has been used in combination with hyaluron oligomers to produce cross-linked elastin scaffolds for tissue implantation α-Elastin and diepoxy cross-linker have been used to produce an elastin based material that shows vascular smooth muscle cell adhesion but decreased proliferation. Compared to a polystyrene equivalent (Leach et al 2005). Bubbling high-pressure CO₂ through an elastin hydrogel is a technique that has been used to increase pore size, improve the swelling ratio and compression modulus, and increase cellular growth and penetration throughout the gel (Annabi et al 2009).

Some elastin based biomaterials can suffer from calcification in which mineral deposits form on the implanted biomaterials (Daamen et al 2007). Calcification may be combated by strategies such as aluminum chloride treatment, or the presence of basic fibroblast growth factor (Daamen et al 2007).
1.9.3 Chitin

Chitin (C8H13O5N)n a derivative of glucose is a long-chain polymer of N-acetyl glucosamine. It is regarded as one of the abundant natural polysaccharide next to cellulose on earth. It is extracted from crustaceans as well as the cell wall of fungi. It is a linear 1, 4-linked polymer composed of N-acetyl-D-glucosamine residues. Medical scientist have made several attempts to apply chitin for various applications like wound dressings and as scaffolds in tissue engineering, wound healing, antibacterial and anti-inflammatory properties.

Structure of Chitin

![Structure of Chitin](image)

**Fig.1.5. Structure of Chitin**

Chitin exhibits characteristic properties of biomaterials like biocompatible, biodegradable, non-toxic, anti-microbial and hydrating agents. Chitin exists in three polymorphic crystalline forms, they are α, β and γ forms. α-chitin the polymorphic form of chitin is found in abundance and is extracted from crabs and shrimps. It exhibits wound healing, antibacterial and anti-inflammatory abilities and can be changed into different forms like hydrogel, fiber etc. β-Chitin, obtained from squid pens, takes the monoclinic form due to the parallel arrangement of the chains. The result of the molecular packing causes intermolecular interactions in β-chitin which make them weaker than those in α-chitin. β-chitin is more liable to dissolution in a
number of solvents and exhibit higher chemical reactivity compared to α-chitin. In α-chitin the polymer chains are arranged antiparallel (Syndiotactic) to each other while in β-chitin they are parallel (Isotactic) to each other whereas randomly in γ-chitin (Atactic). The polymer chains are tightly held by intermolecular hydrogen bonding between N-H and –C=O functional groups.

**1.9.3.1 Chitosan**

Chitosan, a polycation biopolymer, exhibits favorable properties of a biomaterial like non-toxic, biodegradable, and biocompatible. It is a polysaccharide derived from naturally occurring chitin and can be commercially produced by deacetylation of chitin by using sodium hydroxide. The degree of deacetylation of chitosan ranges from 60-100%. Chitosan is positively charged and soluble in acidic, aqueous solution below a pH of 6. These are insoluble in neutral conditions and many organic solvents. Chitosan exhibit several properties such as hemostatic activity, wound healing ability, reducing scars, antimicrobial activity, as well as inhibition of a wide variety of bacteria.

**Structure of Chitosan**

![Structure of Chitosan](image)

**Fig.1.6. Structure of Chitosan**

**1.9.3.2 Various forms of chitin and chitosan.**

Chitin and its derivative chitosan can be prepared into different forms like hydrogels (Nagahama et al 2008, Tamura et al 2010), membranes (Yusof et al 2003,

1.9.4 Gelatin

Gelatin is obtained by partial degradation of water-insoluble collagen fibre, and is widely applied in the biomedical field, because of its numerous qualities like biological origin, biodegradability, hydrogel properties, and commercial availability at low cost. It is biocompatible and exhibits very low antigenicity. It is processed into various forms like adhesive, as absorbent pads in surgical use and in medicine as a wound dressing (Choi and Regenstein 2000). Gelatin exhibits activation of microphage (Wainewright 1977) and high-haemostatic effects (Montero et al 1999). It is used in a wide variety of wound dressings and as a biomaterial in tissue engineering. The drawbacks of gelatin for tissue engineering is its solubility in aqueous media therefore, gelatin containing structures for long-term biomedical applications undergoes cross linking (Venien and Levieux 2005).

1.10 BATCH PROCESS – A METHOD PRESENTLY IN PRACTICE

The method currently in practice to develop thin collagen sheet is batch process. In this study chrome shaving was taken as the raw material to prepare collagen. Chrome shavings is a major solid waste (30-40%) emanated from the leather industry (Kanagaraj et al 2006). The disposal of these leather wastes improperly causes environmental hazards, and an utilization of these wastes in to valuable end products provides a promising solution (Nagai et al 2000). Sastry et al 2010, has reported the preparation of collagen from chrome shavings and this technology has also been commercialized for the treatment of wounds and burns. Ramnath et al 2012, has already reported the extraction of collagen from chrome
containing leather waste. In this method the paste is prepared by treating 100gms of chrome shaving with 5gms of 0.1N NaOH for 24 hours. After the stipulated time it is washed thoroughly with water to remove the chromium present in the sample. It is further treated with concentrated sulfuric acid to remove chromium present, if any, in the sample. It is washed with water thrice and treated with 5ml hydrogen peroxide mixed in 500ml water. The resultant is finally washed with distilled water and ground into a fine paste in a domestic mixie. The paste is spread on a polyethylene tray in the form of a thin sheet shown in the Fig.1.7. The wet sheet is dried under sunlight naturally or under fan. The process of sheet formation is depicted in the following flowchart in Fig.1.7. In all these methods the sheets are prepared manually.

The disadvantages of the process is

- Mass production of the sheets is not possible by this method.
- There is no sterility in the preparation of the sheets.
- It takes more time to dry the sheets.
- The possibility of bacterial contamination of the sheets.

Although this method is the tested and proven, during natural calamities like war and burn victims, when the requirement for bulk production arises, this method cannot be adopted since it takes 7-10 days for preparation of the paste to final end product in sheet form. This is due to the proteinous nature of the material i.e., they have the tendency to retain the water content which takes substantially more time to dry these sheets of wound dressing materials.
Removal of chromium

0.5 hour

pH 7

Fig.1.7. Steps in manual method of sheet formation.
1.11 IMPORTANCE OF AUTOMATION

Automation refers to the Greek word “Auto” (self) and “Matos” (moving). It is also defined as a systems that move by itself or a set of technologies that results in operation of machines and systems without significant human intervention and achieves performance superior to manual operation.

Industrial Automation makes extensive use of Information Technology. It involves significant amount of hardware technologies, related to Instrumentation and Sensing, Actuation and Drives, Electronics for Signal Conditioning, Communication and Display, Embedded as well as Stand-alone Computing Systems etc. As these systems grow more sophisticated in terms of the knowledge and algorithms they use, they encompass larger areas of operation comprising several units or the whole of a factory, or even several of them, and as they integrate manufacturing with other areas of business, such as, sales and customer care, finance and the entire supply chain of the business. However, the lower level Automation Systems that only deal with individual or, at best a group of machines, make less use of IT and more of hardware, electronics and embedded computing. The role of automation in the industry is on the rise owing to the increase in manufacturing processes, which produce finished product from raw/unfinished material using energy, manpower, equipment and infrastructure. The basic objective of any industry is to make profit. Therefore, profit can be maximized by producing good quality products, which may sell at higher price, in larger volumes with less production cost and time. Production systems can be classified into different types as discussed below.

1.11.1 Continuous flow process

In a continuous flow process the manufactured product is in continuous quantities i.e., the product is not a discrete object. Moreover, for such processes, the volume of production is generally very high, while the product variation is relatively low. Typical examples of such processes include Oil Refineries, Iron and Steel Plants, Cement and Chemical Plants.
1.11.2 Mass manufacturing of discrete products

Products are discrete objects and manufactured in large volumes. Product variation is very limited. Typical examples are Appliances, Automobiles etc.

1.11.3 Batch production

In a batch production process, the product is either discrete or continuous. However, the variation in product types is larger than in continuous flow processes. The same set of equipment is used to manufacture all the product types. However for each batch of a given product type a distinct set of operating parameters must be established. Typical examples are Pharmaceuticals, Casting Foundries, Plastic molding, Printing etc.

1.11.4 Job shop production

Typically designed for manufacturing of small quantities of discrete products, which are custom built, generally according to drawings supplied by customers. Any variation in the product can be made. Examples include Machine Shops, Prototyping facilities etc. The Automation systems can be categorized based on the flexibility and level of integration in manufacturing process operations. The classifications are as follows

1.11.4.1 Fixed automation

It is used in high volume production with dedicated equipment, which has a fixed set of operation and designed to be efficient for this set. Continuous flow and Discrete Mass Production systems use this type of automation. e.g. Distillation Process, Conveyors, Paint Shops, Transfer lines etc. A process using mechanized machinery to perform fixed and repetitive operations in order to produce a high volume of similar parts.

1.11.4.2 Programmable automation

It is used for a changeable sequence of operation and configuration of the machines using electronic controls. However, non-trivial programming effort may
be needed to reprogram the machine or sequence of operations. Investment on programmable equipment is less, as production process is not changed frequently. It is typically used in Batch process where job variety is low and product volume is medium to high, and sometimes can be mass produced. Few examples include Steel Rolling Mills, Paper Mills etc.

1.11.4.3 Flexible automation

It is used in Flexible Manufacturing Systems (FMS) which is always computer controlled. High level commands are given by operator's in the form of codes entered into computer identifying product and its location in the order and lower level changes carried out automatically. Each production machine receives settings/instructions from computer. These automatically loads/unloads required tools and carries out the processing instructions. After processing, products are transferred to next machine automatically. It is used in job shops and batch process where product varieties are high and job volumes are medium to low. These types of systems are implemented in Multipurpose CNC machines, Automated Guided Vehicles (AGV) etc.

1.11.4.4 Integrated automation

It denotes complete automation of a manufacturing plant, with all processes functioning under computer control and coordination through digital information processing. It includes technologies such as computer-aided design and manufacturing, computer-aided process planning, computer numerical control machine tools, flexible machining systems, automated storage and retrieval systems, automated material handling systems such as robots and automated cranes and conveyors, computerized scheduling and production control. It may also integrate a business system through a common database.

1.12 CHARACTERIZATION OF BIOMATERIALS

Materials containing nanometer sized crystallites or phases frequently show interesting and useful new properties in comparison to bulk properties of similar
Analyzing and understanding of structural and chemical changes on these extremely small scales are therefore crucial for further success. The current most challenging tasks are property characterizations which are carried out using variety of microscopic and spectroscopic techniques. The basic principles and techniques used for characterization are explained below. The main emphasis of this thesis is to study the characterization of the sheets processed through the new technique for Fourier transform infrared (FTIR), X-Ray Diffraction (XRD), Scanning electron microscopy (SEM) and Atomic force microscopy (AFM).

1.12.1 Fourier Transform Infrared Spectroscopy (FTIR)

The widely used spectroscopic techniques is the Infrared (IR) Spectroscopy adopted by organic and inorganic chemist. Different IR frequencies of a sample are positioned in the path of an IR beam and the absorption measurement is made. The IR spectroscopic analysis identifies the chemical functional group in the sample. The characteristic frequencies of IR radiation are absorbed by different functional groups. IR spectrometer accepts sample which are in the form of solids, gases, liquids. It is a vital tool for explanation and identification of a structure and compound. The IR radiation spans a section of the electromagnetic spectrum having wave lengths from 0.78 to 1000 μm or wave numbers from roughly 13,000 to 10cm⁻¹. At high frequencies they jump to the visible region and at low frequencies in the microwave region. The IR absorption positions are represented as wave number (ν) or wavelength (λ). Wave number is defined as the number of waves per unit length and is directly proportional to frequency and energy of the IR absorption. It's unit is in cm⁻¹ scale. However, wavelengths are inversely proportional to frequencies and their associated energy. presently the unit of wavelength is μm (micrometer). The equation for inter changing the wave number and wavelength is depicted using the following equation:

\[ \nu \text{ (in cm}^{-1} \text{)} = (1/\lambda \text{ (in } \mu \text{m)}}) \times 10^4 \]

The absorption information of IR is presented in the form of a spectrum with wavelength or wavenumber in the x-axis and absorption intensity or percent
transmittances in the y-axis. Transmittance, is the ratio or radiant power transmitted by the sample (I) to the radiant power incident on the sample (I₀). Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance (T).

\[ A = \log_{10}\left(\frac{I}{I_0}\right) = -\log_{10}(T) = -\log_{10}\left(\frac{I}{I_0}\right) \]

The transmittance spectrum gives good contrast between intensities of strong and weak bands since the transmittance ranges from 0 to 100% while absorbance ranges from infinity to zero. The IR regions are categorized into three smaller areas as near IR, mid IR and far IR.

1.12.1.1 Basic principles

The principles of IR can be explained by classical as well as quantum theories. A simple ball and spring model is used, where in a diatomic molecule with two masses \(m_1\) and \(m_2\) are connected by a spring. According to Hooke's law when spring is displaced,

\[ F = -kx \]

Where \(F\) = opposing restoring force
\(k\) = force constant
\(x\) = displacement from equilibrium position

The harmonic equation relation gives the frequency of vibration:

\[ \nu = \frac{1}{2\pi} \sqrt{\frac{k}{\mu}} \]

Where, \(\mu\) is the reduced mass. Using laws of mechanics, a system of masses joined by spring has a number of fundamentals modes of vibration each of which has a particular natural frequency. Consider an oscillator such as the electric vector of electromagnetic radiation coupled to a system of masses such as a polyatomic molecule. By scanning through a range of frequencies some may be ‘tuned’ to the various fundamentals modes of vibration by virtue of a change in dipole moment.
associated with that vibration. So, a series of absorption take place for a polyatomic molecule as we scan through a range of frequencies, radiation is absorbed each time we 'tune-in' or 'comes into resonance' with the natural frequency of a fundamental mode which is capable of dipolar interaction. There is a continuous vibrations in a molecule at temperature above absolute zero. The molecules absorbs radiation when the frequency of a particular vibration is equal to the frequency of the IR radiation directed on the molecule. Three degree of freedom exists for every atom depending on the motions along the three Cartesian coordinate axes (x, y, and z). The total degrees of freedom for a polyatomic molecule of n atoms is $3n$. To explain the translation, three degrees of freedom are required. The rotation of the entire molecule requires three degrees of freedom. Therefore, $3n-6$ degrees of freedom are for fundamental vibrations for nonlinear molecules.

Linear molecules have $3n-5$ basic vibrational modes because they require 2 degrees of freedom to explain the rotation. From the $3n-6$ or $3n-5$ fundamental vibrations, an IR activity may result in those that produce a total change in the dipole moment and that which gives a polarizability change, gives rise to a Raman activity. By Nature, a few vibrations might be IR and Raman active. During this condition the total number of observed absorption bands is mostly different from the total number of fundamental vibrations. Modern IR instruments more commonly use Fourier-transform techniques with a Michelson interferometer. An infrared spectrophotometer consists of an IR light source, a sample container, and a prism to separate light by wavelength, a detector and a record to produce the IR spectrum.
FTIR instruments have distinct advantages;

(a) Better speed and sensitivity. A complete spectrum can be obtained during a single scan of the moving mirror, while the detector observes all frequencies simultaneously.

(b) An FTIR instrument can achieve the same signal-to-noise (S/N) ratio of a dispersive spectrometer in a fraction of the time (1 sec or less versus 10 to 15 min). The S/N ratio is proportional to the square root of the total number of measurements. Because multiple spectra can be readily collected in 1 min or less, sensitivity can be greatly improved by increasing S/N through co addition of many repeated scans.

(c) The interferometer does not require an energy wasting slit since filtering is not required. Alternatively optical aperture is used in the IR system. In the FT instrument the variation in the beam area is mostly 75 to 100
times larger than the slit width of a dispersive spectrometer thus paving way for a large radiation energy. This is an added advantage for many sample or sampling techniques that are limited in energy.

(d) The need for an external calibration is eliminated by using an internal laser reference which uses a helium neon laser. This reference uses an automatic calibration to an accuracy of 0.01 cm\(^{-1}\) in many FTIR systems.

(e) The mechanical design implementation is very simple because there is only a single moving part, moving mirror. therefore less wear and tear and better reliability is ensured.

(f) In FTIR the presence of interferometer modulates all the frequencies and thus eliminates stray light and emission contributions.

(g) The present day spectrometers have a powerful computerized data station which can achieve good data processing tasks like the Fourier transformation, interactive spectral subtraction, baseline correction, smoothing, integration, and library searching.

1.12.2 X-RAY Diffraction (XRD)

In 1895 after discovering X-ray by Rontgen, Von Laude in 1912 demonstrated that X-ray could be diffracted by crystal. In 1935 Le Galley first constructed X-ray power diffractometer. It is primarily used to study structural imperfections.
Fig.1.9. Schematic diagram of the process that contributes to the generation of X-rays

X-ray is an electromagnetic radiation with wavelength of the order of $10^{-10}$ m. These are generated by bombarding a metal with high-energy electrons. The resultant is, high energy penetrates the outer electron shells and interacts with the inner-shell electrons. If the amount of energy is little more than the critical amount of energy, the electron escapes the attractive field of the nucleus, leaving a hole in the inner shell and generates an ionized atom or otherwise the electron is ejected. The ionized atom comes back to its lowest energy (ground state) by filling in the missing electron with one from the outer shells. This transition is accompanied either by the emission of an x-ray or an Auger electron. In 1913, Sir W.H.Bragg and his son Sir W.L.Bragg defined the Bragg's Law ($n\lambda=2d \sin\theta$) have explained the cleavage faces of crystals to reflect X-ray beam at certain angels of incidence ($\theta$). The variable $d$ is the distance between atomic layers in a crystal, and the variable lambda $\lambda$ is the wavelength of the incident X-ray beam, $n$ is an integer. This observation is an example of X-ray wave interference commonly known as X-ray diffraction (XRD).

In 1918 Scherer first observed that small crystallite size could give rise to line broadening, which is called the Scherrer formulating.
Where \( D_v = \frac{k\lambda}{\beta \cos \theta} \)

1.12.3 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy is used to observe the surface directly owing to good resolution and field depth. In scanning Electron Microscope magnified images are created by electrons instead of light waves. It gives a detailed three dimensional image at higher magnitude than the light microscope. SEM works under vacuum in a column where an electron gun emits a beam of high energy electrons. This beam travels downwards through a sequence of magnetic lenses designed to focus the electron to a very fine spot. Near the bottom, a set of scanning coils moves the focused beam back and forth across the specimen, row by row. As the electron beam hits each spot on the sample, it sends a signals to an amplifier. The final images are built up from the number of electrons emitted from each spot on the sample. The functioning of the SEM is summarized below and illustrated in Fig.1.10

1. The Virtual Source represents the electron gun producing a stream of monochromatic electrons.
2. The stream is condensed by the first condenser lens
3. The condenser aperture is used to eliminate the beam by eliminating high-angle electrons.
4. The second condenser lens forms the electrons into a thin, tight, coherent beam and is controlled by the fine probe current knob.
5. A user selectable objective aperture eliminates high-angle electrons from the beam.
6. A set of coils scan the beam in a grid fashion in microseconds range
7. The final lens, the objective, focuses the scanning beam onto the part of the desired specimen.

8. When the beam strikes the sample, interactions take place inside the sample and are detected with various instruments.

9. Before the beam moves to its next dwell point, these instruments count the number of interactions and display a pixel on a CRT whose intensity is determined.

The function of the lens is to form the beam and limit the amount of current in the beam. It works in conjunction with the condenser aperture and eliminates the high-angle electrons from the beam.

![Diagram of Scanning Electron Microscope](image)

**Fig 1.10. Schematic diagram of Scanning Electron Microscope**

### 1.12.3.1 Working Principle

The instrument consists of three major sections: a) Electron optical Column
b) Vacuum system and c) Electronics and display system. A tungsten filament is heated to 2700 K, which produces electrons that are accelerated towards the anode disc. Electrostatic shaping of the electron beam under vacuum gives a beam diameter of about 50µm. Ultimate performance of the SEM is mainly limited by the
diameter of the beam and hence two lenses and condensers de-magnify the beam to around 5nm. The scanning coils deflect this beam and sweep it over the specimen surface. A cathode ray display tube is scanned synchronously with the electron beam. The brightness of the display tube is modulated by the signal, which arises from the interaction of the beam with the surface element which is probed. The strength is translated into image contrast.

Secondary electrons, which the beam probe liberates from the specimen surface, are collected and used as the contrast signal. The yield of collected electrons depends on the nature of the specimen surface and on its inclination with respect to the probing beam. Consequently pictures with a high appearance is obtained. There are different types of interactions of the electron beam possible with the sample. The electrons energetic in the microscope stops the sample and the various reactions that occur are shown in the Fig.1.11.

![Schematic diagram specimen-beam interactions](image)

**Fig.1.11. Schematic diagram specimen-beam interactions**

Backscattered electrons produced by an incident electron colliding with an atom in the specimen, which is nearly normal to the incident's path. The incident electron is then scattered backward 180 degree. The production of backscattered electron varies directly with the specimen's atomic or specimen interaction volume.
Specific interaction volume is the volume inside the specimen in which interactions occurs while being struck with an electron beam.

1.13 ATOMIC FORCE MICROSCOPE

The atomic force microscope (AFM), uses a sharp tip attached to the end of a cantilever raster’s across an area while a laser and photodiode are used to monitor the tip force on the surface. A feedback loop between the photodiode and the piezo crystal maintains a constant force during contact mode imaging and constant amplitude during intermittent contact mode imaging.

![Atomic Force Microscope Diagram](image)

**Fig.1.12. Set up of an Atomic force microscope.**

1.13.1 Working principle

A laser beam is focused on the cantilever that has a highly reflective surface. The laser beam reflected off the cantilever is focused on a position sensitive photodiode quadrant. The cantilever is scanned over the sample surface in a raster pattern. Any deflection in the cantilever as a result of sample interaction causes displacement in the laser spot on the photodiode. This displacement signal is analyzed to calculate the deflection in the cantilever. Imaging can be performed in either constant-force mode (distance between the tip and the specimen is allowed to change) or constant-height mode (force between the tip and the specimen is allowed to change).
An AFM has a pointed probe attached to a rectangular base called a cantilever. The positioning of the cantilever with respect to the specimen is achieved by the piezoelectric elements, called scanners. The piezoelectric element can be connected either to the cantilever or the specimen stage. In the initial AFMs, the piezoelectric element was a piezoelectric tube that is allowed to position the cantilever in the three dimensional space. As the X, Y, and Z scanners in a piezoelectric tube are coupled, there is always some crosstalk between the scanners. AFM instruments therefore use an alternative set of scanners wherein Z-scanner is separated from the X-Y scanner shown in Fig.1.13.
SCOPE OF THE WORK

1. To fabricate a continuous collagen sheet forming machine for the preparation of collagen based wound dressing material.

2. To optimize the sheet formation by incorporating pH Sensor, moisture sensor, IR sensor, temperature sensor and use of microcontroller to automate the process.

3. To characterize the physiochemical and mechanical properties of the wound dressing material prepared using the developed machinery.