CHAPTER 1

INTRODUCTION
1.1. Introduction and Scope of The Study

The knowledge of exploring the advancement in the biomaterial field resulted in development of novel material to accomplish the principles involved in wound healing process. The rapid development of biomaterials are afoot with increases the patient care with the enhanced material properties. The new biomaterial with the biomimetic function of the extracellular matrix (ECM) plays a vital role in the tissue engineering for various tissue damage that occur in various types of wounds like surgical wounds, burns, pressure ulcers and diabetic ulcers (Karukonda et al., 2000). The modern materials evolving at cell to material interaction reduce the healing time to understand the beneficial impact in the healing process. In present scenario the development of novel biomaterial comprising addition of composite materials with enhanced properties results in better functionalities than the existing materials. New materials interact with the surrounding tissue, accomplished by the principles involved in recognition of proteins, proteoglycans, cells and their receptors present in extracellular matrix (ECM) (Sakiyama et al., 2001).

1.2. The Skin

The skin is the largest organ in mammals, which serves as a protective barrier at the interface between the human body and the surrounding environment. It guards the underlying organs and protects the body against harmful microbial, thermal, mechanical and chemical influences. In the past 25 years, great efforts have been made to create substitutes that mimic human skin (MacNeil et al., 2007). Some of the major functions of skin include barricading foreign antigens, controlling and regulating body temperature and acts as sensory organ to external stimuli. The skin is made up of three distinct layers namely epidermis, dermis and fat layer with each layer performing specific functions (Pomahac et al., 1998).

1.2. 1. Wounds

A wound is defined as an injury or tear on the surface by physical, chemical, mechanical or thermal damages. A more scientific definition of a wound is a disruption of a normal anatomic structure and function of the skin (Lazarus et al., 1994). On the basis of wound healing processes, there are two types of wounds: acute and chronic wounds. Acute wounds are caused by mechanical damage induced by sheer, blunting,
and/or stabbing action of hard objects, but the wounds are usually healable within 8 to 12 weeks. (Percival et al., 2002) Chronic wounds are those injuries which are produced as a result of specific diseases such as diabetes, tumors, and severe physiological contaminations, but these wounds could take more than 12 weeks (Moore et al., 2006; Harding et al., 2002).

1. 2. 2. Wound healing

Wound healing involves complex and dynamic processes that result in the restoration of the anatomical continuity and function (Lazarus et al., 1994). There are four basic responses that occur following an injury (Diegelmann et al., 2004).

- Normal repair is the response where there is a re-established equilibrium between scar formation and scar remodeling. This is the typical response that most humans experience following injury.
- Excessive healing where there is too much deposition of connective tissue that results in altered structure and loss of function. Fibrosis, strictures and contractures are examples of excessive healing. Keloids and hypertrophic scars in the skin are examples of fibrosis. Contraction is part of the normal process of healing but if excessive, it becomes pathologic and is known as contracture.
- Deficient healing exists when there is insufficient deposition of connective tissue matrix and that tissue is weakened to the extent that it can fall apart. Chronic non-healing ulcers are the examples of deficient healing.
- Regeneration is the elegant process that occurs when there is loss of structure and function but the organism has the sophisticated capacity to replace that structure by replacing an exact structure before the injury. The liver, epidermis and to some extent, nerves can be partially regenerated after injury.

1. 2. 3. Wound healing cascades

Wound healing is a special biological process which is related to physiological parameters. Selection of a suitable wound dressing material for a specific type of wound requires comprehensive knowledge of the wound healing process. There are numerous published reports in the open literature describing the various biological and physiological stages of the healing process of a wound (Strodtbeck et al., 2001; Clark
1996; Russell et al., 1999; Mast 1999). These can be summarized into five consecutive cascades of events of hemostasis, inflammation, migration, proliferation, and maturation were given in the Figure 1. 1. The first stage includes hemostasis and inflammation, which occurs soon after there is damage to the skin. Fibrinogen, which is one of the major components of the skin’s connective tissues, leads to the coagulation of exudates (blood without cells and platelets), and together with the formation of a fibrin network, produces a clot in the wound which stops (Boateng et al., 2008). Therefore, both hemostasis and inflammatory stages play an important role in the healing process of a wound (Ratner et al., 1996). The inflammatory phase occurs simultaneously with the hemostasis phase usually takes more than 24 h. At this stage, blood neutrophils followed by phagocytes enter the wound, medium and penetrate inside the dead cells. In the migratory phase, the new and live cells (epithelial cells) move towards skin injury to replace dead cells. The proliferation stage consists of the complete coverage of wound by epithelium. At this stage, new stromas usually known as granulating tissues are formed.

**Figure 1. 1. Phases of wound healing, major types of cells involved in each phase and selected specific events (Adapted from Falanga 2005)**
after about 4 days. Microphages, fibroblasts, and blood vessels move toward the wound environment and form a single unit (Martin et al., 1997). The completion of this stage takes about 2 weeks. During the growth of migration phase, a reduction in the inflammatory phase of the wound is gradually observed. The final stage in the healing process of a wound is tissue remodeling. At this stage fibroblasts completely covers the surface of the wound as a new layer of the skin and there is no evidence of the wound. This stage is also known as maturation phase in the healing process of wounds (Hunt et al., 1980).

1.3. Biomaterials

Biomaterials are defined as materials of natural or man-made origin that are used to interface with biological systems to evaluate, treat, supplement or replace any tissue, organ or function of the body (Best et al., 2008). As the field of biomaterial research expands the term includes substances that are designed to control the biological environment of cells and tissues. More than being simply compatible with the host and serving a structural role, biomaterials can now direct cells through micro environmental cues. The uses of biomaterials include replacement of a body part that has lost function due to disease or trauma, to assist in healing, or to improve function, and to correct abnormalities. Performance of materials in the body can be viewed from several perspectives.

1.3.1. Role of biomaterials in tissue engineering and wound healing

Tissue engineering is an approach to reconstitute and regenerate lost or damaged tissue and biomaterials play a vital role (Bhattarai et al 2004; Hubell et al., 1995). Construction of biocompatible scaffolds is one of the leading areas in the field of tissue engineering (Shabani et al., 2009). Biomimetic synthetic polymers have been created to elicit specific cellular functions with direct cell-cell interactions. However, both in implants were initially cell-free, which may serve as matrices to conduct tissue regeneration, and in implants to support cell transplantation (Hubell et al., 1995). Native extracellular matrix is composed of nanoscale fibers that offer structural integrity to tissues (Maretschek et al., 2007, Huang et al., 2005). A major part of tissue engineering involves the creation of scaffolds that mimic both the structure and function of the native
extracellular matrix. The implanted materials play a vital role in tissue damage that occurs in wounds of various types like surgical wounds, burns, pressure ulcers and diabetic ulcers. In this approach, the implanted material can function both as a mechanical and functional scaffold for tissue regeneration (Sell et al., 2007). The most suitable method applied for complete closure/healing of wounds and burns is autografting. Inadequate donors for large wounds has led to the search for an alternative new tissue source. (Matthew et al., 2005) Biological dressings are natural dressings with keratin based material dressings aids faster healing.

1. 4. Ideal Requirements for Wound Dressings

An ideal wound dressing material should be

- Nontoxic
- Biocompatible
- Enhance cellular interaction and tissue development
- Biodegradable and bioresorbable (Huang et al., 2010).

Moreover, the ideal properties of wound dressing are:

- Providing a moist environment
- Creating a protective mechanical barrier and thermal isolation
- Protecting against secondary infections
- Absorbing the exudate and bacteria
- Promoting debridement
- Contributing to simple gas exchange
- Decreasing or removing trauma in the defected area
- Being acceptable for patient
- Not possessing any toxic, irritant or allergic properties
- Cost-effectiveness (Goossens et al., 2010).

1. 5. Methods to Prepare Wound dressing

Many methods have so far been taken up for the preparation of fibers namely:

- Self Assembly (Zhang et al., 2005)
- Mechanical drawing (Nain et al., 2005)
• Template synthesis (Wang et al., 2010)
• Phase separation (Zhao et al., 2001)
• Electrospinning (Naveen et al., 2010)

1.5.1. Self assembly

Self-assembly is a process in which individual, pre-existing components organize themselves through weak, non-covalent (H-bonding, electrostatic interactions) forces into desired patterns and structures were depicted in Figure 1.2. It is a ‘bottom-up’ method and offers novel properties and functionalities, which cannot be achieved by conventional organic synthesis. The main disadvantage of this method is complex, long and an extremely elaborate technique with low productivity.

1.5.2. Mechanical drawing

Drawing is a process capable of producing individual long nanofibers (Figure 1.2). However, only a viscoelastic material that can undergo strong deformations while being cohesive enough to support the stresses developed during pulling, can be made into nanofibers through drawing. It requires a minimum amount of equipments and is a discontinuous process. A micropipette is dipped into a droplet near the solution-solid surface contact line via a micromanipulator, then the micropipette is withdrawn from the liquid at a certain speed, yields nanofibers from each droplet. The viscosity of the polymer has to be assisted with an increase with solvent evaporation and some fiber breaking occurs due to instabilities that occur during the process. The drawing process is disadvantageous since the fiber size is dependent on the orifice size of the extrusion mould.

1.5.3. Template synthesis

In template synthesis, as the name suggests, a nanoporous membrane is used as a template to make nanofibers of solid (fibril) or hollow (tube) shape. A wide variety of materials such as electronically conducting polymers, metals, semiconductors and carbons can be fabricated. Discontinuity of fibers proves to be a major drawback in template synthesis. Extrusion of the polymer solution through the porous membrane is achieved through water pressure. The polymer comes into contact with the solidifying
solution; fibers with diameter dependent on the template pore size were produced were shown in Figure 1. 2.

1.5.4. Phase separation

The phase separation method of preparing fibers consists of dissolution, gelation, and extraction using a different solvent, freezing and drying, resulting in nanoscale porous foam. The process takes relatively long periods of time to transfer the solid polymer into the nanoporous foam. The polymer is dissolved in an appropriate solvent at the desired concentration and stirred at an appropriate temperature until a homogeneous solution is obtained. This is followed by cooling the solution to the gelation temperature of the polymer. The resultant gel is immersed in water several times to allow solvent exchanges were given in Figure 1. 2. Finally, the gel is frozen and lyophilized.

Figure 1. 2. Schematic representation of the different methods to prepare the wound dressing
1.5.5. Electrospinning

The term “Electrospinning” derived from “electrostatic spinning”, was used relatively recently (around 1994), but its fundamental idea dates back to more than 60 years. A polymer solution applied with the electrostatic force between the two electrodes bearing opposite charges. One of the electrodes was placed in the solution and the other onto a collector. One ejected out of a metal spinneret with a small hole, the charged solution jets evaporated to become fibers which were collected on the collector. The fiber characteristics were optimized by changing the viscosity of the polymer solution, distance between the tip and the collector, voltage applied, flow rate and the speed of the collecting drum were depicted in Figure 1.3. (Formhals 1934, 1939, 1940).

Ko-Chung Yen, Ching-Yun Chen (2016) were able to produce keratin and fibrin as a composite nanofiber with different microstructures and mechanical strength resembling the native blood vessels for cardiac tissue engineering application. The fabricated nanofiber mimic the cells in the aorta under maximum shear stress with tissue regeneration and reorganization (Ko-Chung Yen et al., 2016).

![Figure 1.3. Schematic representation of the Electrospinning process](image-url)
1. 6. Antimicrobial Wound Dressing Incorporated With Pharmaceutical Ingredients

So-called active dressings or medicated dressings have been developed by incorporating antimicrobials, growth factors, or supplements such as minerals and vitamins into the system. Incorporation of cleansing or debriding agents aid in the removal of necrotic tissue, antimicrobials will act against infection, while growth factors supplements help in regeneration of tissue (Boateng et al., 2008). Wound dressings have developed over the years from crude applications of plant herbs, animal fat and honey to tissue engineered scaffolds. Nature has been a source of medicinal treatments for thousands of years and plant-based systems continue to play an essential role in the primary health care of 80% of the world’s underdeveloped and developing countries (Kalpana et al., 2011). In the plant kingdom, each plant has its own potential to produce primary and secondary metabolites that are bioactive compounds. These compounds play a vital role in curing many diseases. Bioactive compounds from plant sources have a broad spectrum of anti-bacterial, anti-fungal and anti-oxidant activities.

1. 6. 1. Mupirocin in wound healing

Wound dressing material containing mupirocin as an antimicrobial agent, can be used in the treatment of wounds to prevent infection. Mupirocin calcium is the calcium salt of pseudomonic acid, an antibiotic produced by fermentation of Pseudomonas fluorescens. It inhibits bacterial protein synthesis by binding to the enzyme, isoleucyl-transfer-RNA synthase (Winkelman et al., 1989; Lamb, 1991). This binding results in blocking of incorporation of isoleucine into proteins (Winkelman and Gratton, 1989). The unique chemical structure and mechanism of action, cross resistance with other antibacterial drugs is not a concern (Lamb, 1991). Due to the low affinity of mupirocin for the mammalian enzyme it does not lead to toxicity in humans (does not show toxicity to human fibroblast or keratinocytes, or to cultured human skin graft), (Williford, 1999). At lower concentrations mupirocin acts as a bacteriostatic agent, while at higher concentration it becomes bactericidal. In addition to activity against gram-positive organisms, mupirocin has activity against certain gram-negative organisms, including Haemophilus influenza, Neisseria gonoeehoeae and Pasteurella multocida and it also inhibits the fungi Candida albicans (Williford, 1999). When mupirocin was administered in to the skin, the systemic absorption of the drug was minimal, promoting it as an ideal
drug for topical treatment (Williford, 1999) on application to skin with damaged barrier properties, mupirocin is expected to penetrate through deeper layers potentially leading to being absorbed into systemic circulation. The important fact that mupirocin has the ability to reside in the skin for longer periods of time, up to several days. This makes it as potential drug for topical administration, as dermal treatment depends on the sufficient residence time of the pharmaceutical formulation on the area to be treated. Studies have reported that mupirocin also accelerates wound healing (Williford, 1999).

1.6.2. Curcumin in wound healing

Curcumin (diferuloylmethane) is an orange-yellow component of perennial herb *Curcuma longa*, which belongs to Zingiberaceae family in India. The plant is distributed throughout tropical and subtropical regions of the world, being widely cultivated in south east Asian countries. Turmeric, i.e., the ground rhizomes of *Curcuma longa* L., has a long history of use in food as a spice, mainly as an ingredient in many varieties of curry powders and sauces, where curcumin from turmeric is a main colouring substance (Strasser et al., 2005).

Curcumin (diferuloylmethane) is the same as curcumin I. Curcumin II is desmethoxycurcumin and curcumin III is bis-desmethoxycurcumin. The structures written in the article for desmethoxycurcumin and bis-desmethoxycurcumin are in no way different from those of curcumin II and curcumin III. The structure of curcumin was elucidated as early as 1910 by Milobedeska et al., (Milobedeska et al., 1910).

Curcumin is a naturally occurring multifunctional polyphenolic phytoconstituent which presents anti-inflammatory (Strasser et al., 2005), antimicrobial (Han Sand Y. Yang 2005), antiviral (Opa et al., 2005), anticancer (Aggarwal et al., 2003), antioxidant (Park et al., 2008), and wound healing activities (Panchatcharam et al., 2006). Despite all these promising features, a common problem with curcumin is the very low solubility in aqueous solutions, which limits its bioavailability and clinical efficacy. To overcome the problems of solubility and bioavailability of curcumin, the development of novel delivery systems is attracting significant attention in recent past (a). One of the method is topical formulation of curcumin to support dermal wound healing (Gopinath et al., 2004). Topical applications of curcumin provides antibacterial, anti-inflammatory, antioxidant (free radical scavenging activity) and protection against degenerative disease in patients.
have shown to significantly improve wound healing, which and protect the tissues from oxidative damage (Gopinath et al., 2004). Therefore, development of novel curcumin delivery systems is required.

Turmeric has been used in Ayurvedic medicine since ancient times, with various biological applications. Although some work has been done on the possible medicinal applications, no studies for drug-development have been carried out as yet. Although the crude extract has numerous medicinal applications, clinical applications can be made only after extensive research on its bioactivity, mechanism of action, pharmacotherapeutics and toxicity studies. However, as curcumin is now available in pure form, which shows a wide spectrum of biological activities, it would be easier to develop new drugs from this compound after extensive studies on its mechanism of action and pharmacological effects. Recently, an increased enthusiasm in treating various diseases with natural products. Curcumin is a non-toxic, highly promising natural antioxidant compound having a wide spectrum of biological functions. It is expected that curcumin may find application as a novel drug in the near future to control various diseases, including inflammatory disorders, carcinogenesis and oxidative stress-induced pathogenesis (Chattopadhyay et al., 2004).

1.7. Growth Factors and Cytokines in Wound Healing

The wound healing process begins immediately followed by injury. Wound repair requires close control of degradative and regenerative processes involving numerous cell types and complex interactions between multiple biochemical cascades. Growth factors released in the traumatized area to promote cell migration into the wound area (chemotaxis), stimulate the growth of epithelial cells, and fibroblasts (mitogenesis). Which thereby, initiate the formation of new blood vessels (angiogenesis) and stimulate matrix formation and remodeling of the affected region (Komarcevic, 2000). Growth factors, therefore affect the inflammatory, proliferation and migratory phases of wound healing (Dijke et al., 1989). There are approximately 30 growth factors that have been identified to date, out of which 7 have been shown to be important in normal dermal wound repair. Those 7 important growth factors are basic fibroblast growth factor (bFGF); epidermal growth factor (EGF); keratinocyte growth factor (KGF), also known as fibroblast growth factor–7; platelet-derived growth factor (PDGF); transforming
growth factor -α and -β (TGF-α and -β); and vascular endothelial cell growth factor (VEGF). Regardless of the nature of inciting events, the wound healing response follows a predictable pattern that can be divided into three overlapping phases: acute inflammation; fibroblast, endothelial, and epithelial cell proliferation; and remodeling leading to scar formation (Christine et al., 2001).

1.7.1. Basic fibroblast growth factor (bFGF)

bFGF (molecular weight 150 kd) are a family of homologous peptides originally derived from their ability to stimulate fibroblast proliferation in vitro (Gospodarowicz, 1974). bFGFs consist a group of regulatory molecules with a high affinity for heparin. The release of FGFs occurs due to the action of the enzyme heparinase found in platelets by dissolving the heparin binding. FGFs are found in many tissues, including endothelial cells, macrophages, and fibroblasts, and are both chemotactic toward endothelial cells and leukocytes as well as mitogenic for endothelial cells. bFGF plays a prominent role in angiogenesis, initiating release of basement membrane degrading enzymes that liberate endothelial cells before new vessel formation (Rodiand et al., 1990).

1.7.2. Epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α)

EGF (molecular weight 6 kDa) was first isolated from mice submandibular gland. It was reported to be a potent mitogen and maturation factor for epidermal cells (Ullrich et al., 1990). EGF is present in body fluid, and it is expressed at very low concentration in normal tissue. EGF induces epithelial cells to migrate, divide, and differentiate. Receptors for this very influential growth factor have been localized on the migrating and proliferating epithelial tips present at the wound edges and on epithelial islands arising from the remnant epidermal appendages (Wenczak et al., 1992). EGF and TGF-α are closely related (35% homology) and possess very similar activities. Wound macrophages contain significant amounts of TGF-α that add to the significance of this cell in the initial tissue response to injury. The main function of these growth factors appears to be on granulation tissue development, with epidermal re-growth and modulation of angiogenesis being unique features of TGF-α activity (Whitman et al., 1989).
1. 7. 3. Transforming growth factor-β

Three separate isoforms of TGF-β (molecular weight 25 kDa) super family exist in humans. They differ in the area of distribution, and share similar but not identical functional properties. TGF-α and TGF-β were first isolated from tumors and named for their ability to transform normal cells into malignant phenotypes. TGF-β has been identified in a wide variety of cells, including platelets, macrophages, bone cells, monocytes and lymphocytes. TGF-α β is coordinately upregulated with various integrin receptors in the leading tip of epidermal cells migrating over provisional matrix during reepithelialization (Gailit et al., 1994), suggesting a role in the enhancement of migration. In contrast, this growth factor drives the proliferative potential of epithelial cells to negligible levels (Sarret et al., 1992).

1. 7. 4. Keratinocyte growth factor (KGF)

KGF is a potent mitogen for a variety of epithelial cells. KGF and TNF-α also stimulates epithelial cell migration, which is thought to occur indirectly via the expression of KGF (Brauchle et al., 1994). The release of EGF, TGF-α and FGF stimulates epithelial cell migration and proliferation, resulting in the start of reepithelialization within hours of injury. This process begins with the dissolution of cell-cell and cell–substratum contacts followed by migration of keratinocytes over the provisional extracellular matrix (ECM). When complete wound closure occurs, keratinocytes undergo stratification and differentiation to restore the barrier.

1. 7. 5. Platelet-derived growth factor (PDGF)

PDGF (molecular weight 30 kDa), the major growth factor released from stimulated platelets at the site of vascular insults is mitogenic for most mesenchymally derived cells, including fibroblasts, smooth muscle cells, and osteoblasts, and for some neoplastic cells. Not surprisingly, PDGF has been implicated in several physiological and pathological processes, including wound repair, embryogenesis, atherosclerosis, and tumor growth (Ross et al., 1986; Ross et al., 1988), where the active formation of new capillary vessels is frequently observed. PDGF is stored in the granules of platelets and released after activation of the platelets at sites of tissue injury. PDGF is also expressed by macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts. It is the
most potent competence factors present in wounds and exerts effects during the first two phases of repair. The production of PDGF at wound sites is not constant, and increase in concentration has been correlated with the augmented connective tissue formation (Heldin et al., 1996).

1.7.6. Vascular endothelial cell growth factor (VEGF)

VEGF is a potent stimulator of migration, proliferation and survival in endothelial cells (Spyridopoulos et al., 1997). VEGF is critical to proper wound repair in stimulating angiogenesis to supply nutrients and oxygen needed for skin regrowth (Wilgus et al., 2005). It appears that bacterial cell wall products are able to inhibit VEGF production in wounds, thereby preventing revascularization of the tissue (Grose et al., 2004).

1.7.7. Interleukin-1 (IL-1)

IL-1 is synthesized by epithelial cells in response to injury, and stimulates both chemoattraction and growth of epithelial cells (Sauder et al., 1990). The autocrine nature of epithelial cell–derived IL-1 is emphasized by the fact that it additionally induces the cell to synthesize IL-1 and TGF-β (the latter factor is also known to induce epithelial cell motility).

1.7.8. Tumor necrosis factor-α

Macrophages can stimulate angiogenesis by expressing TNF-α a substance first associated with the necrosis and regression of certain solid tumors. IL-1 and TNF-β are crucial inflammatory cytokines. Cytokines are now being successfully used as beneficial topical agents in healing wounds (Karukonda et al., 2000). TNF-β in human dermal wounds can be used as a marker for wound age estimation by the use of the multiplex beaded array system.

1.8. Keratin Based Wound Dressing Materials

The keratin based research led to the development of many keratin-based biomaterials for use in tissue engineering applications. This development leads to explore the solid foundation in exploring the several key properties of keratins as overall physical, chemical and biological behavior of these biomaterials. Moreover, the keratin proteins were extracted first and to self-assemble and polymerize into porous, fibrous scaffolds. The spontaneous self-assembly of keratin solutions, has been studied
extensively at both the microscale (Thomas et al., 1987; Steinert et al., 1976; Thomas et al., 1986) and macroscale levels (Ikkai et al., 2002).

The self-assembly phenomenon is evident in the highly conserved superstructure of the hair fiber, when processed correctly, is responsible for the reproducible architecture, dimensionality and porosity of keratin-based materials. In addition, all the keratin biomaterials possess cell binding motifs, such as leucine-aspartic acid-valine (LDV) and glutamic acid-aspartic acid-serine (EDS) binding residues, which are capable of supporting cellular attachment and proliferation (Tachibana et al, 2002; Verma et al., 2008). However, keratin as three dimensional matrix that allows for cellular infiltration, attachment and proliferation that prove more advantageous in a variety of tissue engineering applications (Magin et al., 2007; Izawa et al., 2006). Much has been done to both fabricate and characterize new keratin-based products such as films, sponges, scaffolds and fibers. In many cases, these novel keratin materials showed to possess excellent biocompatibility. Many researchers have discovered methods for modulating the physical and mechanical properties of keratins in order to create biomaterials that have appropriate characteristics for their application of interest.

1.8.1. Keratin films

The preparation of protein films from keratin extracted from wool and human hair has been used for a number of years to explore the structural and biological properties of self-assembled keratins. Yamauchi et al., (Yamauchi et al., 1996) were among the first to begin to investigate the properties of products made from extracted wool keratins and in doing so described the physiochemical and biodegradational properties of solvent-cast keratin films. Although pure keratin films were too fragile for practical use, the addition of glycerol resulted in a transparent, relatively strong, flexible, and biodegradable film (Yamauchi et al., 1996). Fujii et al also demonstrated that hair keratins were useful as protein films by rapid casting method. This research also revealed the feasibility of incorporating such bioactive molecules (Fujii. Tet al., 2004). Like many natural-derived biomaterials, keratin-based products were ultimately focused on the optimization of the physical strength and flexibility of films while maintaining their excellent biological activities. Several approaches have been considered by blending both natural and synthetic polymers to keratin. The biological activity of keratin films was also increased.
by incorporating a cell adhesion peptide, Arg-Gly-Asp-Ser (RGDS), at the free cysteine residues of reduced keratin extracts. RGDS-carrying keratin films proved to be excellent substrates for mammalian cell growth, and this work again demonstrated the potential and versatility of keratin biomaterials (Yamauchi et al., 2003). As a biomaterial much attention has been explored by blending with silk fibroin (Lee, 2001), chitosan (Tanabe et al., 2002) and poly(ethylene oxide) (Aluigi et al., 2007) to check its biological, mechanical and biodegradable properties. More effect has been taken to produce keratin films with most stable shapes with the addition of sulfur groups. Thereby increases the water content of the film, biocompatibility and also attachment and proliferation of fibroblast on the keratin substrates (Katoh et al., 2004).

1.8.2. Keratin sponges and scaffolds

The ability of extracted keratin proteins to self-assemble and polymerize into complex three dimensional structures has led to their development as scaffolds for tissue engineering. Fabrication of wool keratin scaffold matrices were created by lyophilization of aqueous wool keratin solutions after controlled freezing, which resulted in a rigid and heat-stable structure with a homogenously porous microarchitecture. The keratins contain RGD and LDV cell binding motifs aids in cell adhesion with good cell compatibility and supported the attachment and proliferation of fibroblasts with potential modification sites for the immobilization of bioactive substances (Tachibana, et al., 2002). The functionalization of free active thiol group in keratin was linked with various processes as disulfide-linked lysozyme, iodoacetic acid, 2-bromoethylamine, and iodoacetamide to produce a more hybride structure to mimic the function of the ECM. The keratin sponge as a rigid combination with hydroxyapatite in bone tissue engineering application (Tachibana et al., 2005; 2006).

1.8.3. Keratin fibers

The research on the electrospinning of biocompatible polymeric materials has greatly increased due to the abundance of potential biomedical applications for nanofibrous materials. Electrospinning is a technique that utilizes a high voltage to create an electrically charged jet of polymer that is drawn toward a grounded collection plate or mandrel. The resulting fibers have diameters in the nano- to micro-scale range and are
randomly arranged to form a non-woven fibrous mat. The enhanced physical configuration \(i.e.,\) small pore size, high porosity, three-dimensional features, and high surface area-to-volume ratio) of nanostructured nonwovens promotes cell adhesion and growth. This lead to the development of electrospun membranes for such uses as bandages for wound healing and scaffolds for tissue engineering. Recently, keratin based fibers with fibroin / keratin fibers (Zoccola et al., 2008), PVA/keratin (Katoh et al., 2004) and cellulose/keratin (Wrześniewska-Tosik et al., 2007) were fabricated with different optimization techniques. Although, some materials were introduced for the keratin fiber material, still research is going on to make an efficient keratin based material.
The utilization of the keratin from the bovine horn (Waste) into value added dressing material (Wealth). The attentions towards layered dressing material make the dressing material with more advantage by merging the disadvantage of the individual scaffolds. Even though here are many applications of keratin in the biomedical field, main attention in the present study was focused on the potential wound dressing material as a film and sponge material for biomedical applications.

1. Single layered wound dressing materials
2. Dual layered wound dressing materials

The prepared materials were characterized for their physiochemical properties; in vitro and in vivo evaluation was also carried out using the prepared material.

1. 9. Scope of the Present Work

The experimental drivers of this study are as follows

1. Preparation of mupirocin incorporated keratin based wound dressing material and its
   a) Characterization
   b) In vitro evaluation using fibroblasts and keratinocyte cell lines

2. Preparation of electrospun nanofibrous scaffold sandwiched as a dual layered keratin based wound dressing material and their
   a) Characterization
   b) In vitro evaluation using fibroblasts and keratinocyte cell lines and
   c) In vivo evaluation using rats as animal model.

3. Preparation of mupirocin incorporated keratin-fibrin-gelatin 3D sponge as a wound dressing material and its
   a) Characterization
   b) In vitro evaluation using fibroblasts and keratinocyte cell lines

4. Preparation of curcumin loaded nanofibrous scaffold sandwiched as a dual layered Keratin- fibrin-gelatin 3D sponge incorporated with mupirocin as a wound dressing material and their
   a) Characterization
   b) In vitro evaluation using fibroblasts and keratinocyte cell lines and
   c) In vivo evaluation using rats as animal model.
References


