CHAPTER 1
INTRODUCTION

1.1 Wound

Wound is a reversible or irreversible outcome of injury in which the part effected is torn, cut or punctured. This may be due to trauma, surgery or health disorders. The wounds are generally classified according to the depth of tissue loss. The classification is as follows, wounds with tissue loss and without tissue loss. The wounds with tissue loss include burn wound (second and third degree burn wounds), diabetic foot ulcer, etc and wounds without tissue loss include laceration, first degree burn wound etc. The classification of wound is done according to its depth into the skin, which indicates whether it is a superficial, partial thickness or a full thickness wound. A superficial wound involves epidermis only whereas partial thickness wound include epidermis and dermis. A full thickness wound consisted of epidermis, dermis and subcutaneous tissue. Figure 1.1 shows the schematic representation of structure of skin.

Fig. 1.1. Schematic representation of structure of skin
Wounds can even be classified based on the time at which it heals as acute and chronic. An acute wound is an injury that causes a break in the skin and it can happen suddenly, last a short time, and may heal on its own. For example, a puncture wound is usually made by a sharp, round, and pointed object, such as a needle or nail. There are principally two types of acute wound; traumatic wounds and surgical wounds. A traumatic wound such as a minor cut, leads to extensive tissue injuries when a force exceeds the strength of the skin or the underlying supporting tissues. A traumatic wound is classified by whether or not it is tidy or untidy. A surgical wound is either incised and sutured or lay open to heal by a surgeon. The wound breaks the integrity of the skin including the epidermis and dermis. Surgical wounds are classified in relation to the potential for infection in the wound: they are considered to be either clean, clean contaminated, contaminated or dirty.

A chronic wound is a wound that does not heal in orderly set of stages and in a predictable amount of time the way most wounds do; wounds that do not heal within three months are often considered chronic. Chronic wounds seem to be detained in one or more of the phases of wound healing. Diabetic foot ulcer is an example for chronic wound. Figure 1.2 shows various types of wounds.

Wound infection is the major difficulty in the field of wound care management, because such infections can cause exudate formation, delay the wound healing, facilitate improper collagen deposition, etc. Microbes are the major reason for infection and the prevalence of the same is high in and around us. The major infection causing bacteria were Staphylococcus aureus (S. aureus), Staphylococcus epidermidis (S. epidermidis), Pseudomonas aeruginosa (P. aeruginosa) and Escherichia coli (E. coli). Once it gains entry into the body, these microbes grow immediately and start to form colonies. The microbes can easily enter the body through the wounds and can reach into deeper portions of the tissue. Furthermore, it can lead to internal infection.
1.2 Stages of wound healing

Wound healing, a normal biological process in the human body, is achieved through four precisely and highly programmed phases: hemostasis, inflammation, proliferation, and remodeling. For a wound to heal successfully, all four phases must occur in the proper sequence and time frame. Many factors can interfere with one or more phases of this process, thus causing improper or impaired wound healing. Figure 1.3 shows the different phases of wound healing.

1.2.1 Hemostasis

The first phase of hemostasis begins immediately after wounding, with vascular constriction and fibrin clot formation. The clot and surrounding wound tissue release 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).

1.2.2 Inflammation

Once bleeding is controlled, inflammatory cells migrate into the wound (chemotaxis) and promote the inflammatory phase, which is characterized by the sequential infiltration of
neutrophils, macrophages and lymphocytes. A critical function of neutrophils is the clearance of invading microbes and cellular debris in the wound area. Macrophages play multiple roles in wound healing. Macrophages release cytokines that promote the inflammatory response by recruiting and activating additional leukocytes. Macrophages are also responsible for inducing and clearing apoptotic cells (including neutrophils), thus paving the way for the resolution of inflammation. As macrophages clear these apoptotic cells, they undergo a phenotypic transition to a reparative state that stimulates keratinocytes, fibroblasts, and angiogenesis to promote tissue regeneration. In this way, macrophages promote the transition to the proliferative phase of healing. T-lymphocytes migrate into wounds following the inflammatory cells and macrophages, and peak during the late-proliferative/early-remodeling phase. T-cells regulate many aspects of wound healing, including maintaining tissue integrity, defending against pathogens, and regulating inflammation.

1.2.3 Proliferation

The proliferative phase generally follows and overlaps with the inflammatory phase, and is characterized by epithelial proliferation and migration over the provisional matrix within the wound. In the reparative dermis, fibroblasts and endothelial cells are the most prominent cell types present and support capillary growth, collagen formation, and the formation of granulation tissue at the site of injury. Within the wound bed, fibroblasts produce collagen as well as glycosaminoglycans and proteoglycans, which are major components of the extracellular matrix (ECM).
1.2.4 Remodelling

Following robust proliferation and ECM synthesis, wound healing enters the final remodelling phase, which can last for years. In this phase, regression of many of the newly formed capillaries occurs so that vascular density of the wound returns to regular pattern. One critical feature of the remodelling phase is ECM remodelling to an architecture that approaches
that of the normal tissue. The wound also undergoes physical contraction throughout the whole wound healing process, which is believed to be mediated by contractile fibroblasts that appear in the wound.

1.3 Wound healing methods

Wound healing can be achieved through various methods and they are given below.

1.3.1 Wound dressing

Wound dressings are the most commonly used therapeutic agents, for wound healing. An ideal wound dressing should have the following features and they included the ability to: (1) absorb exudates and toxic components from the wounds surface; (2) maintain a high humidity at the wound/dressing interface; (3) allow gaseous exchange; (4) provide thermal insulation; (5) protect the wound from bacterial penetration; (6) be nontoxic; and (7) be removed easily without trauma to the wound; (7) have acceptable handling qualities (resistance to tear and disintegration when wet or dry); leaves no foreign particles in wound and (9) be sterilizable. The wound dressing should be non-allergic and non-irritant to skin. If the dressing is biodegradable, then the by-products promote better healing and this kind of dressing will be helpful in treating wounds where its removal is difficult. A wound with necrotic eschar needs a dressing that facilitates debridement. An ideal wound dressing should conform to wound contours. A wound dressing with sustainable release of antimicrobial agents is required for the treatment of infected wounds. The features required for an ideal wound dressing is represented pictorially in the figure given below (Fig. 1.4).
1.3.2 Wound healing therapies
There are many wound healing therapies available currently and the details of them are given below.

1.3.2.1 Hyperbaric oxygen therapy
Hyperbaric oxygen therapy (HBOT) is breathing 100% oxygen while under increased atmospheric pressure. HBOT can be done in single-person chambers or chambers that can hold
more than a dozen people at a time. A single-person chamber, or monoplace, consists of a clear plastic tube about seven feet long. The patient lies on a padded table that slides into the tube. The chamber is gradually pressurized with pure oxygen. Patients are asked to relax and breathe normally during treatment. Chamber pressures typically rise to 2.5 times the normal atmospheric pressure. Patients may experience ear popping or mild discomfort, which usually fades if the pressure is lowered a bit. At the end of the session, which can last from thirty minutes to two hours, technicians slowly depressurize the chamber.

1.3.2.2 Vacuum assisted closure

Vacuum assisted closure (also called vacuum therapy, vacuum sealing or topical negative pressure therapy) is a sophisticated development of a standard surgical procedure, the use of vacuum assisted drainage to remove blood or serous fluid from a wound or operation site. The therapy involves the controlled application of sub-atmospheric pressure to the local wound environment. The technique will help to remove chronic edema, leading to increased localized blood flow, and the applied forces result in the enhanced formation of granulation tissue. Even then the technique is appeared costly and inconvenient for patients.

1.3.2.3 Low level laser therapy

Low level laser therapy (LLLT) is a medical procedure used to treat pain and speed up wound healing. This treatment uses low-level lasers to alter the healing process at a cellular level. It will diminish inflammation by reducing the number of cellular chemicals and enzymes linked to pain and inflammation. Another mechanism is the stimulation of the cells to increase the production of certain enzymes which affect cell proliferation or cell division, thus increasing the healing speed of a wound. The effects of low-level laser therapy is depends on the specific wavelength of the laser treatment itself. The ideal wavelength, dosage, duration and location of treatment are specific to each ailment and wound.

1.3.2.4 Shock wave therapy

Shock wave therapy has been investigated as an adjuvant therapy in the treatment of acute and chronic wounds. There are several devices with focused and unfocused shock waves that have been administered to a heterogeneous group of wounds. Shock wave therapy promotes wound healing with little or no adverse events. It affects the cellular function and leads to the
expression of several genes and elaboration of growth factors known to promote wound healing. It triggers biochemical effect that initiates growth in blood vessels and releases growth factors, thereby causing chronically aggrieved tissue to heal.

1.3.3 Skin graft

A skin graft is a section of epidermis and dermis which has been completely separated from its blood supply in one part of the body, the donor site, before being transplanted to another area of the body, its recipient site. A skin graft is used to permanently replace damaged or missing skin or to provide a temporary wound covering. This covering is necessary because the skin protects the body from fluid loss, aids in temperature regulation, and helps prevent disease-causing bacteria or viruses from entering the body. Skin that is damaged extensively by burns or non-healing wounds can compromise the health and well-being of the patient. Skin grafts can be classified as partial, full-thickness grafts and pedicle skin grafts.

A split-skin or partial-dermal skin graft involves excision of the epidermis and part of the dermis, but leaves behind sufficient reticular (deep) dermis in the wound bed to enable the skin to regenerate itself. The most common donor site areas for split-skin grafts include the thigh, buttock, back, upper arm, forearm and abdominal wall. A full-thickness graft consists of the epidermis and the full-thickness of the dermis. Since none of the reticular dermis remains to allow spontaneous regeneration of skin, the wound must be directly closed to heal by primary intention. Common donor site areas for full-thickness skin grafts include the pre- and post-auricular (ear), supraclavicular and antecubital (inner elbow) areas, the upper eyelid, scalp, groin and areola. A pedicle graft is one in which a part remains attached to the donor site, whereas the remainder is transferred to the recipient site. Its own blood supply remains intact, and it is not detached until the new blood supply has fully developed. This type is often used on the face, neck, or hand. Skin grafts are divided into many types depends on the donor.

1) Autologous or autograft: An autograft is a graft taken from one part of an individual’s body that is transferred to a different part of the body of that same individual. The most common donor site is the anterior or lateral aspect of the thigh. Use of the posterior thigh as a donor site is a bit more painful and difficult for the patient to care for postoperatively.
2) **Isogeneic:** The donor and recipient individuals are genetically identical. Isogenic grafts are either graft between animals of a single highly inbred strain, between the F1 hybrids produced by crossing inbred strains, or between identical twins.

3) **Allogeneic:** An allogeneic graft or allograft is a graft between genetically disparate individuals of the same species. More specifically, it is a graft in which the grafted tissue carries a histocompatibility allele or alleles, and hence presumably an alloantigen or alloantigens, foreign to the recipient.

4) **Xenogeneic:** Tissue or organs from an individual of one species transplanted into or grafted onto an organism of another species, genus, or family. A common example is the use of pig heart valves in humans.

1.4 Different types of wound dressings

1.4.1 Passive dressings

   Passive dressings can be sub classified into absorbing and non-absorbing. Gauze, lint, non-stick and tulle are come under the passive dressing category. They fulfill very few of the properties of an ideal dressing and have very limited use as primary dressings, but some are useful as secondary dressings.

1.4.2 Interactive dressings

   Interactive dressings comprise of polymeric films and foams, which are mostly transparent, permeable to water vapor and oxygen but impermeable to bacteria. These films are recommended for low exuding wounds. Interactive dressings use the environment provided by the body to encourage normal healing and stimulate the healing cascade.

1.4.3 Bioactive dressings

   Bioactive dressing is capable to deliver substances active in wound healing; either by delivery of bioactive compounds or dressings is constructed from material having endogenous activity. These materials include proteoglycans, collagen, non-collagenous proteins, alginates, gelatin, chitosan, etc.

1.4.4 Hydrogel based dressings

   Hydrogel is a network of polymer chains that are water-insoluble, sometimes found as a colloidal gel in which water is the dispersion medium. Hydrogels are superabsorbent (they can contain over 99% water) natural or synthetic polymers. Hydrogels possess also a degree of
flexibility very similar to natural tissue, due to their significant water content. Current uses of hydrogels include scaffolds for tissue engineering, drug delivery, wound dressings, etc. The advantages of using hydrogel dressings are retention of moist atmosphere, high swelling capacity, provide cooling sensation and healing of wound without scar formation.

1.5 Polymers for wound dressing

There are different polymers mainly synthetic and natural polymers used for wound dressings. Synthetic polymers include poly(vinyl alcohol), poly(caprolactone), poly(urethane), poly(ethylene oxide), poly(lactic-co-glycolic acid), etc. Natural polymers include alginate, gelatin, collagen, chitin, chitosan etc. These materials are having their own advantages and disadvantages. Among these materials chitin and its de-acetylated derivative chitosan have shown tremendous advancement in the biomedical field including tissue engineering, drug delivery, wound dressing, etc.

1.5.1 Chitin

Chitin (C$_8$H$_{13}$O$_5$N)$_n$ is a long-chain polymer of a N-acetylglucosamine, a derivative of glucose. Chitin is the second most abundant natural polysaccharide after cellulose on earth. Chitin is a readily available biological material obtained 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. Attempts have been made to use chitin for various applications like wound dressings and as scaffolds in tissue engineering due to their wound healing, antibacterial and anti-inflammatory properties. Chitin and its derivative, chitosan, are biocompatible, biodegradable, non-toxic, antimicrobial and hydrating agents. The three polymorphic crystalline forms of chitin are α, β and γ. These forms of chitin differ from one another based on the arrangement of polymer chains. The polymer chains are arranged antiparallel (Syndiotactic) to each other in α-chitin, parallel (Isotactic) to each other in β-chitin whereas randomly in γ-chitin (Atactic). These polymer chains are tightly held by intermolecular hydrogen bonding between N-H and –C=O functional groups.
Fig. 1.5. Structure of Chitin

Fig. 1.6. Polymer chain arrangements in α-Chitin (Syndiotactic)

Fig. 1.7. Polymer chain arrangements in β-Chitin (Isotactic)
1.5.1.1 α-Chitin

α-chitin is the most abundant polymorphic form of chitin and is mainly isolated from crabs and shrimps. α-Chitin possesses wound healing, antibacterial and anti-inflammatory abilities [1]. It has been reported that α-chitin enhances the apatite layer deposition, which is essential for tissue engineering applications [2]. It leads to the migration of keratinocytes towards the wound site to enhance wound healing. α-Chitin can be converted into different forms like hydrogel, fiber etc [2,3]. This material could also be used to make scaffold for tissue engineering and wound dressing applications.

1.5.1.2 β-Chitin

β-Chitin, obtained from squid pens, takes the monoclinic form in which the chains are arranged in a parallel fashion. As a result of the molecular packing, the intermolecular interactions in β-chitin are weaker than those in α-chitin, making the β-chitin more susceptible to dissolution in a number of solvents and exhibiting higher chemical reactivity compared to α-chitin.

Fig. 1.8. Polymer chain arrangements in γ-Chitin (Atactic)
1.5.1.3 Chitosan

Chitosan, a polycation biopolymer, is a non-toxic, biocompatible, and biodegradable polysaccharide derived from naturally occurring chitin. Chitosan is produced commercially by deacetylation of chitin by using sodium hydroxide. The degree of deacetylation of chitosan will be in the range of 60-100%. Positively charged chitosan is soluble in acidic aqueous solution below pH 6, while generally insoluble in neutral conditions and most organic solvents. Chitosan has many useful and advantageous 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](image)

**Fig. 1.9. Structure of chitosan**

Chitosan was found to enhance the functions of polymorphonuclear leukocytes, macrophages and fibroblasts. As a result, chitosan promotes granulation and organization, and therefore it is beneficial for open wounds; certain polymorphonuclear functions are enhanced, such as phagocytosis and the production of chemical mediators. The peculiarity of chitosan is the ability to promote sufficient granulation tissue formation accompanied by angiogenesis and regular deposition of thin collagen fibers.

1.6 Limitations of chitin and chitosan at neutral pH

At neutral pH, chitin and chitosan do not show antimicrobial activity; in order to impart that activity, it is necessary to incorporate some antimicrobial agents into it. Antibiotics can be used to perform this function. But the drug resistance of microbes against these antibiotics and
specificity of antibiotics towards microbes limits the use of antibiotics. Hence, people started to look at various antimicrobial particles which were prepared based on nanotechnology.

1.7 Nanotechnology

Nanotechnology is expected to open some new aspects to fight and prevent diseases using atomic scale tailoring of materials. The ability to uncover the structure and function of biosystems at the nanoscale stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both in vivo and in vitro biomedical research and applications. The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles. In all the nanomaterials with antibacterial properties, metallic nanoparticles and metallic oxides are the best. Nanoparticles increase chemical activity due to crystallographic surface structure with their large surface to volume ratio. The importance of bactericidal nanomaterials study is because of the increase in new resistant strains of bacteria against most potent antibiotics.

1.7.1 Nanoparticles

A nanoparticle is a particle with at least one dimension less than 100 nm. Nanoparticles have attracted much attention for their distinct characteristics that are unavailable in conventional macroscopic materials. Their uniqueness arises specifically from higher surface-to-volume ratios and an increased percentage of atoms at the grain boundaries. The ongoing worldwide nanotechnology revolution is predicted to impact several areas of biomedical research and other science and engineering applications [4]. Nanoparticle-assisted drug delivery, cell imaging, and cancer therapy are important biomedical applications of nanotechnology. Progress in utilizing inorganic nanoparticles for biomedical applications has advanced rapidly as a result of the extensive amount of work done in the synthesis and modification of the particles.

The advantage of using the inorganic oxides for biomedical applications is that they contain mineral elements essential to humans and exhibit strong activity even when administered in small amounts. Among inorganic materials, metal oxide nanoparticles are of special interest. ZnO belonging to a group of metal oxides is characterized by photocatalytic and photo-oxidizing ability against chemical and biological species [5, 6]. Currently lots of antibacterial nanoparticles
are there in use for various biomedical applications. Among those materials, zinc oxide nanoparticles (nZnO) are capable of providing antimicrobial activity and it enhances wound healing [7].

1.7.2 ZnO nanoparticles (nZnO)

ZnO is an inorganic compound with a wide band gap of 3.37eV and it is a semiconducting material. In nature, it occurs as zincite and crystallizes preferentially in a hexagonal structure. ZnO is unique as it exhibits piezoelectric, optical absorption and emission, and pyroelectric properties. Zinc oxide crystallizes in two main forms, hexagonal wurtzite and cubic zinc blende. The wurtzite structure is most stable at ambient conditions and thus most common. The zinc blende form can be stabilized by growing ZnO on substrates with cubic lattice structure. In both cases, the zinc and oxide centers are tetrahedral.

![Crystal structure of ZnO](image)

**Fig. 1.10.** Crystal structure of ZnO

ZnO has attracted wide interest because of its good photocatalytic activity and high stability [8]. ZnO has received tremendous attention for its use in thin film transistors and solar cells. Crystalline ZnO-based thin films exhibit wide range electrical conductivity and high transmittance in the visible range. ZnO-based transparent oxide semiconductor (TOS) films have been considered for use as the active layer in thin film transistors. The possibility of engineering the band gap and influencing the physical, chemical, and electronic properties has provided a strong thrust to study ZnO on the nanoscale.
nZnO has become a nano-functional material, widely concerned following carbon nanotubes. It is well-known that nZnO possess antibacterial activity and it is currently used in many devices, pigments, and components for the pharmaceutical and cosmetic industries. nZnO can be used for photodynamic cancer therapy, selective destruction of tumor cells, for providing antibacterial surface coating, cell labeling applications, as DNA biosensors, etc. It is well known that ZnO-based transparent conductive oxide (TCO) films are now available for utilization as transparent electrodes in liquid crystal displays and solar cells. In addition, ZnO-based transparent oxide semiconductor (TOS) films have been considered for use as the active layer in thin film transistors (TFTs) and sensing layer in ultraviolet (UV) photodetectors.

1.8 Literature Review

1.8.1 α-Chitin

α-Chitin is the most abundant polymorphic form of chitin and is mainly isolated from crabs and shrimps [9, 10]. α-Chitin is highly hydrophobic and is insoluble in water and common organic solvents. Its highly crystalline structure accounts for its poor solubility [11]. α-Chitin has got variety of application in the biomedical field [12, 13]. α-Chitin can be easily converted into nanofibers, gels, scaffolds, membranes, powders, beads and composite [14-24]. Previous reports have shown that this material is capable of enhancing the wound healing process [23-25]. The main biochemical activities of chitin based materials in wound healing are polymorphonuclear cell activation, fibroblast activation, cytokine production, giant cell migration and simulation of type IV collagen synthesis [17-19]. Enzymatic cleavage by lysozyme results in formation of N-acetyl glucosamine and glucosamine [26-28]. For α-chitin, the hemostatic effect is believed to be due to the mobilization of erythrocytes, clotting factors, and platelets to the site of injury [29-31]. α-Chitin can be used as a supporting material for tissue engineering application owing to their porous structure, gel forming properties, ease of chemical modification and high affinity to in vivo macromolecules [16, 21, 32-34]. Chow et al made a series of porous chitin matrixes by producing chitin gels from chitin solutions followed by lyophilization to give porous chitin matrixes [35]. Mouse and human fibroblast cell cultures exposed to these chitin matrixes were found to be growing and proliferating indicating the feasibility of using these porous chitin matrixes for cell transplantation applications to regenerate tissues.
Wan et al combined chitin with hydroxyapatite to produce a hydroxyapatite-chitin composite material by dispersing hydroxyapatite in chitin to give an intimately blended material [36]. Tamura et al reported the preparation of α-chitin hydrogel and the development of membranes from the hydrogel [15]. They have analyzed the mineralization potential of the same by immersing in simulated body fluid. The obtained data revealed the membranes were capable of depositing minerals on top of it. The cell viability data showed that the cells were viable towards the tested cells and showed no sign of toxicity. Jayakumar et al reported the development of scaffolds and membranes from the α-chitin hydrogel and evaluated the osteoblast cell viability and attachment on the scaffolds and membranes [37]. Nagahama et al reported the preparation of α-chitin/gelatin and α-chitin/gelatin/N-acetyl glucosamine composite membranes and evaluated the cell attachment as well as bioactivity of the same [20, 21]. Sudheesh Kumar et al reported the development and in vitro evaluation of α-chitin hydrogel/nano hydroxyapatite composite scaffolds for bone tissue engineering applications [38]. The prepared composite scaffolds showed excellent mineralisation potential, biodegradable nature and cell viability. The MG63 cells attached and proliferated well on the scaffolds. The incorporation of nano-bioactive glass into α-chitin hydrogel was done and the scaffold prepared using that showed tremendous mineralization potential as well as excellent cell proliferation abilities [22]. The antibacterial potential of silver nanoparticle incorporated α-chitin hydrogel composite scaffold was studied for wound healing applications [39]. The composite bandages showed excellent antibacterial potential against gram positive S. aureus and gram negative E. coli. The incorporation of silica nanoparticle into α-chitin hydrogel was done and the bioactivity of the composite scaffold has been investigated [2]. The data showed the composite scaffold was capable of deposition of minerals while immersing in simulated body fluid.

The increasing interest in chitin and its deacetylated forms is due to the biological activity resulting from its susceptibility to degradation under the influence of enzymes present in body fluids such as lysozyme and N-acetyl glucosaminidase. The degradation products, being chito-oligomers, are able to stimulate macrophages and enhance collagen deposition, thus accelerating the wound healing process [1]. Chitin will gradually depolymerize to release N-acetyl-β-D-glucosamine, which initiates fibroblast proliferation and helps in ordered collagen deposition and stimulates increased level of natural hyaluronic acid synthesis at the wound site [40]. It helps in
faster wound healing and scar prevention. Chitin–Poly (acrylic acid) blend was prepared and further treated with glycyltrimethylammonium chloride (GTMAC) for wound healing applications and the obtained data was promising that the materials were capable of enhanced wound healing [41, 42]. The development of a bilayer wound dressing material composed of carboxymethyl chitin hydrogel based upper layer and chlorhexidine gluconate impregnated chitosan acetate foam based lower layer was reported [43]. The prepared material showed excellent antibacterial activity as well as adequate swelling capacity. Kifune et al patented a wound dressing made of a non-woven fabric of α-chitin fibers [44]. The dressing with compatibility to living body and good adherence property to the surface of wounds was found suitable for the protection of skin defect wounds. Chitin artificial skin, Beschitin W, has been proved to be beneficial in clinical practice [45].

Commercial dressings based on α-chitin are available in the market. α-Chitin dressings in sponge form (Chitopack S®) and non-woven made of chitin-modified PET (Chitopack P®) are commercial wound remedies offered by Eisai Co. in Japan. The dressing Beschitin is recommended for successful and fast healing of burns, skin abrasions, postoperative wounds, bed sores, ulcers, and several other injuries.

1.8.2 β-Chitin

The β-chitin adopts a monoclinic unit cell where the polysaccharide chains are arranged in parallel pattern [46]. The main sources of β-chitin are squid pen and cuttlefish pen. Previous studies have shown that β-chitin is biodegradable under the influence of lysozyme and the degradation products were beneficial to the human body [47]. Ratanajiajaroen et al reported the fabrication of porous β-chitin structure by supercritical CO₂ drying [48]. Nata et al reported the preparation of self-sustaining hydrogel from β-chitin nanofibrils [49]. The prepared hydrogel showed adequate mechanical strength as well. Composite of β-chitin was developed by incorporation of octacalcium phosphate into β-chitin and the prepared composite showed excellent mechanical strength [50]. Composites of β-chitin with calcium carbonate polymorphs were prepared by precipitation of the mineral into a β-chitin scaffold by means of a double diffusion system [51]. The prepared scaffolds showed excellent cell viability and cell attachment abilities. β-Chitin based wound dressing material has been developed with the incorporation of silver sulfadiazine for antibacterial wound dressing [52]. This material found to be effective
against *P. aeruginosa* upto 7 days. *In vivo* evaluation in wistar rats revealed that the prepared material was capable of faster healing. Histological studies confirm the proliferation of fibroblasts in the wound bed and a distinct reduction in infectious cells. β-chitin/nano silver composite scaffolds have been made and *in vitro* evaluation were completed for wound dressing applications [53]. The obtained data showed that the scaffolds inhibited the growth of *S. aureus* and *E. coli*. The scaffolds were biocompatible and Vero cells attached and proliferated well on the scaffold. It has been reported that osteoblast cells attached and proliferated on the β-Chitin membranes under *in vitro* conditions [37]. The membrane showed mineral deposition after 21 days of immersion in simulated body fluid.

β-Chitin-hydroxyapatite composite membranes were prepared by alternate soaking of β-chitin membranes in CaCl$_2$ (pH 7.4) and Na$_2$HPO$_4$ solutions for 2 h in each solution. The minerals were deposited on the membranes were hydroxyapatite and that have been confirmed by SEM, FT-IR, EDS and XRD analysis [54]. The bioactivity of β-chitin scaffold was investigated by immersing in simulated body fluid and found that the scaffolds were capable of deposition of minerals [55]. The prepared scaffolds showed excellent cell viability and the cells attached and started proliferating on the scaffolds. These scaffolds will be useful for tissue engineering as well as wound dressing applications. Suzuki et al reported the preparation of scaffold using β-chitin and chitosan [56]. The prepared scaffolds promoted the adhesion and proliferation of rabbit chondrocytes. Ratanajiajaroen et al reported the incorporation of curcumin in β-chitin non-woven fibrous sheet [57]. They also investigated the release of curcumin from curcumin loaded fibrous sheet. It was found that the amounts of loaded curcumin affected the release characteristics of it from the β-chitin sheet. Seong et al reported the development of semi-interpenetrating network composed of β-chitin and poly(ethylene glycol). The prepared membranes showed excellent tensile strength and can be used for various biomedical applications [58]. Another study reported the development of β-Chitin hydrogel/nano hydroxyapatite composite scaffolds for wound dressing and tissue engineering applications. The prepared scaffolds showed enhanced biomineralization potential and human dermal fibroblast cells attached and proliferated on the scaffolds [59].
1.8.3 Chitosan

Chitosan has been widely used as a wound dressing material due to its properties. The notable properties of chitosan include its non-toxicity, hemostatic action, anti-inflammatory effect, biodegradability, biocompatibility, antimicrobial activity, retention of fibroblast growth factors, release of glucosamine, N-acetyl glucosamine monomers, oligomers, and stimulation of human dermal fibroblast cellular activities [17, 60-62]. It stimulates cell adhesion and proliferation and helps in the organization of the extracellular matrix [63-65]. It has been reported that the chitosan mesh membrane possesses potential wound-healing effects by enhancing the re-epithelialization and granular layer [13]. Another study reported the use of composite hydrogel sheets made from chitosan, honey, and gelatin for burn wound healing [66]. An animal burn model was performed on the back of New Zealand rabbit, and treated with the hydrogel sheet. The macroscopic image and histopathology were examined. The results showed that hydrogel sheet had a significant effect on wound contraction with the shortest treatment duration of 12 days compared to controls. Histological examination revealed that hydrogel sheet treated burn wound was repaired with intact epidermis on day 12, but the control wound did not completely heal. A study on chitosan film enriched with an antioxidant agent reported that chitosan can stimulate fibroblasts and promote collagen deposition [67].

Tran et al reported rutin-releasing chitosan hydrogels as injectable dressings for dermal wound healing [68]. Rutin was employed to enhance production and accumulation of extracellular matrix in the healing process. In vitro study demonstrates that released rutin significantly enhanced cell proliferation as compared with media without rutin. In vivo wound healing study was performed by injecting hydrogel on rat dorsal wounds and histological results demonstrated that rutin-conjugated hydrogel exhibited enhancement of wound healing as compared with controls. Fast in situ forming supramolecular hydrogels consisted of the tyramine-conjugated supramolecular structures and chitosan derivative were prepared via an enzymatic reaction [69]. The hydrogels showed a good cytocompatibility in vitro. These hydrogels could be promising injectable biomaterials with variable degradation times to control both the cellular behaviors as a regenerative cell matrix and the drug release behavior as a drug delivery vehicle.
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Human keratocyte-reprogrammed induced pluripotent stem cells (iPSCs), were combined with chitosan hydrogel and used as a rapid delivery system to efficiently enhance corneal wound healing [70]. Lou et al reported the preparation of carboxymethyl chitosan/oxidized dextran hydrogel [71]. The developed biodegradable hydrogel showed potential application in the prevention of postoperative adhesion. Chiu et al evaluated the controlled delivery of thymosin β4, which supports cardiomyocyte survival by inducing vascularization and upregulating Akt activity, in the treatment of myocardial infarction [72]. Zhu et al reported the attachment and proliferation of human fibroblast cells on chitosan membranes which were pre-treated with argon-plasma [73]. The plasma treated membranes showed enhanced attachment and proliferation of cells compared to the untreated chitosan membranes. Nacer et al reported the preparation and in vivo evaluation of poly(vinyl alcohol)/chitosan hydrogel for deep second degree burn wound in rat model [62]. The wounds treated with poly(vinyl alcohol)/chitosan hydrogel healed on the 9th day, while those treated with paraffin gauze dressing and cotton gauze healed on the 16th day. Histological analysis showed that new granulation tissue and epithelialization progressed better in wound treated with hydrogel of poly(vinyl alcohol)/chitosan.

Yen et al reported the wound healing effect of layered hydrogel dressing comprised of chitosan, alginate and polyglutamic acid [74]. The prepared materials showed enhanced wound healing potential and were non-toxic to cells. Takei et al reported the use of hydrogel dressing made of chitosan derivative for full thickness wound treatment in rats [61]. Murakami et al reported the development and in vivo evaluation of hydrosheet composed of alginate, chitin/chitosan, and fucoidan for treating and healing of impaired wounds [75]. The histological examinations showed significantly advanced granulation tissue and capillary formations in the healing-impaired wounds treated with the hydrosheet material. A drug delivery system of chitosan-collagen hydrogel incorporated with lysostaphin based on the lysostaphin gauze was developed for methicillin-resistant S. aureus infected burn wounds. After two weeks of treatment, the wounds started healing and no bacteria could be detected on the wounds [76]. The wound dressing developed from carboxymethyl chitosan hydrogel and chitin-poly(acrylic acid) hydrogel were evaluated for their dermal irritation potential response. The data showed that the prepared materials showed no signs of irritation [77].
Mizuno et al reported the development and in vivo evaluation of basic fibroblast growth factor (bFGF) loaded chitosan membranes for effective wound healing in genetically diabetic mice [78]. Full-thickness wounds were created on the backs of diabetic mice, and chitosan film or bFGF-chitosan film was applied to the wound. The wound was smaller after 5 days in both groups, but the wound size reduced further on day 20 only in the bFGF-chitosan group. Proliferation of fibroblasts and an increase in the number of capillaries were observed in both groups, but granulation tissue was more abundant in the bFGF-chitosan group. Chitosan sponges were prepared and the ability to deliver antibiotic was evaluated and the obtained data revealed that the prepared material has potential as local delivery systems for adjunctive therapy for infection control [79]. The PNIPAM/genipin crosslinked sponges exhibited the most sustained release of biologically active antibiotics, with an average antibiotic release 63% higher than uncrosslinked and 37% higher than genipin crosslinked sponges, after 96 h. No significant cytotoxic effects from the sponge or eluates were exhibited with NIH 3T3 fibroblasts. A blend film containing chitosan and gelatin showed promising results to serve as a biomaterial for human adipose derived stem cell (ASC) based cell therapy [80]. The researchers placed porous collagen matrix on ASC-seeded C/G blends to simulate the application of ASC-seeded chitosan/gelatin (C/G) films onto injured tissue and found that a C/G film composed of 75% chitosan could facilitate significantly more cell transfer into the overlying collagen sponge. Kim et al reported the use of chitosan gel for sustained local delivery of bone morphogenetic protein-2 for osteoblastic differentiation [81].

Sun et al reported the development of collagen/chitosan scaffold for the sustained release of recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) [82]. In vivo investigation revealed that the heparinized scaffolds loaded with rhGM-CSF (H(1)E/rhGM-CSF) had the best cellular adhesion and migration, new vessel formation, and highest expression of VEGF and TGF-β1, indicating promoted angiogenesis. Another study reported the development and in vivo evaluation of cross-linked gelatin/alginate, gelatin/hyaluronate and chitosan/hyaluronate sponges for full thickness wound healing application in wistar rats [83]. They found that the wounds treated with these materials showed enhanced healing in comparison to the control. Chitosan/gelatin hydrogel scaffolds were prepared and their biostability was evaluated. The prepared scaffolds were porous in nature and were capable of absorbing large
volume of liquid [84]. Chen et al reported the preparation of injectable chitosan hydrogel [85]. In vitro cytotoxicity and hemolysis analysis revealed that the gel was biocompatible. In vivo subdermal injection into mice models further confirmed the non-cytotoxicity of the hydrogel and the hydrogel was highly resistant to degradation.

There are a handful of reports on the use of curcumin for wound healing by combining with chitosan and its derivatives [86-88]. An in situ injectable nano-composite hydrogel composed of curcumin, N, O-carboxymethyl chitosan and oxidized alginate (CCS-OA) as a novel wound dressing was successfully developed for the dermal wound repair application [89]. In vivo wound healing study was performed by injecting hydrogels on rat dorsal wounds. Histological study revealed that application of nano-curcumin/CCS-OA hydrogel could significantly enhance the re-epithelialization of epidermis and collagen deposition in the wound tissue. DNA, protein and hydroxyproline content in wound tissue from each group were measured on 7th day of post wounding and the results also indicated that combined usage of nano-curcumin and CCS-OA hydrogel could significantly accelerate the process of wound healing. Curcumin nanoformulation loaded methoxy poly(ethylene glycol)-graft-chitosan film (curcumin-MPEG-chitosan film) was developed and its applicability on the wound healing was investigated [90]. In vitro cytotoxicity test showed that the developed MPEG-chitosan film was non-cytotoxic. Tests to determine the antioxidant efficiency revealed that curcumin in the film did not show any significant difference compared to that of unmodified curcumin. Furthermore, in vivo wound healing test showed that the rate of wound reduction was greatly elevated with the rapid re-epithelialization in curcumin-MPEG-chitosan film group. Masson's trichrome staining and the hydroxyproline measurement in the wound tissue also suggested that application of curcumin-MPEG-chitosan film could greatly increase the collagen synthesis compared with that of MPEG-chitosan film treatment [90].

1.8.4 Chitin and chitosan hydrogel based wound dressings

Hydrogels are insoluble polymers that expand in water and are available in sheet, amorphous gel or sheet hydrogel-impregnated dressings [91]. They provide a moist environment for cell migration and absorb exudate. Autolytic debridement without harm to granulation or epithelial cells is another advantage of hydrogel dressings [92]. Hydrogels have marked cooling and soothing effect on the skin, which is valuable in burns and painful wounds. Hydrogel
dressings are seen as an essential component in many different types of wound care. This is because hydrogel dressing is designed to hold moisture in the surface of the wound, providing the ideal environment for both cleaning the wound, and allowing the body to get rid of necrotic tissue [92, 93]. The moisture in the wound is also essential in pain management for the patient, and these dressings are very soothing and cooling. With their high moisture content they also help to prevent bacteria and oxygen from reaching the wound, providing a barrier for infections [93].

Application of the chitosan hydrogel significantly induced wound contraction and accelerated wound closure and healing. Histological examinations also have demonstrated an advanced granulation tissue formation and epithelialization in the chitosan hydrogel treated wounds [94]. Application of the chitosan hydrogel significantly induced wound contraction and accelerated wound closure in both db/db and db/+ mice. The addition of fibroblast growth factor-2 (FGF-2) in the chitosan hydrogel further accelerated wound closure in db/db mice, although not in db/+ mice. Histological examination also has demonstrated an advanced granulation tissue formation, capillary formation and epithelialization in wounds treated with FGF-2-incorporated chitosan hydrogels in db/db mice [95]. The capabilities of the chitosan/non-anticoagulant heparin (periodate-oxidized (IO4-) heparin) hydrogel to immobilize fibroblast growth factor (FGF)-2, as well as the controlled release of FGF-2 molecules from the hydrogel in vitro and in vivo were evaluated. The prepared hydrogel was biodegraded in about 20 days after subcutaneous injection into the back of a mouse. When the FGF-2-incorporated hydrogel was subcutaneously injected into the back of both mice and rats, a significant neovascularization and fibrous tissue formation were induced near the injected site. These results indicate that the controlled release of biologically active FGF-2 molecules is caused by biodegradation of the hydrogel, and that subsequent induction of the vascularization occurs [96].

Interpenetrating polymer network (IPN) hydrogels were prepared by UV irradiation of solutions in mild aqueous acid media of poly(ethylene glycol) macromer (PEGM) with chitosan in the presence of glutaraldehyde as a crosslinking agent. The prepared PEGM can be used for various biomedical applications [97]. Another study reported the preparation of cross-linked hydrogel films with polyvinyl alcohol (PVA) and chitosan using the freeze-thawing method. They used the prepared hydrogel for loading minocycline. In wound healing test, this
minocycline-loaded PVA-chitosan hydrogel showed faster healing of the wound made in rat dorsum than the conventional product or the control (sterile gauze) due to antifungal activity of chitosan. In particular, from the histological examination, the healing effect of minocycline-loaded hydrogel was greater than that of the drug-loaded hydrogel [98]. Another study reported, in situ-formed hydrogel membrane through ultraviolet cross-linking of a photocross-linkable azidobenzoic hydroxypropyl chitosan aqueous solution. The hydrogel membrane was stable, flexible, and transparent, with a bulk network structure of smoothness, integrity, and density [99]. The hydrogel membrane also exhibited barrier function, as it was impermeable to bacteria but permeable to oxygen. In vitro experiments using two major skin cell types (dermal fibroblast and epidermal keratinocyte) revealed the hydrogel membrane have neither cytotoxicity nor an effect on cell proliferation. The wound dressing synthesized from carboxymethyl chitosan hydrogel (CM) and chitin-(polyacrylic acid) hydrogel (PAA) were examined for their dermal irritation potential response using the Draize test and obtained data showed no marked irritation on the skins in presence of the material tested [100].

Polyacrylic acid grafted chitin (Chitin-PAA) hydrogel was prepared and wound healing potential was evaluated [101]. The obtained data revealed that the wounds treated with the prepared hydrogel healed faster compared to lipido-colloid absorbent dressing and alginate wound dressing [102]. N, O-(carboxymethyl)chitosan (NOCC) was incorporated into the backbone of a collagen (COL) matrix without or with chondroitin sulfate (CS) or an acellular dermal matrix (ADM). The result of a cell migration study demonstrated that the migration of fibroblasts was significantly enhanced by NOCC in a concentration-dependent manner. Results of the in vivo wound healing study showed that matrixes incorporating NOCC showed markedly enhanced wound healing compared with the control [103, 104].

1.8.5 Wound dressings with nanocrystalline silver

Chang et al reported the incorporation of silver nanoparticles into chitosan hydrogel/2-glycerophosphate mixture to develop an antibacterial wound dressing [105]. The developed hydrogel showed bacterial growth inhibition against P. aeruginosa and S. aureus. The antibacterial activity was found dose dependent. The prepared dressing material showed cytotoxicity to HS68 cells (Human skin fibroblast) at concentrations of silver nanoparticles at 6
and 12 ppm. The dressing materials showed low cytotoxicity when the concentration of silver nanoparticles was less than 6 ppm.

Singh et al reported the evaluation of chitin membranes containing silver nanoparticles for use as an antimicrobial wound dressing [106]. Silver nanoparticles were synthesized by gamma irradiation in the presence of sodium alginate as stabilizer. The prepared silver nanoparticles were characterized by UV-Vis absorption spectra, XRD and Transmission electron microscopy (TEM). In vitro antimicrobial tests were assessed on P. aeruginosa and S. aureus to determine the antimicrobial efficiency of the chitin membranes containing 30, 50, 70 and 100 ppm nanosilver. No viable counts for P. aeruginosa were detected with 70 ppm silver nanoparticles dressing after 1h exposure. A 2-log CFU/ml reduction in viable cell count was observed for S. aureus after 1 h and a 4-log CFU/ml lessening after 6 h with 100 ppm nanosilver chitin membranes.

Thomas et al reported the development of chitosan/silver nanoparticle films by a simple photochemical method of reduction of silver ions in an acidic solution of AgNO3 and chitosan [107]. The presence of silver nanoparticles was confirmed from the transmission electron microscopy (TEM), X-ray diffraction (XRD) and thermo-gravimetric analysis (TGA) of the film. The surface plasmon resonance (SPR) obtained at 400 nm also confirmed the presence of nanosilver in the chitosan film. The developed chitosan-nanosilver films demonstrated tremendous antibacterial action against E. coli and Bacillus. These films can be used as antimicrobial packaging materials, as wound dressings and can also be used for various implants.

Lu et al reported the preparation of a wound dressing composed of nano-silver and chitosan [108]. Sterility and pyrogen testing assessed biosafety, and efficacy was evaluated using Sprague-Dawley rats with deep partial-thickness wounds. Silver sulfadiazine and chitosan film dressings were used as controls. At intervals wound areas were measured, wound tissues biopsied and blood samples taken. Compared with the controls, the silver nanocrystalline chitosan dressing significantly increased the rate of wound healing and was associated with silver levels in blood and tissues lower than levels associated with the silver sulfadiazine dressing. Sterility and pyrogen tests of the silver nanocrystalline chitosan dressing were negative. Thus this dressing can be used for wound dressing applications.
Madhumathi et al and Sudheesh et al reported the preparation and evaluation of silver nanoparticle incorporated α-chitin hydrogel and β-chitin hydrogel based composite scaffolds respectively [39, 109]. They evaluated the antibacterial potential of the prepared material and proved the excellent antibacterial potential of this material against S. aureus and E. coli. Apart from that, the same material showed enhanced hemostatic potential which would be beneficial for wound dressing applications.

1.8.6 nZnO

nZnO have been widely used for antibacterial and UV protective applications [110-117]. Previous studies had shown that in the size scale less than 100 nm and at the appropriate concentration nZnO possess potent antibacterial activity but has no adverse effect on normal cells [118, 119]. Further, the zinc ions released from ZnO can enhance keratinocyte migration towards the wound site and promote healing [120]. Hirto et al reported the preparation and antibacterial activity of nZnO [121]. The prepared nZnO showed sustainable antibacterial activity against E. coli. Nanocomposites consisting of genipin-crosslinked chitosan, poly(ethylene glycol), ZnO and silver nanoparticles were prepared and antibacterial activities of nanocomposite films were tested toward the bacterial species E. coli, P. aeruginosa, S. aureus, and B. subtilis [122]. The prepared nanocomposite film showed higher antibacterial activity compared to the control. Another study reported the preparation of electrospun mat composed of sodium alginate, poly(vinyl alcohol) and nano ZnO. They have evaluated the antibacterial potential of the prepared mats in vitro [123]. Tamar et al reported the preparation of nanocomposite composed of ZnO and iron oxide [124]. The prepared nanocomposite showed adequate colloidal aqueous stability and enhanced antibacterial activity against S. aureus and E. coli.

Salehi et al reported the preparation of a nanocomposite material containing nZnO and chitosan [125]. The prepared nanocomposite was capable of adsorbing dyes from colored solutions. Justin et al reported the reduced S. aureus growth on nZnO coated poly(vinyl chloride) film [126]. Live/dead bacteria assays provided images to confirm the reduced presence of active bacteria on samples with nZnO. Vyom et al, reported that sub-acute oral exposure to nZnO in mice leads to an accumulation of nanoparticles in the liver causing oxidative stress mediated DNA damage and apoptosis [127]. Shakeel et al reported the surface modification of nZnO with
concanavalin A. The prepared nanoparticles were capable for binding significant amount of enzyme [128]. Paszek et al reported the toxicity of nZnO on human umbilical vein endothelial cells above a concentration of 30 μg/ml [129]. nZnO were found to induce intracellular reactive oxygen species generation accompanied with the mitochondrial membrane potential reduction in human bronchial epithelial cells [130, 131]. Hackenberg et al reported that nZnO causing toxicity towards human keratinocyte as well as fibroblast cells [132]. nZnO caused toxicity towards human head and neck squamous cell carcinoma at a concentration of 2 μg/ml, but the same concentration was found non-toxic to normal cells [133].

Hackenberg et al recently reported that nZnO was able to kill human squamous carcinoma cells in vitro and the cytotoxicity was further increased when the nanoparticles were combined with paclitaxel or cisplatin [134]. Flow cytometry revealed a combination of apoptosis and necrosis. In another study, some researchers (Hackenberg at al) reported the toxicity of nZnO at a concentration above 50 μg/ml towards human nasal mucosa cells in vitro [135, 136]. Studies revealed the toxicity of nZnO towards human liver cells and rat neuronal cells in vitro [137, 138]. The decrease in cell viability and the mode of cell death induced by nZnO was apoptosis. They also induced DNA damage which was mediated by oxidative stress. Another study reported the toxicity of nZnO towards mouse alveolar macrophages and data showed that at a concentration above 10μg/ml nZnO caused toxicity and the cell death was due to apoptosis [139]. Akthar et al investigated the selective toxicity of nZnO towards human liver cancer cells whereas they did not impart any sign of toxicity on rat astrocytes and hepatocytes [140]. It has been reported that ZnO nanoparticle were toxic to mammalian cells and human keratinocytes [141, 142].

The potential of nZnO for making solar cells have been investigated by many researchers [143-148]. They reported the preparation of dye sensitized solar cell using nZnO. UV resistance of wooden material can be enhanced by coating nZnO on the wooden surfaces [149]. This was due to the photostability of nZnO.

Literature review of nZnO showed that not much work was done on biomedical applications of nZnO in vivo. The toxicity of any material will be different in in vivo conditions compared to the in vitro conditions. In the in vivo condition, 3 dimensional environments will play a significant role and other factors like electric stimulation, stress, presence of enzymes, etc
will also play crucial roles. So, we need to explore the potential of nZnO for wound healing as well as antimicrobial activity under *in vivo* conditions.

### 1.9 Thesis Scope and Objectives

Wound infection is the major difficulty in the field of wound care management, because such infections can cause exudate formation, delay the wound healing, facilitate improper collagen deposition, etc. Microbes are the major reason for infection and the prevalence of the same is high in and around us. The major infection causing bacteria were *S. aureus* and *E. coli*. Once enter it gains access into the body these microbes grow immediately and start to form colonies. The microbes can easily enter the body through the wounds and can reach into deeper portions of the tissue and, furthermore, can lead to internal infection. The remedy for the above-mentioned harms would be the use of wound dressing with antibacterial activity.

Kaltostat™ is the leading wound dressing bandage available in the market and it is made of alginate. Even though people are using this material since long it has drawbacks like adherence nature on to the wound, poor swelling ability and no antibacterial activity. Apart from Kaltostat, there are other dressings also available (Nu Derm™, Duoderm™, Tegaderm™, etc) and these dressings have de-merits like less flexibility, poor mechanical strength, lack of porosity, tendency of dressings to adhere onto the wound surface and majority of the dressings did not possess antibacterial activity. The adherence of wound dressing material on the wound surface cause the trauma to the healing wound when it is peeled off.

Previous studies have reported that chitosan films and membranes were used to treat patients with deep burns, orthopedic injuries, etc. The reasons for choosing chitosan was the ease of availability, hemostatic potential, biodegradability and the biodegradation product was N-acetyl glucosamine, which was already present in the human body and will enhance the re-epithelization. The so-called membranes and films have poor mechanical strength, less flexibility, poor wound healing potential and has no antibacterial activity.

Hydrogel-based wound dressings would be helpful to provide a better healing effect on these types of wounds. Hydrogel based wound dressing materials provide cooling sensation and a moisture environment, as well as act as a barrier to microbes at the wound surface. These materials were capable of absorbing large volume of wound exudate from the wound surface and hence reduce the chance for infection. Further, the hydrogel based materials would be helpful to
promote the cell adhesion and proliferation. Chitin and chitosan did not show antibacterial activity at neutral pH so, in order to impart antibacterial activity it is necessary to incorporate some antibacterial agents into it. ZnO nanoparticle is one of the most widely used engineered nano materials in commercial products due to its UV light absorption, antimicrobial, catalytic, semi-conducting, and magnetic properties. ZnO nanoparticle is, therefore, widely applied to personal care products, sunscreen, paints, electronic materials, rubber manufacture, food additives, and medicine. So, we selected nZnO for this purpose owing to its antibacterial activity as well as wound healing enhancing ability by migrating keratinocytes cells towards the wound site. In this work, we developed, characterized and evaluated in vitro and in vivo, the wound healing potential of nZnO incorporated chitosan, α-chitin and β-chitin hydrogel based nanocomposite bandages.

1.10 Objectives of the study
1. To synthesize and characterize nZnO, chitosan, α- chitin and β-chitin hydrogel.

2. To develop and characterize nZnO incorporated chitosan, α-chitin and β-chitin hydrogel nanocomposite bandages.

3. To evaluate the porosity, swelling capacity, biodegradation, hemostatic potential, antimicrobial activity and cytocompatibility of the prepared composite bandages under in vitro conditions.

4. To evaluate the human dermal fibroblast cell viability, attachment, proliferation and infiltration on the composite bandages.

5. To evaluate the in vivo excisional wound healing potential of the β-chitin hydrogel/nZnO and chitosan hydrogel/nZnO composite bandages in animal model (Sprague-Dawley rats).

6. To evaluate the in vivo antibacterial potential of the β-chitin hydrogel/nZnO and chitosan hydrogel/nZnO composite bandages at the wound site in Sprague-Dawley rats.
1.11 Research Questions

1. How can chitinous hydrogel be developed into a wound dressing bandage?

2. What would be the effect of incorporating nZnO into the chitinous hydrogel bandages?

3. How does the nZnO incorporation into the chitinous bandages affect its cytocompatibility towards normal cells?

4. How do the chitinous hydrogel/nZnO composite bandages enhance wound healing when compared to the Kaltostat™ when assessed *in vivo*?

1.12 Research Hypothesis

1. Chitinous hydrogel by lyophilisation technique can be developed into a flexible, porous bandage with adequate swelling, and blood clotting abilities making it suitable for wound dressing applications.

2. The small proportion of nZnO, when added to chitinous hydrogel will not alter its structural and functional properties. nZnO being a proven antimicrobial agent its incorporation into the chitinous hydrogel bandages will significantly reduce microbial growth.

3. The concentration of nZnO used in the bandages will be in a range that does not minimize the cytocompatibility towards normal cells.

4. Chitinous hydrogel/nZnO composite bandage will enhance the wound healing by promoting re-epithelialisation and collagen deposition compared to Kaltostat™ under *in vivo* conditions.