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Novel Chitin and Chitosan Materials in Wound Dressing

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1. Introduction

Chitin is the second most abundant natural polysaccharide after cellulose on earth. It is a high molecular weight linear homopolymer of β-(1, 4) linked N-acetylglucosamine (N-acetyl-2-amino-2-deoxy-D-glucopyranose) units. Chitosan, a copolymer of glucosamine and N-acetyl glucosamine units linked by 1-4 glucosidic bonds, is a cationic polysaccharide obtained by alkaline deacetylation of chitin. The role of chitin and chitosan as biomaterials are amazing as evidenced by the published scientific papers and patents. Chitin and chitosan are attracting increasingly more attention recently due to its biological and physicochemical characteristics. Chitin and chitosan with beneficial biological and antimicrobial properties and high valuable potential for wound healing are attractive for wound care. Healing restores integrity of the injured tissue and prevents organisms from deregulation of homeostasis. The treatment of the wounds has evolved from the ancient times. Initially, application of dressing material was aimed at inhibition of bleeding, protection of the wound from environmental irritants as well as water and electrolyte disturbances. Skin plays an important role in homeostasis and the prevention of invasion by microorganisms. Skin generally needs to be covered with a dressing immediately after it was damaged. At present, there are three categories of wound dressing: biologic, synthetic and biologic-synthetic. Alloskin and pigskin are biologic dressings commonly used clinically, but they have some disadvantages, such as limited supplies, high antigenicity, poor adhesiveness and risk of cross contamination. Synthetic dressings have long shelf life, induce minimal inflammatory reaction and carry almost no risk of pathogen transmission. In recent years, researchers have focused on biologic-synthetic dressings (Bruin et al., 1990; Suzuki et al., 1990), which are bilayered and consist of high polymer and biologic materials. These three categories of wound dressing are all used frequently in the clinical setting, but none is without disadvantages. An ideal dressing should maintain a moist environment at the wound interface, allow gaseous exchange, act as a barrier to microorganisms and remove excess exudates. It should also be non-toxic, non-allergenic, nonadherent and easily

removed without trauma, and it should be made from a readily available biomaterial that requires minimal processing, possesses antimicrobial properties and promotes wound healing. In recent years, a large number of research groups are dedicated to produce a new, improved wound dressing by synthesizing and modifying biocompatible materials (Shibata et al., 1997; Draye et al., 1998; Ulubayram et al., 2001).

Recent reports are also aiming on the acceleration of the wound repair by systematically designed dressing materials. In particular, efforts ware focused on the use of biologically derived materials such as, chitin and its derivatives, which are capable of accelerating the healing processes at molecular, cellular, and systemic levels. Chitin and its derivative, chitosan, are biocompatible, biodegradable, nontoxic, anti-microbial and hydrating agents. Due to these properties, they show good biocompatibility and positive effects on wound healing. Previous studies have shown that chitin-based dressings can accelerate the repair of different tissues and facilitates contraction of wounds and regulates secretion of the inflammatory mediators such as interleukin 8, prostaglandin E, interleukin 1 β, and others. Chitosan provides a non-protein matrix for 3D tissue growth and activates macrophages for tumoricidal activity. It stimulates cell proliferation and histoarchitectural tissue organization. Chitosan is a hemostat, which helps in natural blood clotting and blocks nerve endings and hence reducing pain. Chitosan 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. It helps in faster wound healing and scar prevention (Paul & Sharma, 2004). The advantage of chitin and chitosan is easily can processed into hydrogels (Nagahama et al., 2008a; Nagahama et al., 2008b; Tamura et al., 2010), membranes (Yosof, Wee, Lim & Khor, 2003; Marreco et al., 2004; Jayakumar et al., 2007; Jayakumar et al., 2008, Jayakumar et al., 2009; Madhumathi et al., 2009), nanofibers (Shalumon et al., 2009; Shalumon et al., 2010; Jayakumar et al., 2010), beads (Yosof, Lim & Khor, 2001; Jayakumar et al., 2006), micro/nanoparticles (Prabaharan & Mano, 2005; Prabaharan, 2008; Anitha et al., 2009; Anitha et al., 2010; Dev et al., 2010), scaffolds (Peter et al., 2009; Peter et al., 2010; Prabaharan & Jayakumar, 2009; Maeda et al., 2008) and sponges (Muramatsu, Masuda, Yoshihara & Fujisawa, 2003; Portero, 2007) for various types of biomedical applications such as drug and gene delivery (Prabaharan & Mano, 2005; Jayakumar et al., 2010a), wound healing (Jayakumar et al., 2005; Jayakumar et al., 2007; Jayakumar et al., 2010b; Jayakumar et al., 2010c; Tamura et al., 2010) and tissue engineering (Jayakumar et al., 2005; Jayakumar et al., 2010d; Tamura et al., 2010). Various forms of wound dressings materials based on chitin and chitosan derivatives are commercially available. The ordered regeneration of wounded tissues requires the use of chitin and chitosan in the form of non-wovens, nanofibrils, composites, films, scaffolds and sponges. So far a number of research works have been published on chitin and chitosan as wound dressing materials. However, only a few review articles have been reported about chitin and chitosan-based wound dressings with limited information (Ueno, Mori & Fujinaga, 2001; Ravi Kumar, 2000; Kim et al., 2008; Muzzarelli, 2009; Tamura et al., 2010). In this paper, we reviewed a recent development and applications on wound dressing materials based on chitin, chitosan and their derivatives.

2. Applications of chitin and chitosan materials in wound dressing

Chitin and chitosan have an accelerating effect on the wound healing process. A number of studies have demonstrated that chitin and chitosan accelerated wound healing. Chitin and

chitosan have been used as nanofibers, gels, scaffolds, membranes, filaments, powders, granules, sponges or as a composite. The main biochemical activities of chitin and chitosan-based materials in wound healing are polymorphonuclear cell activation, fibroblast activation, cytokine production, gaint cell migration and simulation of type IV collagen synthesis (Mezzana, 2008). Nanofiber matrices have shown tremendous promise as tissue engineering scaffolds for skin substitutes. The advantages of a scaffold composed of ultrafine, continuous fibers are oxygen-permeable high porosity, variable pore-size distribution, high surface to volume ratio and most importantly, morphological similarity to natural extracellular matrix (ECM) in skin, which promote cell adhesion migration and proliferation. Recent advances in process chemistry have made it possible to make chitin and chitosan nanofibril materials with more flexibility and useful for the development of new bio-related products (Mattioli-Belmonte et al., 2007). Dibutyrylchitin (DBC) is a water-soluble chitin derivative with confirmed biological properties. DBC is obtained in the reaction of shrimp chitin with butyric anhydride, under heterogeneous condition, in which perchloric acid was used as a catalyst. Recently, DBC fibrous materials were used for wound healing applications (Chilarski et al., 2007). In this study, DBC non-woven fabrics after γ-sterilisation were applied to a group of nine patients with different indications. Satisfactory results of wound healing were achieved in most cases, especially in cases of burn wounds and postoperative/posttraumatic wounds and various other conditions causing skin/epidermis loss (Chilarski et al., 2007). The effects of DBC on the repair processes and its mechanisms of action were studied by Blasinka & Drobnik (2007). The results showed that DBC implanted subcutaneous to the rats increased weight of the granulation tissue. Increased cell number isolated from the wound and cultured on the DBC films was also revealed. DBC elevates the glycosaminoglycans (GAG) level in the granulation tissue. This study documents the beneficial influence of DBC on the repair, which could be explained by the modification of the extracellular matrix and cell number (Blasinka & Drobnik, 2007). The effectiveness of three chitin nanofibril/chitosan glycolate-based preparations, a spray (Chit-A), a gel (Chit-B), and a gauze (Chit-C), in healing cutaneous lesions was assessed macroscopically and by light microscopy immunohistochemistry (Mattioli-Belmonte et al., 2007). These evaluations were compared to the results obtained using a laser co-treatment. The wound repair provided by these preparations are clearly evident even without the synergistic effect of the laser co-treatment. These results confirmed the effectiveness of chitin nanofibril/chitosan glycolate-based products in restoring subcutaneous architecture.

A biocompatible carboxyethyl chitosan/poly(vinyl alcohol) (CECS/PVA) nanofibers were prepared by electrospinning of aqueous CECS/PVA solution (Zhou et al., 2008) as wound dressing material. The potential use of the CECS/PVA electrospun fiber mats as scaffolding materials for skin regeneration was evaluated *in vitro* using mouse fibroblasts (L929) as reference cell line. Indirect cytotoxicity assessment of the fiber mats indicated that the CECS/PVA electrospun mat was non-toxic to the L929 cell. Cell culture results showed that fibrous mats were good in promoting the L929 cell attachment and proliferation (Zhou et al., 2008). This novel electrospun matrix would be used as potential wound dressing for skin regeneration. It is known that chitosan derivatives with quaternary ammonium groups possess high efficacy against bacteria and fungi. It is now widely accepted that the target site of these cationic polymers is the cytoplasmic membrane of bacterial cells (Tashiro, 2001). The photo cross-linked electrospun mats containing quaternary chitosan (QCS) were efficient in inhibiting growth of Gram-positive bacteria and Gram-negative bacteria (Ignatove et al., 2007). These results suggested that the cross-linked QCS/PVP electrospun

mats are promising materials for wound-dressing applications. Similarly, the photo-cross-linked electrospun nano-fibrous QCS/PVA mats had a good bactericidal activity against the Gram-negative bacteria *E. coli* and Gram-positive bacteria *S. aureus* (Ignatove et al., 2006). These characteristic features of the electrospun mats reveal their high potential for wound-dressing applications. A remarkable wet spun alginate composite containing 0.15-2.0% chitin nanofibrils was also characterized in view of its use as a wound dressing material (Turner et al., 1986; Watthanaphanit, Supaphol, Tamura, Tokura, & Rujiravanit, 2008). The result showed that the overall susceptibility to lysozyme was improved by the tiny amounts of chitin nanofibrils. Moreover, the release of chitin oligomers as a consequence of the enzymatic hydrolysis is a significant contribution to the efficacy of the calcium-alginate dressings. The best biomaterials for wound dressing should be biocompatible and promote the growth of dermis and epidermis layers. Chen et al. (2008) reported composite nanofibrous membrane of chitosan/collagen, which are known for their beneficial effects on wound healing. The membrane was found to promote wound healing and induce cell migration and proliferation. From animal studies, the nanofibrous membrane was found to be better than gauze and commercial collagen sponge in wound healing.

A wound dressing system with high liquid absorbing, biocompatibility, and antibacterial properties was designed based on chitosan/collagen (Wang, Su & Chen, 2008). Various solution weight ratios of collagen to chitosan were used to immobilize on the polypropylene nonwoven fabric, which were pre-grafted with acrylic acid (AA) or *N*-isopropyl acrylamide (NIPAAm) to construct a durable sandwich wound dressing membrane with high water absorbing, easy removal, and antibacterial activity. Swelling properties and antibacterial activity of the membranes were measured, and wound healing enhancement by skin full-thickness excision on animal model was examined. The results indicated that NIPAAm-grafted and collagen/chitosan-immobilized polypropylene nonwoven fabric (PP-NIPAAm-collagen-chitosan) showed a better healing effect than AA-grafted and collagen/chitosanimmobilized polypropylene nonwoven fabric (PP-AA-collagen-chitosan). The wound treated with PP-NIPAAm-collagen-chitosan demonstrated the excellent remodeling effect in histological examination with respect to the construction of vein, epidermis, and dermis at 21 days after skin injury. The values of water uptake and water diffusion coefficient for PP-NIPAAm-collagen-chitosan were higher than that for PP-AA-collagen-chitosan under a given solution weight ratio of collagen/chitosan. Both PP-NIPAAm-collagen-chitosan and PP-AA-collagen-chitosan demonstrated antibacterial activity (Wang, Su & Chen, 2008). A novel genipin cross-linked chitosan film, was prepared as a wound dressing material (Liu, Yao & Fang, 2008). This study examined the *in vitro* properties of the genipin-cross-linked chitosan film and the bi-layer composite. Furthermore, *in vivo* experiments were conducted to study wounds treated with the composite in a rat model. Experimental results showed that the degree of cross-linking and the *in vitro* degradation rate of the genipin-cross-linked chitosan films can be controlled by varying the genipin contents. In addition, the genipin contents should exceed 0.025 wt% of the chitosan-based material if complete cross-linking reactions between genipin and chitosan molecules are required. Water contact angle analysis shows that the genipin-cross-linked chitosan film is not highly hydrophilic; therefore, the genipin-cross-linked chitosan layer is not entangled with the soybean protein non-woven fabric, which forms an easily stripped interface layer between them. Furthermore, this wound dressing material provides adequate moisture, thereby minimizing the risk of wound dehydration and exhibits good mechanical properties. The in vivo histological assessment results revealed that

epithelialization and reconstruction of the wound are achieved by covering the wound with the composite, and the composite is easily stripped from the wound surface without damaging newly regenerated tissue (Liu, Yao & Fang, 2008).

Chitin and chitosan hydrogels are also used as wound dressing materials. Water-soluble chitin hydrogel was prepared with the desired deacetylation degree of 0.50 and molecular weight of 800 kDa as wound dressing material. The resulting hydrogel was found to be more susceptible to the action of lysozyme than chitosan. Full-thickness skin incisions were made on the backs of rats and then chitin, chitosan, chitin powders and the chitin hydrogel were embedded in the wounds. The chitin powder was found to be more efficient than chitin or chitosan as a wound healing accelerator: the wounds treated with chitin hydrogel were completely re-epithelialized, granulation tissues were nearly replaced by fibrosis and hair follicles were almost healed with in 7 days after initial wounding. Also, the chitin hydrogel treated skin had the highest tensile strength and the arrangement of collagen fibers in the skin was similar to normal skins. The chitin hydrogel was considered to be a suitable wound-healing agent due to its easy application and high effectiveness. It is likely that the superior enzymatic degradability and hydrophilicity of water-soluble chitin enhances its activity as a wound-healing accelerator (Cho et al., 1999). Topical formulations based on water soluble chitin were prepared and their effects on wound healing were evaluated on a rabbit ear model (Han, 2005). Full-thickness, open skins wound were made on the ears of rabbits and water soluble chitin ointments were embedded in the open wound. The application of water soluble chitin ointments significantly accelerated wound healing and wound contraction. The areas of epithelialization and granulation tissues in water soluble chitin ointment group were found to be remarkably larger than those in control group (no treatment) and in placebo group (treated with ointment-base materials). A large number of grown granulation tissues including dense fibroblast deposition were observed under the thickened epithelium of the wound treated with water soluble chitin ointments. The number of inflammatory cells in water soluble chitin ointment group was significantly decreased compared with those in control and placebo groups, indicating that water soluble chitin would give low stimuli to wounds and prevent excessive scar formation. Overall results demonstrated that the topical formulation based on water soluble chitin is considered to become an excellent dressing as a wound-healing assistant (Han, 2005).

Pietramaggiori et al. (2008) demonstrated that treatment of full-thickness cutaneous wounds in a diabetic mouse model with chitin-containing membranes results in an increased wound closure rate correlated with impressive rise of angiogenesis. Serum starved endothelial cells were treated with vascular endothelial growth factor (VEGF) or with different concentrations of chitin. As compared with the total number of cells plated (control), at 48 h after serum starvation, there was a twofold reduction of the number of cells, but this reduction was compensated upon addition of VEGF or chitin at either 5 or 10 mg/ml. These results indicate that like VEGF, chitin treatment prevents cell death induced by serum deprivation. However, chitin does not result in a higher metabolic rate (by MTT assays), suggesting that this polymeric material is not causing marked increases in cellular proliferation but is rescuing cells from dying by serum deprivation. To overcome current limitations in wound dressings for treating mustard-burn induced septic wound injuries, a non-adherent wound dressing with sustained anti-microbial capability has been developed (Loke et al., 2000). The wound dressing consists of two layers: the upper layer is a carboxymethyl chitin hydrogel material, while the lower layer is an anti-microbial impregnated biomaterial. The hydrogel layer acts as a mechanical and microbial barrier, and

is capable of absorbing wound exudate. In physiological fluid, the carboxymethylated chitin hydrogel swells considerably, imbibing up to 4 times its own weight of water and is also highly porous to water vapor. The moisture permeability of the dressing prevents the accumulation of fluid in heavily exudating wounds seen in second-degree burns. The lower layer, fabricated from chitosan acetate foam, is impregnated with chlorhexidine gluconate. From the in vitro release studies, the loading concentration was optimized to deliver sufficient anti-microbial drug into the wound area to sustain the anti-microbial activity for 24 h (Loke et al., 2000).

β-Chitin grafted poly(acrylic acid) (PAA) was prepared with the aim of obtaining a hydrogel suitable for wound dressing application. In this study, acrylic acid was first linked to chitin, via ester bonds between the chitin primary alcohol groups and the carboxyl groups of acrylic acid, as the active grafted moiety that was further polymerized upon addition of an initiator to form a network. The chitin-PAA films were synthesized at various acrylic acid contents: the degree of swelling of the chitin-PAA films was in the range of 30-60 times of their original weights depending upon the monomer feed content. The chitin-PAA film with 1:4 weight ratio of chitin: acrylate, possessed optimal physical properties. The cytocompatibility of the film was tested with L929 mouse fibroblasts that proliferated and adhered well onto the film. The morphology and behavior of the cells on the chitin-PAA film were found to be normal after 14 days of culture (Tanodekaew et al., 2004).

Skin repair is an important field of the tissue engineering, especially in the case of extended third-degree burns, where the current treatments are still insufficient in promoting satisfying skin regeneration. Bio-inspired bi-layered physical hydrogels only constituted of chitosan and water were processed and applied to the treatment of full-thickness burn injuries (Boucard et al., 2007). A first layer constituted of a rigid protective gel ensured good mechanical properties and gas exchanges. A second soft and flexible layer allowed the material to follow the geometry of the wound and ensured a good superficial contact. To compare, highly viscous solutions of chitosan were also considered. Veterinary experiments were performed on pig's skins and biopsies at days 9, 17, 22, 100 and 293, were analyzed by histology and immuno-histochemistry. Only one chitosan material was used for each time. All the results showed that chitosan materials were well tolerated and promoted a good tissue regeneration. They induced inflammatory cells migration and angiogenetic activity favouring a high vascularisation of the neo-tissue. At day 22, type I and IV collagens were synthesised under the granulation tissue and the formation of the dermal-epidermal junction was observed. After 100 days, the new tissue was quite similar to a native skin, especially by its aesthetic aspect and its great flexibility (Boucard et al., 2007). Ribeiro et al. (2009) developed chitosan hydrogel for wound dressing. In this study, fibroblast cells isolated from rat skin were used to assess the cytotoxicity of the hydrogel. The results showed that chitosan hydrogel was able to promote cell adhesion and proliferation. Cell viability studies showed that the hydrogel and its degradation by-products are non-cytotoxic. The evaluation of the applicability of chitosan in the treatment of dermal burns in Wistar rats was performed by induction of full-thickness transcutaneous dermal wounds. From macroscopic analysis, the wound beds of the animals treated with chitosan were considerably smaller than those of the controls. Histological analysis revealed lack of a reactive or a granulomatous inflammatory reaction in skin lesions with chitosan and the absence of pathological abnormalities in the organs obtained by necropsy, which supported the local and systemic histocompatibility of the biomaterial. This study suggested that chitosan hydrogel may aid the re-establishment of skin architecture (Ribeiro et al., 2009).

Application of ultraviolet light irradiation to a photocrosslinkable chitosan aqueous solution resulted in an insoluble and flexible hydrogel (Ishihara et al., 2001; 2002). In order to evaluate its accelerating effect on wound healing, full-thickness skin incisions were made on the backs of mice and subsequently a photocross-linkable chitosan aqueous solution was added into the wound and irradiated with ultraviolet light for 90 seconds. Application of the chitosan hydrogel significantly induced wound contraction and accelerated wound closure and healing compared with the untreated controls. Histological examination showed an advanced contraction rate on the first 2 days and tissue fill rate on days 2 to 4 in the chitosan hydrogel-treated wounds. Furthermore, in cell culture studies, chitosan hydrogel culture medium supplemented with 5% fetal-bovine serum was found to be chemo attractant for human dermal fibroblasts in an invasion chamber assay using filters coated with Matrigel and in a cell migration assay. Due to its ability to accelerate wound contraction and healing, chitosan hydrogel may become accepted as an occlusive dressing for wound management (Ishihara et al., 2001; 2002).

For effective wound healing accelerator, water-soluble chitosan/heparin complex was prepared using water-soluble chitosan with wound healing ability and heparin with ability to attract or bind growth factor related to wound healing process (Kweon, Song & Park, 2003). To study the wound healing effect, full thickness skin excision was performed on the backs of the rat and then water-soluble chitosan and water-soluble chitosan/heparin complex ointments were applied in the wound, respectively. After 15 days, gross and histologic examination was performed. Grossly, untreated control group revealed that the wound had well defined margin and was covered by crust. The second group treated with water-soluble chitosan ointment revealed small wound size with less amount of covering crust and ill-defined margin, which appeared to regenerate from margin. The third group treated with water-soluble chitosan/heparin complex ointment appeared to be nearly completely healed. The third group (water-soluble chitosan/heparin) showed nearly complete regeneration of appendage structure similar to normal in the dermis in contrast to control and second group with absence and less number of skin appendages, respectively (Kweon, Song & Park, 2003). For rapid wound healing, a hydrogel sheet composed of a blended powder of alginate, chitin/chitosan and fucoidan (ACF-HS; 60:20:2:4 w/w) has been developed as a functional wound dressing (Murakami et al., 2010). On application, ACF-HS was expected to effectively interact with and protect the wound in rats, providing a good moist healing environment with exudates. In addition, the wound dressing has properties such as ease of application and removal and good adherence. In this work, full-thickness skin defects were made on the backs of rats and mitomycin C solution (1 mg/ml in saline) was applied onto the wound for 10 min in order to prepare healing-impaired wounds. After thoroughly washing out the mitomycin C, ACF-HS was applied to the healing-impaired wounds. Although normal rat wound repair was not stimulated by the application of ACF-HS, healing-impaired wound repair was significantly stimulated. Histological examination demonstrated significantly advanced granulation tissue and capillary formation in the healing-impaired wounds treated with ACF-HS on day 7, as compared to those treated with calcium alginate fiber (Kaltostat; Convatec Ltd., Tokyo, Japan) and those left untreated (Murakami et al., 2010).

PVA, water-soluble chitosan and glycerol based hydrogel was made by irradiation followed by freeze-thawing was evaluated as wound dressing (Yang et al., 2010). MTT assay suggested that the extract of hydrogels was nontoxic towards L929 mouse fibroblasts. Compared to gauze dressing, the hydrogel based on PVA, water-soluble chitosan and

glycerol can accelerate the healing process of full-thickness wounds in a rat model. Wounds treated with hydrogel healed at 11th day postoperatively and histological observation showed that mature epidermal architecture was formed. These results indicate that it is a good wound dressing material (Yang et al., 2010). Sung et al. (2010) developed minocycline-loaded wound dressing with an enhanced healing effect. The cross-linked hydrogel films were prepared with PVA and chitosan using the freeze-drying method. Their gel properties, *in vitro* protein adsorption, release, *in vivo* wound healing effect and histopathology were then evaluated. Chitosan decreased the gel fraction, maximum strength and thermal stability of PVA hydrogel, while it increased the swelling ability, water vapour transmission rate, elasticity and porosity of PVA hydrogel. Incorporation of minocycline did not affect the gel properties, and chitosan hardly affected drug release and protein adsorption. Furthermore, the minocycline-loaded wound dressing composed of 5% PVA, 0.75% chitosan and 0.25% drug was more swellable, flexible and elastic than PVA alone because of relatively weak cross-linking interaction of chitosan with PVA. 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, indicating the potential healing effect of minocycline. Thus, the minocycline-loaded wound dressing composed of 5% PVA, 0.75% chitosan and 0.25% drug is a potential wound dressing with excellent forming and enhanced wound healing (Sung et al., 2010).

Hydrophilic biopolymeric membranes having a high swellability and permeability for water vapor and gases, good fluid transport via the membrane, and a high selectivity for the transport of polar substances. These properties in combination with an adequate mechanical strength make them highly desirable for the treatment of wounds as a coverage material. Flexibility, softness, transparency and conformability permit to use chitin films as occlusive, semi-permeable wound dressings. The chitin films are generally non-absorbent, exhibiting a total weight gain of only 120-160% in physiological fluid. Dry chitin films transpire water vapor at a rate of about 600 g/m^2/24 h, (similar to commercial polyurethane-based film dressings), that rises to 2400 g/m^2/24 h when wet (higher than the water vapor transmission rate of intact skin): the chitin films are non-toxic to human skin fibroblasts, maintaining 70-80% cell viability. Wound studies using a rat model showed no signs of allergenicity or inflammatory response. The chitin films displayed accelerated wound healing properties. Wound sites dressed with the chitin films healed faster and appeared stronger than those dressed with Opsite and gauze (Yusof, Wee, Lim, & Khor, 2003). Chitin accelerates macrophage migration and fibroblast proliferation, and promotes granulation and vascularization. While some chitin and chitosan derivatives have biochemical significance, some other is rather inert, as it is the case for dibutyryl chitin; in general, however, they are biocompatible. The high biocompatibility of dibutyryl chitin in the form of films and non-wovens has been demonstrated for human, chick and mouse fibroblasts by various methods: this water-insoluble modified chitin was also tested in full-thickness wounds in rats with good results (Muzzarelli et al., 2005). Traumatic wounds in a large number of patients were treated with chitosan glycolate dressings; in all cases they healed with satisfactory results (Muzzarelli et al., 2007).

Asymmetric chitosan membrane has been prepared by immersion precipitation phase-inversion method and evaluated as wound covering material (Mi et al., 2001). The top layer which contains skin surface and interconnected micropores was designed to prevent

bacterial penetration and dehydration of the wound surface but allows the drainage of wound exudate. The sponge-like sublayer was designed to achieve high adsorption capacity for fluids, drainage of the wound by capillary and enhancement of tissue regeneration. The thickness of the dense skin surface and porosity of sponge-like sublayer was controlled by the modification of phase-separation process using per-evaporation method. The asymmetric chitosan membrane showed controlled evaporative water loss, excellent oxygen permeability and promoted fluid drainage ability. Moreover, this material inhibited exogenous microorganisms invasion due to the dense skin layer and inherent antimicrobial property of chitosan. Wound covered with the asymmetric chitosan membrane was hemostatic and healed quickly. Histological examination confirmed that epithelialization rate was increased and the deposition of collagen in the dermis was well organized by covering the wound with this asymmetric chitosan membrane. The results in this study indicate that the asymmetric chitosan membrane could be adequately employed in the future as a wound dressing material (Mi et al., 2001). Chitosan membranes have been tested as wound dressing at the skin-graft donor site in patients (Azad et al., 2004). Bactigras, a commonly used impregnated tulle gras bandage, served as a control. Chitosan membrane, prepared with a 75% degree of deacetylation and a thickness of 10 μm, was used in non-mesh or mesh form. The progress in wound healing was compared by clinical and histological examination. Itching and pain sensitivity of the wound dressed area was scored with the use of a visual analogue scale. Mesh chitosan membrane in contrast to the nonmesh membrane allowed blood to ooze into the surrounding gauze. After 10 days, the chitosan-dressed area had been healed more promptly as compared with the Bactigras dressed area. Moreover, the chitosan mesh membrane showed a positive effect on the re-epithelialization and the regeneration of the granular layer. The data confirm that chitosan mesh membrane is a potential substitute for human wound dressing (Azad et al., 2004).

Fibroblast growth factor (bFGF) has been shown to stimulate wound healing (Mizuno et al., 2003). However, consistent delivery of bFGF has been problematic. Mizuno et al. (2003) studied the stability of bFGF incorporated into a chitosan film as a delivery vehicle for providing sustained release of bFGF. The therapeutic effect of this system on wound healing in genetically diabetic mice was determined as a model for treating clinically impaired wound healing. A chitosan film was prepared by freeze-drying hydroxypropyl chitosan in acetate buffer solution. Growth factor was incorporated into films before drying by mixing bFGF solution with the hydroxypropyl chitosan solution. bFGF activity remained stable for 21 days at 5 °C, and 86.2% of activity remained with storage at 25 °C. Full-thickness wound 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 was smaller 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. These results suggest that chitosan itself facilitates wound repair and that bFGF incorporated into chitosan film is a stabile delivery vehicle for accelerating wound healing (Mizuno et al., 2003).

Surface modification of biomaterials is another way to tailor cell responses whilst retaining the bulk properties. Silva et al. (2008) prepared chitosan membranes by solvent casting and treated with nitrogen or argon plasma at 20W for 10-40 min. Atomic Force Microscopy analysis (AFM) indicated an increase in the surface roughness as a result of the etching process. X-ray photoelectron spectroscopy (XPS) and contact angle measurements showed different surface elemental compositions and higher surface free energy on the surface

modified chitosan membrane. The MTS test and direct contact assays with an L929 fibroblast cell line indicated that the plasma treatment improved the cell adhesion and proliferation. Overall, the results demonstrated that such plasma treatments could significantly improve the biocompatibility of chitosan membranes and thus improve their potential in wound dressings and tissue engineering applications (Silva et al., 2008).

HemCon® bandage is an engineered chitosan acetate preparation designed as a hemostatic dressing, and is under investigation as a topical antimicrobial dressing (Burkatovskaya et al., 2008). The conflicting clamping and stimulating effects of chitosan acetate bandage on normal wounds were studied by removing the bandage from wounds at times after application ranging from 1 hour to 9 days. The results showed that three days application gave the earliest wound closure, and all application times gave a faster healing slope after removal compared with control wounds. Chitosan acetate bandage reduced the number of inflammatory cells in the wound at days 2 and 4, and had an overall beneficial effect on wound healing especially during the early period where its antimicrobial effect is most important (Burkatovskaya et al., 2008). The hydrophilic polymer membranes based on macromolecular chitosan networks have been synthesized and characterized (Clasen, Wilhelms & Kulicke, 2006). The structure of the membrane has been altered in several ways during the formation to adjust the properties, particularly with regard to the elasticity, tensile strength, permeability and surface structure. An alteration of the network structure was achieved by addition of flexibilizer, cross-linking with dialdehydes, simplex formation of the chitosan with the polyanion sulfoethyl cellulose, and the introduction of artificial pores on the micro- and nanometer scale into the chitosan matrix with silica particles or poly(ethylene glycol) (PEG). In this study, the impact of the network structures on physical properties of the membranes, the water vapor and gas permeability and the tensile strength was reported to evaluate possible application of the membranes as a wet wound dressing material with microbial barrier function that actively assists the healing process of problematic wounds (Clasen, Wilhelms & Kulicke, 2006).

Chitosan derivative sheets and pastes were evaluated *in vitro* for possible utilization in wound dressing applications (Rasad et al., 2010). In this study, the cytotoxicity of oligo chitosan, *N, O*-carboxymethyl-chitosan (*N, O*-CMC) and *N*-carboxymethyl-chitosan (*N*-CMC) derivatives in sheet like and paste forms were evaluated using primary normal human dermal fibroblast cultures and hypertrophic scars; a fibrotic conditions representing a model of altered wound healing with overproduction of extracellular matrix and fibroblast hyperproliferative activity. Cytotoxicities of these chitosan derivatives were assessed using MTT assay. The results indicated that both chitosan derivative sheets and pastes have appropriate cytocompatibility and appear promising as safe biomaterials with potential wound healing applications. *N, O*-CMC sheet exhibited highest cytocompatibility property and may be regulated by matrix metalloproteinase-13 (MMP-13) in controlling the cell growth and its expression level (Rasad et al., 2010).

In situ photopolymerized hydrogel dressings create minimally invasive methods that offer advantages over the use of preformed dressings such as conformability in any wound bed, convenience of application and improved patient compliance and comfort. An in situ-hydrogel membrane was prepared through ultraviolet cross-linking of a photocross-linkable azidobenzoic hydroxypropyl chitosan aqueous solution (Lu et al., 2010). The prepared hydrogel membrane is stable, flexible, and transparent, with a bulk network structure of smoothness, integrity, and density. 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 in situ photocross-linked azidobenzoic hydroxypropyl chitosan hydrogel membrane has a great potential in the management of wound healing and skin burn (Lu et al., 2010). A wound dressings film composed of chitosan and minocycline hydrochloride was prepared using commercial polyurethane film (Tegaderm) as a backing (Aoyagi, Onishi & Machida, 2007). Various formulations were applied to severe burn wounds in rats in the early stage, and the wound status and change in the wound surface area were examined. The use of 10 mg of minocycline hydrochloride and complete sealing with Tegaderm had a negative effect. Minocycline hydrochloride ointment was not effective, but Geben cream was fairly effective. However, chitosan (83% degree of deacetylation) with a cutting of Tegaderm film containing 2mg of minocycline hydrochloride and chitosan (83% degree of deacetylation) films showed an excellent effect (Aoyagi, Onishi & Machida, 2007). To accelerate wound healing by stimulating the recruitment of fibroblasts and improve the mechanical properties of collagen matrixes, *N*, *O*-CMC was incorporated into the backbone of a collagen matrix without or with chondroitin sulfate or an acellular dermal matrix (Chen et al., 2006). The result of a cell migration study demonstrated that the migration of fibroblasts was significantly enhanced by *N*, *O*-CMC in a concentration-dependent manner. In the analysis with a dynamic mechanical analyzer, *N*, *O*-CMC-chondroitin sulfate/collagen matrixes presented higher tensile strengths than *N*, *O*-CMC/acellular dermal matrix/collagen matrixes. Skin fibroblasts cultured on the matrixes containing did *N*, *O*-CMC showed increased proliferation and secretion of three kinds of cytokines compared with the control. Results of the in vivo wound healing study showed that matrixes incorporating *N*, *O*-CMC showed markedly enhanced wound healing compared with the control. These results clearly suggest that *N*, *O*-CMC/collagen matrixes containing chondroitin sulfate or acellular dermal matrix can be used as potential wound dressings for clinical applications (Chen et al., 2006).

Biocompatible chitosan/polyethylene glycol diacrylate (PEGDA) blend films were successfully prepared by Michael addition reaction with different weight ratios as wound dressing materials (Zhang, Yang & Nie, 2008). The mechanical properties and the swelling property of chitosan were found to be enhanced after the chemical modification. Indirect cytotoxicity assessment of films with mouse fibroblasts (L929) indicated that the material showed no cytotoxicity toward growth of L929 cell and had good in vitro biocompatibility. SEM observation indicated that the microporous surface structure of the chitosan/PEGDA films was good to grow, proliferate, and differentiate of L929 cell. These chitosan/PEGDA films have the potential to be used as wound dressing material (Zhang, Yang & Nie, 2008). To create a moist environment for rapid wound healing, a chitosan-PVA-alginate film with sustained antibacterial capacity had been developed by the casting/solvent evaporation method (Pei et al., 2008). This new type of chitosan-PVA-alginate film consists of a chitosan top layer and sodium alginate sublayer separated by an ornidazole (OD)-incorporated PVA layer, exhibited perfect binding characteristics among the three layers. Physical characterization of the chitosan-PVA-alginate film showed that the triple-layer film had excellent light transmittance, control of water vapor transmission rate and fluid drainage ability promotion, compared with the single-layer film. From the *in vitro* release studies, about 90% of OD was released from the composite films within 60 min, and no significant difference was observed in cumulative release percentage with increases in the drug content. The composite film at low concentration of OD (1.0 mg/cm^2) showed effective antimicrobial activity in the cultures of *Staphylococcus aureus* and *Escherichia coli* in agar

plates. The results indicated that chitosan-PVA-alginate composite film incorporated with OD has the potential for wound dressing application (Pei et al., 2008).

Chitosan/hyaluronic acid composite films with high transparency could be fabricated for wound dressing material on glass or poly(methyl methacrylate) substrates (Xu et al., 2007). Along with the increase of hyaluronic acid amount, the resulting films became rougher as detected by AFM. However, increase of the hyaluronic acid amount weakened the water vapor permeability, bovine albumin adsorption and fibroblast adhesion, which are desirable characteristics for wound dressing. *In vivo* animal test revealed that compared with the vaseline gauge the chitosan/ hyaluronic acid film could more effectively accelerate the wound healing and reduce the occurrence of re-injury when peeling off the dressing again. These results demonstrate that the chitosan mixed hyaluronic acid may produce inexpensive wound dressing with desired properties (Xu et al., 2007). Membranes made of chitosan in combination with alginates as polyelectrolyte complexes have also been prepared. They display greater stability to pH changes and are more effective as controlled release membranes than either the chitosan or alginate separately. The membranes based on chitosan/alginate could be used on highly exuding wounds and prevented the bacterial infections (Rodrigues, Sanchez, Da Costa, & Moraes, 2008).

A polyelectrolyte complex (PEC), which consists of chitosan as a cationic and γ-poly (glutamic acid) (γ-PGA) as an anionic polyelectrolyte, was developed as a wound dressing material (Tsao et al., 2010). The physical and chemical properties of the chitosan/γ-PGA PECs were fully investigated. Experimental results showed that the physical and chemical properties and the *in vitro* degradation of the chitosan/γ-PGA PECs directly reflect the degree of complex formation. In addition, chitosan/γ-PGA PECs provide suitable moisture content and exhibit good mechanical properties, both favorable for allowing dressing to be easily stripped off from the wound surface without damaging newly regenerated tissue. Histological examinations revealed that, more than 50% of re-epithelialization and regeneration of the wound are achieved by dressing it with the chitosan/γ-PGA PECs. On the basis of wound-healing efficacy, chitosan/γ-PGA PECs can be potentially applied as a wound dressing material (Tsao et al., 2010). A membrane composed of an alginate layer and a chitosan layer with sustained antimicrobial efficacy was prepared (Dong et al., 2010). In this study, ciprofloxacin HCl was incorporated into the alginate layer. Water uptake capacity, in vitro drug release, and in vitro antimicrobial activity were evaluated. The composite membrane exhibited perfect binding characteristic between the two layers. The water uptake capacity of all the membranes was above 800%. The ciprofloxacin HCl was found to be released from the composite membranes for 48 h. The membrane was found to control the bacterial growth persistently. The results suggested that this chitosan/alginate composite membrane incorporated with ciprofloxacin HCl had the potential for wound dressing application (Dong et al., 2010). The biocompatible and microbiologically safe composite membranes based on chitosan and 2-hydroxyethyl methacrylate (HEMA) have been prepared by gamma irradiation (Casimiro, Gil & Leal, 2010). The antimicrobial activity of obtained membranes against several reference strains was evaluated after inoculation. Sub-lethal gamma radiation doses were also applied in artificially contaminated membranes and the D values of microorganisms in use were determined in order to predict which radiation dose could guarantee membranes microbiological safety. *In vitro* haemolysis tests were performed using drug loaded membranes irradiated at different doses. Results point out that those membranes naturally exhibit antimicrobial properties. Also show that, over the studied range values, drug loaded irradiated membranes display a non-significant level of haemolysis (Casimiro, Gil & Leal, 2010).

Antibiotic resistance of microorganisms is one of the major problems faced in the field of wound care management resulting in complications such as infection and delayed wound healing. Currently a lot of research is focused on developing newer antimicrobials to treat wounds infected with antibiotic resistant microorganisms (Rai et al., 2009). Ag has been used as an antimicrobial agent for a long time in the form of metallic silver and silver sulfadiazine ointments. Recently Ag nanoparticles have come up as a potent antimicrobial agent and are finding diverse medical applications ranging from silver based dressings to silver coated medical devices (Rai et al., 2009). It is well known that membranes with asymmetric structures are of industrial importance. The top skin layer renders the membrane selectivity, whereas the porous support layer provides the membrane with mechanical strength. Ag sulfadiazine-incorporated asymmetric chitosan membranes with sustained antimicrobial capability have been developed by a dry/wet phase separation method to overcome current limitations in Ag sulfadiazine cream for treating acute burn wounds (Mi et al., 2003). The asymmetric chitosan membrane consists of a dense skin and sponge-like porous layer, which can fit the requirements (oxygen permeability, controlled water vapor evaporation and the drainage of wound exudates) for this membrane to be used as a wound dressing. Silver sulfadiazine cream is a traditionally-used antibacterial for the prevention of wound infection; however, it has raised concern of potential silver toxicity. The asymmetric chitosan membrane acts as a rate-controlling wound dressing to incorporate silver sulfadiazine, and release sulfadiazine and Ag ions in a sustained way. The release mechanism depends on the mass-transfer resistance for the release of sulfadiazine and silver ions from the dense and sponge-like porous layers, and the chemical resistance for the interaction of silver ions with chitosan polymeric chains, respectively. The bacteria-cultures (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) and cell-culture (3T3 fibroblasts) assay of the Ag sulfadiazine-incorporated asymmetric chitosan membrane showed prolonged antibacterial activity and decreased potential silver toxicity (Mi et al., 2003).

Hemorrhage remains a leading cause of early death after trauma, and infectious complications in combat wounds continue to challenge caregivers. Although chitosan dressings have been developed to address these problems, they are not always effective in controlling bleeding or killing bacteria. Ong et al. (2008) aimed to refine the chitosan dressing by incorporating a procoagulant (polyphosphate) and silver. Chitosan containing different amounts and types of polyphosphate polymers was fabricated, and their hemostatic efficacies evaluated *in vitro*. The optimal chitosan-polyphosphate formulation accelerated blood clotting, increased platelet adhesion, generated thrombin faster, and absorbed more blood than chitosan. Silver-loaded chitosan-polyphosphate exhibited significantly greater bactericidal activity than chitosan-polyphosphate *in vitro*, achieving a complete kill of *Pseudomonas aeruginosa* and a >99.99% kill of *Staphylococcus aureus* consistently. The Ag dressing also significantly reduced mortality from 90% to 14.3% in a *P. aeruginosa* wound infection model in mice. This study demonstrated for the first time, the application of polyphosphate as a hemostatic adjuvant, and developed a new chitosan-based composite with potent hemostatic and antimicrobial properties (Ong et al., 2008). Wound dressing composed of nano Ag and chitosan was fabricated using chitosan films preliminarily sterilized by immersion in 75% alcohol solution overnight, exposed to ultraviolet light for 1h on each side and rinsed with sterile water (Lu, Gao, & Gu, 2008). Sterile chitosan films were immersed in nano Ag solution at 4 °C for 12 h for self-assembly of the nano- Ag chitosan films via Ag-N bonding and to obtain 0.35% w/w Ag in the dressing material. In this study, AFM was used to examine the dressing, and SEM identified

the nano Ag immobilized on the chitosan film. Sterility and pyrogen testing assessed biosafety, and efficacy was evaluated using Sprague-Dawley rats with deep partial-thickness wounds. Ag sulfadiazine and chitosan film dressings were used as controls. At day 13, the healing rate of the Ag-chitosan dressing group was higher than the rates of the control groups at 99% compared with 82% for the Ag sulfadiazine group. The healing time was 13.51 ± 4.56 days for the Ag-chitosan group, and 17.45 ± 6.23 days for the Ag sulfadiazine group, respectively. Observations made on the histological sections on day 9 indicated that in the nano Ag dressing group a continuous epithelial lamina was formed, together with some sebaceous glands. The Ag sulfadiazine group showed no epithelial laminae, whilst the chitosan film group exhibited patchy epithelial laminae with a few sebaceous glands. At 13 days the blood silver content was 5 times normal and on the 45th day post-operatively, the Ag content of liver, kidney and brain had increased in both nano-silver and Ag sulfadiazine groups but more so in the latter, where the liver silver content was 100 times greater than normal. Due to its distinct antimicrobial action towards a broad range of bacteria, yeast, fungi and viruses, the Sprague-Dawley rats of the nano- Ag group showed less post-operative infection than the control groups. The wound environment far less affects nano-Ag than the ionic form of Ag: therefore at the same concentration, the bactericidal activity of nano-Ag is greater than that of the ionic form (Lu, Gao, & Gu, 2008).

Thomas et al. (2009) developed chitosan/Ag nanoparticles films by a simple photochemical method of reduction of silver ions in an acidic solution of silver nitrate and chitosan. Chitosan used here is a natural polymer and acts as a very good chelating and stabilizing agent; thus, this approach of formation of chitosan/silver nanoparticle films is proved to be an excellent 'green approach' for the synthesis of metal nanoparticle composites. The developed chitosan-nano Ag films demonstrated excellent antibacterial action against model bacteria, *Escherichia coli* and *Bacillus*. These films can be used as antimicrobial packaging materials, as wound dressings and can also be grafted onto various implants (Thomas et al. 2009). Vimala et al. (2010) prepared porous chitosan-Ag nanocomposite films in view of their increasing areas of application in wound dressing, antibacterial application, and water purification. The entire process consists of three-steps including Ag ion-poly(ethylene glycol) (PEG) matrix preparation, addition of chitosan matrix, and removal of PEG from the film matrix. Chitosan films having uniform pores were impregnated with Ag nanoparticles were fabricated by this approach. The embedded silver nanoparticles were clearly observed throughout the film in SEM and the extracted Ag nanoparticles from the porous chitosan-Ag nanocomposite showed ~12nm in TEM. Improved mechanical properties were observed for porous chitosan-Ag nanocomposite than for chitosan blend and chitosan-Ag nanocomposite films. Further, the examined antibacterial activity results of these films revealed that porous chitosan-Ag nanocomposite films exhibited superior inhibition (Vimala et al. 2010).

ZnO has attracted wide interest because of its good photocatalytic activity, high stability, antibacterial property and non-toxicity (Cohen, 2000; Wang, 2004; Sharma et al., 1995). Novel chitosan/Ag/ZnO blend films were prepared via a new method of sol-cast transformation (Li et al., 2010). The results revealed that ZnO and Ag nanoparticles with spherical and granular morphology had uniform distribution within chitosan polymer. The product had excellent antimicrobial activities against *B. subtilis*, *E. coli*, *S. aureus*, *Penicillium*, *Aspergillus*, *Rhizopus* and *yeast*. Chitosan/Ag/ZnO blend films had higher antimicrobial activities than chitosan/Ag and chitosan/ZnO blend films. Moreover, the blend films almost maintained the initial color of chitosan, which have potential application as

antibacterial materials (Li et al., 2010). In another study ZnO nanoparticles were prepared by the Pechini method from polyester by reacting citric acid with ethylene glycol in which the metal ions are dissolved, and incorporated into blend films of chitosan and PVA with different concentrations of polyoxyethylene sorbitan monooleate, Tween 80 (Vicentini, Smania Jr & Laranjeira, 2010). The antibacterial activity of the films was tested, and the films containing ZnO nanoparticles showed antibacterial activity toward the bacterial species *S. aureus*. The observed antibacterial activity in the composite films prepared in this work suggests that they may be used as hydrophilic wound and burn dressings (Vicentini, Smania Jr & Laranjeira, 2010). The above studies indicated that ZnO nanoparticles incorporated with chitosan enhanced the antibacterial activity.

Collagen-chitosan spongy skin was developed as a scaffold for the reconstruction of skin *in vitro*: this artificial skin promoted the remodeling of an extracellular matrix similar to normal dermis (Berthod et al., 1996). Smad3 mediates the intracellular signaling of TGF-b1 superfamily and plays a critical role in the cellular proliferation, differentiation and elaboration of matrix pivotal to cutaneous wound healing. Smad3 antisense oligonucleotides (ASOs) impregnated polyelectrolyte complex scaffold containing chitosan and sodium alginate was prepared for accelerated wound healing (Hong et al., 2008). Physicochemical properties of polyelectrolyte complex were characterized by zeta potential, SEM and bioadhesive test. Full-thickness, excisional wounds were made on the dorsum of C57BL6 mice. Then, smad3 ASOs-polyelectrolyte complex, polyelectrolyte complex alone, smad3 ASOs and gauze dressing were applied to determine concentration of TGF-b1 and collagen in tissues. Zeta potentials and bioadhesive strengths of ASOs-polyelectrolyte complex were increased as the chitosan ratio in polyelectrolyte complex. In smad3 ASOs-polyelectrolyte complex, the healing process suggested by wound closure and histological observation was faster than other groups because collagen contents increased and level of TGF-b1 decreased. These results demonstrate that the smad3 ASOs-polyelectrolyte complex composed of chitosan and sodium alginate scaffold could be applied for accelerated wound healing (Hong et al., 2008). The gold colloid/chitosan film scaffold, accelerate proliferation of newborn mice keratinocytes, was fabricated by self-assembly technology (Zhang et al., 2009). In this study, the keratinocytes were cultured and observed on three different extracellular matrices: gold colloid/chitosan film scaffold, chitosan film and cell culture plastic (control groups). 6, 12 and 24 h after inoculation, the cell attached ratios were calculated respectively. In comparison to control groups, gold colloid/chitosan film scaffold could significantly increase the attached ratio of keratinocytes and promote their growth. Meanwhile, there were not any fusiform fibroblasts growing on this scaffold. The rapidly proliferating keratinocytes were indentified and characterized by immunohisto chemistry and TEM, which showed the cells maintain their biological activity well. The results indicated that gold colloid/chitosan film scaffold was nontoxic to keratinocytes, and was a good candidate for wound dressing in skin tissue engineering (Zhang et al., 2009).

Recently, a few studies have been reported on chitin scaffolds with Ag nanoparticles to treat patients with deep burns, wounds etc. Madhumathi et al. 2010 developed and characterized novel *α*-chitin/nano Ag composite scaffolds for wound healing applications. The antibacterial, blood clotting and cytotoxicity of the prepared composite scaffolds were studied. These *α*-chitin/nano Ag composite scaffolds were found to be bactericidal against *S.aureus* and *E.coli* with good blood clotting ability. Sudheesh Kumar et al. (2010) also reported about *β*-chitin/nano Ag composite scaffolds for wound healing applications using *β*-chitin hydrogel with Ag nanoparticles. The antibacterial, blood clotting, swelling, cell

attachment and cytotoxicity studies of the prepared composite scaffolds were evaluated. The prepared β-chitin/nano Ag composite scaffolds were found to be bactericidal against *Escherichia coli* and *Staphylococcus aureus* and they showed good blood-clotting ability as well. Cell attachment studies using vero (epithelial) cells showed that the cells were well attached on the β-chitin/nano Ag scaffolds. These results suggested that both *a* and β-chitin/nano Ag composite scaffolds could be a promising candidate for wound dressing applications.

A porous sponge-type wound dressing materials based on β-chitin were prepared (Lee et al., 2000). Oxygen permeabilities of the samples were found to be relatively good. These sponge-type samples contained antimicrobial agents, silver sulfadiazine, in order to prevent bacteria infection on a wound surface. Anti-microbacterial tests on agar plate were carried out to confirm the bactericidal capacity of wound dressing materials. These materials impregnating silver sulfadiazine had the complete bactericidal capacity against *Pseudomonas aeruginosa* up to 7 days. Finally, a wound healing effect of β-chitin-based semi-interpenetrating polymer networks was evaluated from the animal test using the wistar rat *in vivo*. Histological studies confirmed the proliferation of fibroblasts in the wound bed and a distinct reduction in infectious cells (Lee et al., 2000). Denkbas et al. (2004) prepared and characterized chitosan sponges encapsulated with a model antibiotic drug, norfloxacin, as wound dressing material. The cross-linked chitosan sponges were prepared by a solvent evaporation technique. The results indicated that the chitosan sponges were in the fibrillar structure. In this study, the swelling behavior, norfloxacin loading, *in vitro* release characteristics, and antibacterial activity were determined. The effects of cross-linker concentration, norfloxacin/chitosan ratio, chitosan molecular weight, and base concentration were also investigated. The most effective parameter was found to be the degree of neutralization. It was also observed that the equilibrium-swelling ratio decreased with increasing cross-linking density. The norfloxacin release was found to be swelling controlled initially and diffusion controlled at the extended release periods. It was found that the antibacterial activity was directly proportional to the release rate. These chitosan based sponges could be used for wound dressing applications (Denkbas et al., 2004).

3. Conclusions

From this review, chitin and chitosan seem to be excellent dressing materials for the wound healing. In this paper, we overviewed the recent progress of chitin and chitosan-based fibrous materials, hydrogels, membranes, scaffolds and sponges in wound dressing. In the area of wound management, the use of chitin, chitosan and its derivative is immense. Chitin and chitosan have excellent properties such as biodegradability, bio-compatibility, non-toxicity and have been shown to enhanced wound healing in animals and humans. To improve the potential of chitin and chitosan for wound dressing applications, further studies are needed.

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