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Physical and Chemical Modification of Chitin/Chitosan for Functional Wound Dressings

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Contents

Abstract This chapter provides insight into the functionalization of chitin and chitosan for general and specific-purpose wound dressings, such as hemorrhage,

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infected, burn, and diabetic ulcer wounds. The understanding of different kinds of wounds, wound healing process, and factors affecting wound healing is essential for the design of well-functioning biomaterials as well as the fabrication of wound dressings. Functionalization of chitin/chitosan, including physical and chemical modification to form functional wound dressings for specific purposes, is described in this chapter.

Keywords Chitin · Chitosan · Functionalization · Modification · Regeneration · Wound dressing · Wound healing

1 Introduction

Skin is the largest organ in our body and serves its primary function as a protective barrier against external impacts. Wounds can present a cause of serious illness and mortality in everyday pathology [1, 2], and so can require complicated clinical intervention. Wounds can be categorized into acute and chronic wounds according to the duration of their healing [3, 4]. The healing of acute wounds proceeds via the normal healing pathway with both anatomical and functional restoration. Acute wounds can be caused by a traumatic loss of tissue, surgical procedure, abrasions, or even laceration (deep cuts on the skin) associated with infection [3–5]. Chronic wounds are associated with diseases, such as diabetic and pressure ulcers [3–5], and can be defined as hard to heal wounds. The current potential wound healing approaches are based on autograft, allograft, cultured epithelial autograft, and wound dressing [6]. Wound dressings function to preserve hydration within the wound in order to optimize regeneration, protect against infection, and avoid disruption of the wound base.

An ideal wound dressing should absorb exudates, maintain a moist environment at the wound interface, act as a barrier to microorganisms, and be breathable or allow gaseous exchange. It also should be non-allergenic, non-toxic, and non-adherent so as to be easily removable without trauma. Many polymeric materials have been investigated for the purpose of wound dressings, including synthetic polymers, such as polyurethane [7, 8], polycaprolactone [9, 10], poly(lactic acid) [11, 12], polyethylene glycol (PEG) [13, 14], silicone rubber [15, 16], etc., as well as natural or bio-based polymers. Increasing attention has been paid to biopolymer-based wound dressing materials because of their inherent properties, such as biocompatibility, biodegradability, hemostatic activity, moist maintaining ability, and the ability to support tissue regeneration and differentiation, leading to an accelerated wound healing [17]. Most of the currently available biodegradable polymer-based wound dressings are made from chitin/chitosan [18, 19], hyaluronic acid [20, 21], collagen [22, 23], alginate [24, 25], and so on.

Interestingly, chitin and chitosan have a high potential for wound healing applications because of their prominent properties, such as exudates absorbability,

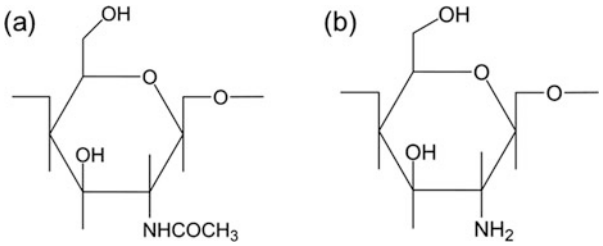

Fig. 1 Monomeric structure of (**a**) chitin and (**b**) chitosan

stimulating hemostasis, and accelerating tissue regeneration [26]. Chitin, [β-(1-4)-2-acetamido-2-deoxy-D-glucose], is the second most abundant natural polysaccharide after cellulose and occurs in nature as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods, mostly in crabs and shrimp shells [27]. Chitin also can be found in the cell walls of fungi and yeast [27]. Chitosan, [β-(1-4)-2-amino-2-deoxy-D-glucose] is the *N*-deacetylated derivative of chitin. Chitin can be extracted by decalcification and deproteinization, while chitosan is obtained by deacetylation of chitin. Therefore, chitin and chitosan are commonly copolymers categorized by degree of acetylation (DA) or degree of deacetylation (DD) representing the amount of *N*-acetyl glucosamine and *N*-glucosamine in their monomeric unit.

The *N*-acetyl glucosamine present in chitin and chitosan is structurally like hyaluronic acid, an extracellular matrix (ECM) component of dermal tissue that is essential for wound repair [28]. Since chitin and chitosan are biodegradable, non-toxic, and biocompatible, they also have received considerable attention in various fields of biomedical and pharmaceutical applications. The monomeric structure of chitin and chitosan is presented in Fig. 1.

Owing to their structures, chitin and chitosan are multifunctional polymers and amenable to chemical modification. Chitin has primary and secondary hydroxyl groups at the C-6 and C-3 positions, respectively, and acetamido groups at the C-2 position, while chitosan has amino groups at C-2 position. Due to the availability of the free amino groups in chitosan, it can be protonated and carries a positive charge. Considering its biodegradability, chitin and chitosan are metabolized by the human enzyme lysozyme, making it biodegradable. The biodegradation activity by lysozyme is mainly controlled by the DA or DD and also by the distribution of *N*-acetylglucosamine residues as well as molecular weight (MW) [29]. Chitin and chitosan are degraded into oligosaccharides of variable length and are non-toxic in nature. These oligosaccharides can be introduced in the metabolic pathways and degraded into *N*-glucosamine and *N*-acetylglucosamine. The amino sugar of *N*-glucosamine can be incorporated into the glycosaminoglycan (GAG) and glycoprotein pathways or otherwise excreted from the system [30]. *N*-Acetyl glucosamine also initiates fibroblast proliferation, supports an ordered collagen deposition, and stimulates an increased level of natural hyaluronic acid synthesis at the wound site, leading to faster wound healing and scar prevention [31]. Besides, chitin and chitosan can be fabricated in various forms, such as hydrogels, membranes, fibers, electrospun mats, sponges, scaffolds, and so on.

In order to design effective chitin and chitosan wound dressings, this chapter gives information on the wound healing process and factors effecting wound healing in Sect. 2, the fabrication methods of chitin and chitosan wound dressings in Sect. 3, commercially available chitin- and chitosan-based wound dressings in Sect. 4, and the physical and chemical modification of chitin and chitosan (Sect. 5) for making functional wound dressings, such as hemostatic, antimicrobial, burn, and diabetic ulcer wound dressings in Sect. 6.

2 Wound Healing Process and Factors Effecting Wound Healing

A wound is defined as a damage or destruction to the normal anatomical structure, biological feature, and function [3]. For skin, the damage can range from a simple tear of the skin surface or deep cuts into the subcutaneous tissue with damage to other surrounding tissues, such as muscles, tendons, vessels, nerves, and bone [5]. Wound healing is a complex and dynamic process starting at the moment of injury. The mechanism underlying this process consists of four continuous, overlapping, and precise programmed phases [5, 32]: (1) hemostasis and coagulation, (2) inflammation, (3) proliferation, and (4) remodeling. The normal wound healing process and biological events are schematically presented in Fig. 2. The individual phases are described below. In addition, those wound healing phases involve multiple cell types, synthesis of the extracellular matrix (ECM) proteins, and the action of mediators including growth factors and cytokines. Therefore, the bio-physiological events and major factors mediating the wound healing process are summarized in Table 1.

2.1 Hemostasis and Coagulation Phase

This phase begins immediately after injury and serves to prevent bleeding, protect the vascular system to allow it to close intact, and provide a matrix for combating invasive cells in the later phase [3, 5]. The injured vessels lightly close due to the contraction of vascular smooth muscle cells. However, the vascular smooth muscle can prevent bleeding only for a few minutes and bleeding resumes again due to the hypoxia and acidosis in the wound wall. Therefore, the coagulation mechanism is activated with platelet aggregation and clot formation to limit bleeding. The platelets and blood components come into contact with the exposed collagen and other ECM components triggering the release of clotting factors (comprised of fibronectin, fibrin, vitronectin, and thrombospondin) [33], which leads to the formation of a blood clot. Numerous proteins are contained in the α-granules of platelets: platelet-derived growth factor (PDGF), transforming growth factor (TGF), platelet factors,

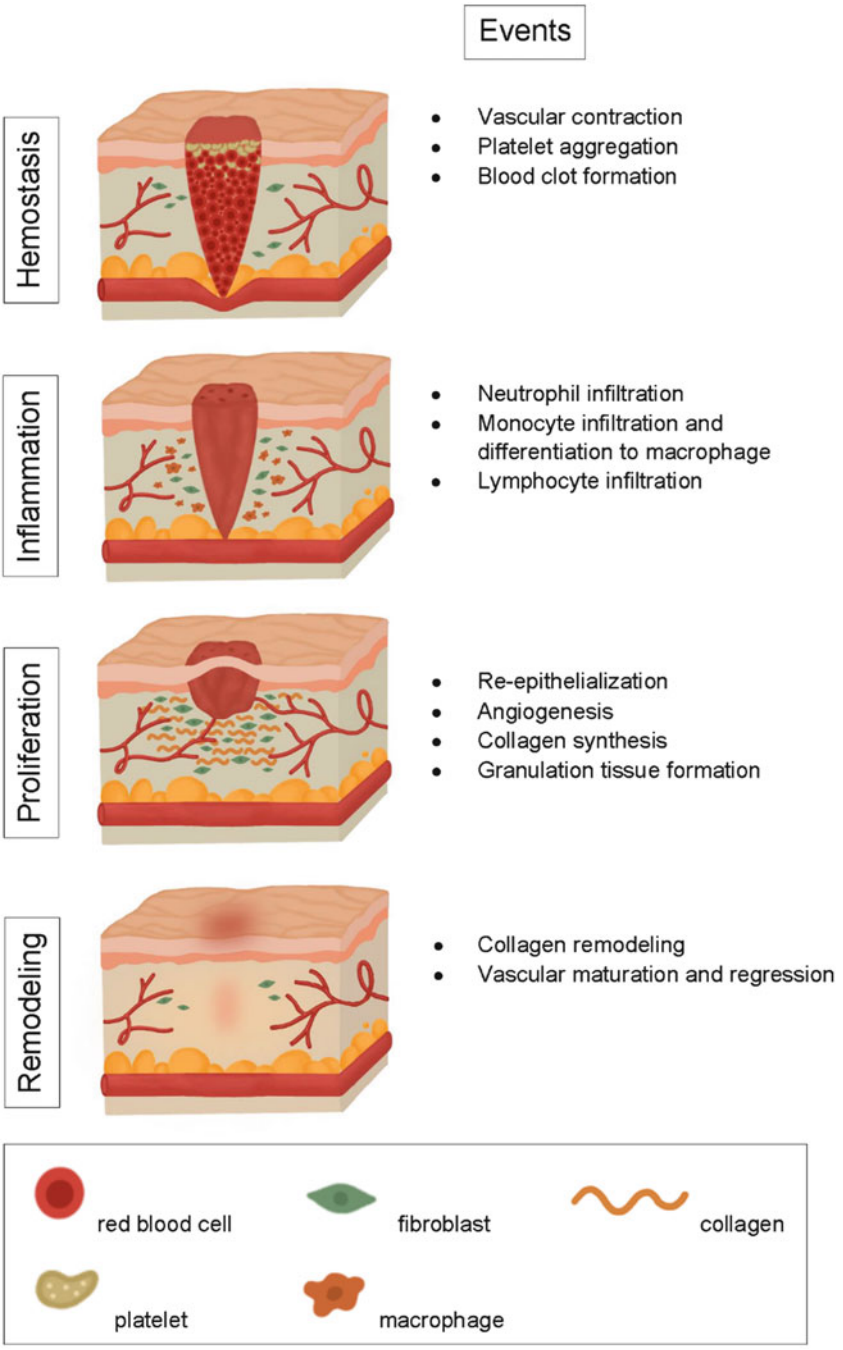

Fig. 2 Schematic illustration of the normal wound healing phases

Table 1 Bio-physiological events and major factors mediated normal wound healing

Phase	Bio-physiological events [32]	Major mediated factors
Hemostasis	1. Vascular contraction 2. Platelet aggregation 3. Blood clot formation	PDGF, TGF, IL-1, PDAF VEGF, EGF, IGF Fibronectin
Inflammation	1. Neutrophil infiltration 2. Monocyte infiltration and differentiation to macrophage 3. Lymphocyte infiltration	TGF, FGF, collagenase IL-1, IgG
Proliferation	1. Re-epithelialization 2. Angiogenesis 3. Collagen synthesis 4. GTF (ECM formation)	PDGF, TGF, FGF, VEGF
Remodeling	1. Collagen remodeling 2. Vascular maturation and regression	PDGF, TGF, FGF

interleukin (IL)-1, platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin-like growth factor (IGF), and fibronectin [34]. These molecules promote the activities of neutrophils, macrophages, endothelial cells, and fibroblasts in later phase.

2.2 Inflammatory Phase

The second (inflammatory) phase aims to establish an immune barrier against invading microorganisms. The early inflammatory phase initiates the infiltration of the wound sites by neutrophils. Neutrophils function to prevent infection by removing foreign substances, bacteria, and tissue debris via phagocytosis. The redundant neutrophils after completing the task of phagocytosis are eliminated to the wound surface by apoptosis without potentiating the inflammatory response or damaging the tissues [35]. Initially, blood monocytes undergo differentiation with phenotypic changes to become macrophages on arrival into the wound. Macrophages appear at wound sites in the late inflammatory phase to continue the process of phagocytosis of apoptotic cells, including neutrophils [35], and so pave the way for the resolution of inflammation. The infiltrating macrophages also provide an abundant amount of potent tissue growth factors and mediators, such as TGF, collagenase, and fibroblast growth factors (FGFs) to stimulate keratinocytes, fibroblasts, and endothelial cells to proliferate, and so promote tissue regeneration [36] towards the end of this second phase and into the third (proliferative) phase.

Lymphocytes, which are the last cells to enter the wound site, arrive during the late inflammatory phase by chemotactic attraction to IL-1 and the breakdown products of immunoglobulin G (IgG) [35, 37]. The IL-1 plays a vital role in collagenase regulation, being needed for collagen production and degradation of the ECM components, and regulating wound healing [35, 37]. If the wound is

associated with various pathological alterations, including an increased protease activity and infection, the inflammation may persist for months or years and it is then classified as a chronic wound.

2.3 Proliferative Phase

The proliferative phase generally follows and overlaps with the inflammatory phase, and starts with fibroblast migration induced by the action of TGF and PDGF and deposition of a newly produced ECM, leading to the replacement of the temporary matrix composed of several proteins of fibronectin, hyaluronan, GAG, proteoglycans, and types I and III collagen [5, 32, 37]. Therefore, the granulation tissue formation (GTF) and wound contraction can be seen macroscopically in this phase of the wound healing process. Endothelial cells are responsive to numerous angiogenic factors, including FGF, PDGF, VEGF, and TGF, to regenerate epithelial tissue (re-epithelialization) and restore the vascular network [5, 6]. Keratinocytes are involved in repairing the epidermal barrier, while endothelial cells and fibroblasts are responsible for angiogenesis and GTF as part of the ECM formation [5, 6].

2.4 Remodeling Phase

The remodeling phase involves reorganization and contraction of the newly formed ECM as well as scar formation [5, 6, 37] and may last for several years. This phase is controlled by regulatory mechanisms that are designed to maintain a balance between synthesis and degradation, leading to normal healing. This process is regulated by a number of factors, including PDGF, TGF, and FGF [5].

It should be noted that exogenous and endogenous factors can modulate such events and influence the healing process. Especially, systemic disorder diseases, including diabetes, immunosuppression, and venous stasis, may disturb the process of wound healing, resulting in chronic or non-healing wounds [5]. In addition, external agents, such as the utilization of corticosteroid, inflection, smoking, alcohol consumption, stress, etc., can hinder the early closure of the wound [32, 38]. These complicating factors may also cause the appearance of keloids and hypertrophic scars [38].

Keloids and hypertrophic scars are fibroproliferative disorders of the skin that result from abnormal healing of injured or irritated skin. They can be called pathological or inflammatory scars. A hypertrophic scar occurs directly after the initial repair and develops into thick skin that does not spread beyond the original wound, whilst a keloid may occur after healing and continues to grow and spread, invading the surrounding healthy tissue. Tension on the skin around the wound results in prolonged and/or repeated inflammation and generates abnormal numbers of blood vessels as well as collagen and nerve fibers in the dermal reticular layer

[39]. The mechanobiology of the dermis and blood vessels, along with genetic and systemic factors, are possible factors that promote pathological scar development by inducing endothelial dysfunction (vascular hyperpermeability) during the inflammatory stage of wound healing. The continued presence of these factors prolongs the influx of inflammatory cells and factors, thereby leading to fibroblast dysfunction [39]. A scar is formed from the overgrowth of granulation tissue (proliferation of collagen fibers).

3 Fabrication of Chitin/Chitosan Wound Dressings in Various Forms

Chitin and chitosan have been widely used in many fields of biomedical applications, such as tissue engineering, drug carriers, and wound dressings, because of their processibility into various forms. The fabrication of chitin/chitosan wound dressings has been performed in different processes depending on the specific purpose. Chitin and chitosan can be formed into various forms, including hydrogels, membranes, scaffolds, hydrocolloids, and so on, by many methods or processing techniques. Chitin is insoluble in common organic solvents as a direct result of the strong intra- and intermolecular hydrogen bonding, while chitosan can be dissolved in dilute acid, making chitin less processable. However, chitin is more favorable than chitosan in certain applications, especially in the biomedical fields. This is because of the fact that the acetamide group present in chitin is similar to the amide linkage of protein in living tissues [40], which makes chitin more biocompatible than chitosan. This chapter describes briefly the main fabrication techniques used for making chitin-/chitosan-based wound dressings for different applications.

3.1 Hydrogels

Hydrogels are interesting for biomedical applications because of their high-water absorbability and biocompatibility. Progress in the synthesis and designing of hydrogels for wound healing has advanced. Hydrogels can absorb tissue extrudates, provide a moist environment to prevent wound dehydration, and allow transportation of oxygen [41]. The most commonly used fabrication methods for chitin- and chitosan-based hydrogels are described as follows.

3.1.1 Chitin-Based Hydrogel

Fabrication of a chitin hydrogel is difficult because of the limited solubility of chitin, making it hard to perform further processing [42]. Among many attempts, chemical

modification of chitin with various chemical reagents to substitute hydrophilic groups onto the surface of the chitin chains seems to be a promising method to enhance its water sorption ability and solubility [43]. Modification of chitin has mostly been performed in a sodium hydroxide (NaOH)/urea solvent system, and then reacted with other reagents, such as acrylamide [42], propylene oxide [44], and 2-aminoethyl chloride hydrochloride (2-AECH) [45]. Typically, chitin or chitin derivatives were cast in a container and allowed to form a gel, perhaps with the addition of some crosslinkers or physical polymerization.

3.1.2 Chitosan-Based Hydrogel

Fabrication of a hydrogel from chitosan is more attractive than that from chitin due to the *N*-deacetyl groups or amino groups of chitosan, making chitosan more hydrophilic than chitin and so it is easy to dissolve in dilute acid. In order to prepare the chitosan hydrogel, most methods have started from chitosan dissolved in acetic acid [46–48]. The addition of additives or bioactive substances into the hydrogel can improve the physiochemical and biological properties of the chitosan hydrogel for specific purposes. Some drugs or hormones, such as thyroxine [46], heparin, and bemiparin [47], have been loaded during the preparation of a chitosan hydrogel with the aim of stimulating angiogenesis and giving anticoagulant properties, respectively. In many reports, silver (Ag), in form of nanoparticles (AgNPs), was incorporated into the chitosan hydrogel in order to enhance other biological activities, such as antimicrobial and anti-inflammatory properties [48, 49]. Moreover, chitosan can be blended with other polymers, such as PEG [48] and lignin-polyvinyl alcohol (PVA) [50] to enhance its water absorbability and mechanical strength. Finally, the chitosan solution mixture was cast on a setting container and frozen overnight to stabilize the hydrogel structure, followed by neutralization. In some cases, a crosslinking agent has been applied instead of the freezing process.

In addition, a water soluble derivative of chitosan, carboxymethyl chitosan (CMCTS), was synthesized by chemical modification of chitosan with monochloroacetic acid [51]. The CMCTS can be easily dissolved in water over a wide range of concentrations [49, 52], and the CMCTS aqueous solution can be fabricated into a hydrogel under room temperature by complexing with polyelectrolytes [49] or chemically crosslinked with a crosslinker, such as genipin [52].

Hydrogels prepared from chitin, chitosan, and their derivatives can function as active substance carriers. Figure 3 illustrates the chitin and chitosan hydrogels as carriers of active substances, such as drugs, Ag, and growth factors, in order to enhance the wound healing process.

3.2 Membranes

A membrane, as a very thin film, has considerable advantages as a wound dressing, including its high surface-to-volume ratio, permeability, and breathability for

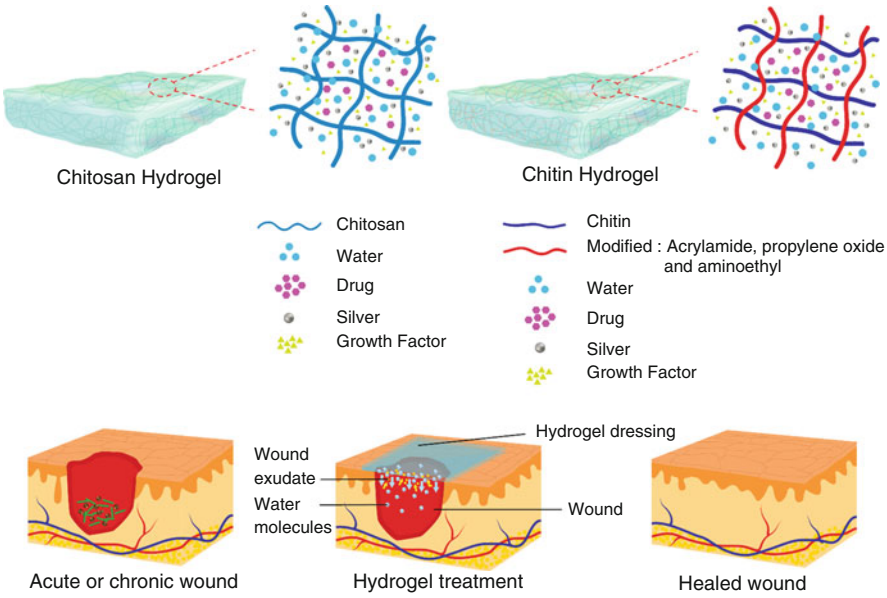

Fig. 3 Schematic illustration of using chitin or chitosan hydrogels as carriers of bioactive agents and applying the hydrogels to enhance wound healing

biological events. The application of a membrane requires the capability to control the transportation, diffusion, or separation of small molecules (such as gas and water) as well as ions. The most commonly used fabrication methods for chitin- and chitosan-based membranes are described as follows.

3.2.1 Chitin-Based Membranes

The casting process has always been used to prepare chitin membranes. The chitin is dissolved in a lithium chloride and dimethylacetamide solvent system [53, 54] and cast on a petri dish, with the dense membrane being obtained by gradual coagulation. Otherwise, after casting in a container, it was moved to soak in different kinds of solvents, washed with water, and dried to obtain the porous chitin membrane [54, 55].

3.2.2 Chitosan-Based Membranes

Currently, one of the most promising approaches for wound healing that mimics the structural similarities of the skin epidermal and dermal layers is the use of asymmetric membranes. The inner layer is a porous structure that functions as an exudate absorption matrix and supporting matrix for cell adhesion, migration, and

proliferation as well as allowing gaseous exchange and nutrients flow. Whereas, the outer layer is a dense layer mimicking the epidermis of the skin that functions as barrier to protect the wound from external threats (physical, chemical, and infection). Several techniques, such as electrospinning, bioprinting, wet-phase inversion method, dry/wet method, and supercritical carbon dioxide ($scCO_2$)-assisted phase inversion technique, have been explored to produce asymmetric membranes [56].

The fabrication of a chitosan-titanium dioxide composite membrane [57] and a membrane of gelatin/chitosan/cinnamaldehyde crosslinked with glutaraldehyde [58] have been obtained using the wet-phase inversion method. Briefly, the chitosan mixture is cast and then immersed in a non-solvent coagulant bath. The procedure is similar to the wet-phase inversion method, but the dry/wet method adds one more step of pre-evaporation (drying) of the cast membrane before immersion of the polymer matrix into the coagulation bath. This pre-evaporation stage increases the concentration of the polymeric solution and creates a dense outer top layer that acts as barrier to protect the wound against external contamination [59]. The preparation of a silver sulfadiazine (AgSD)-incorporated asymmetric chitosan membrane [59] and fabrication of a sponge-like asymmetric chitosan [60] are examples of those fabricated by the dry/wet method.

Figure 4a illustrates asymmetric chitin and chitosan membranes with the incorporation of some active substances, while Fig. 4b presents the wet-phase inversion method and dry/wet method. The $scCO_2$-assisted phase inversion method was proposed for the first time as an alternative technique to produce an asymmetrical membrane for skin wound healing. Being a green technology, it can form solvent-free membranes with short processing times and no collapse of the structure. Furthermore, $scCO_2$-assisted phase inversion allows the production of dry, clean, and ready-to-use membranes with a highly controlled morphology (by changing the pressure, temperature, and/or depressurization rate) and reduces the solvent recovery costs. The process does not require additional post-treatments or any potential organic solvents [61].

A traditionally famous method is the freeze-dried technique, in which samples are frozen and dried under vacuum to obtain porous membranes. Chitosan containing polyurethane modified with *N*-isopropyl acrylamide membrane [62] and bacterial cellulose-chitosan composite membrane [63] are examples of wound dressing membranes fabricated by the freeze-dry method.

3.3 Fiber and Electrospun Mat

Electrospinning is a simple and inexpensive method for producing nanofibers. It has become a popular fabrication method for porous fibrous materials that can be applied for tissue engineering scaffolds and wound dressings. The advantages of an electrospun mat made from ultrafine polymer fibers are its high porosity and nano-scale diameters, variable pore-size distribution, high surface to volume ratio, and, most importantly, its morphological similarity to the natural ECM in the skin

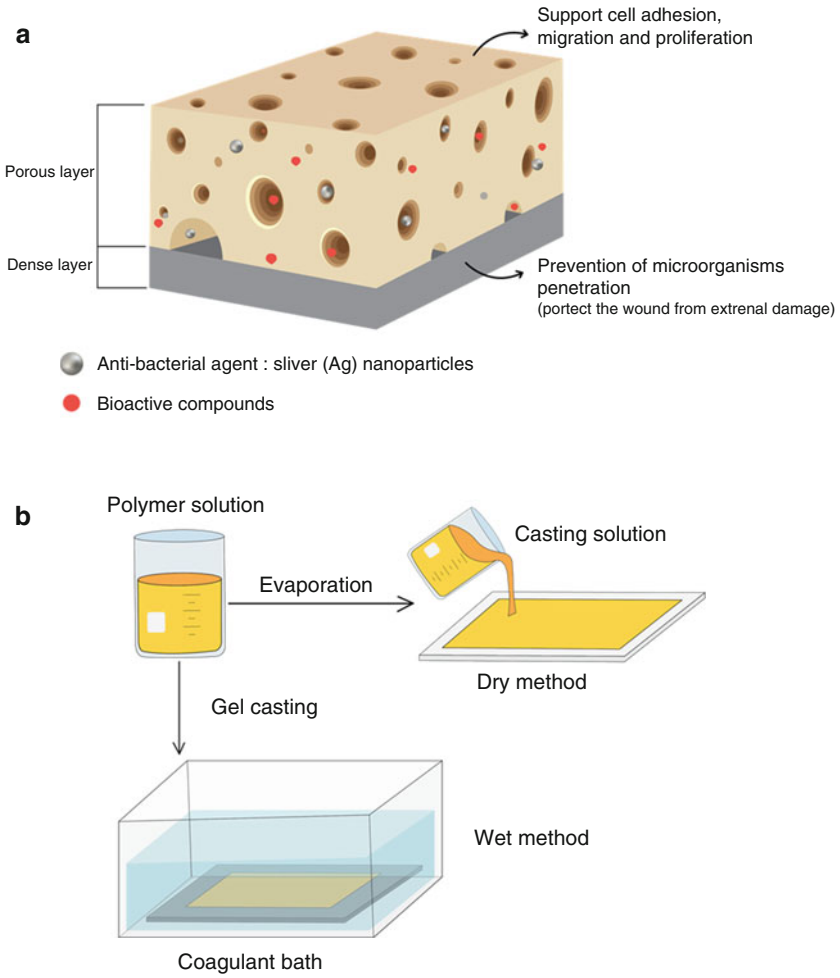

Fig. 4 Schematic illustration of (**a**) asymmetric chitin and chitosan membranes and (**b**) wet-phase inversion method and dry/wet method

[64]. The electrospun mat promotes cell attachment and proliferation. For this reason, the electrospinning technique has been used in many studies to prepare chitin and chitosan fibrous mats for wound dressing applications. However, electrospinning of natural polymers, such as chitosan and alginate, is more difficult than that of synthetic polymers. Chitosan presents a very low solubility in most organic solvents, and the use of dilute or concentrated acid solutions seems to be the most reliable option to properly dissolve it [65], since the protonation of amine groups of chitosan greatly contributes to its solubility. The spinnability of pure chitosan is challenging due to its polycationic nature and high viscosity in solution, and specific intra- and inter-molecular interactions. Indeed, formation of three-

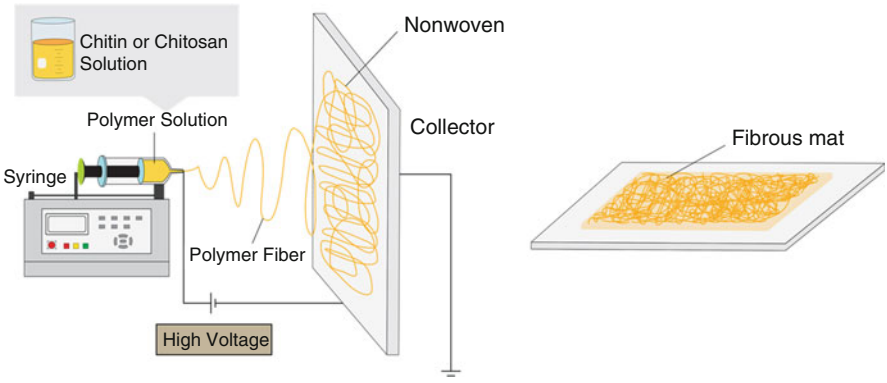

Fig. 5 Schematic illustration of the electrospinning fabrication of a chitin or chitosan fibrous mat

dimensional (3D) strong hydrogen bonds prevents the free movement of the polymeric chain segments exposed to the electrical field, making the formation of a stable jet problematic. In order to overcome these issues, blending with synthetic polymers, such as polyethylene oxide [65–67], PVA [64, 68], and poly (vinyl pyrrolidone) [69], are generally used as both a spinning enabler/enhancer and a hydrophilicity improver [65, 66]. The electrospinning fabrication of chitin and chitosan fibrous mat is represented in Fig. 5.

3.4 Sponges and Scaffolds

Sponge dressings with a porous structure exhibit a huge porosity, high strength, and high specific surface area, beneficial to meet the demands of a higher gas permeability, tunable water vapor evaporation, more wound exudate absorption, presence of good hemostatic properties, and protecting the wound from infection and dehydration [70]. While polymeric scaffolds are also a 3D porous structure that provides support for cell attachment, proliferation, and differentiation, they must possess biocompatibility, high porosity, and a good mechanical strength [17]. There are many processes to fabricate chitosan sponges, including gas foaming and particulate leaching, but the most common method is through the process of lyophilization. Lyophilization, also called freeze-drying, sublimates liquid from frozen materials, which helps to reduce damage to the material and preserves the structural and chemical integrity of proteins and natural substances. In the case of chitosan sponges, lyophilization creates pores, which in turn creates a greater surface area than dense forms of chitosan [71].

Many researchers have used the lyophilization method in their studies [72–76]. Some researchers have added the natural product *Aloe vera* extract [72, 73], metallic particles (AgNPs [73], aluminium monostearate [74], and AgSD [75]), or blended with other polymers (PVA [76]), in order to obtain an antibacterial property

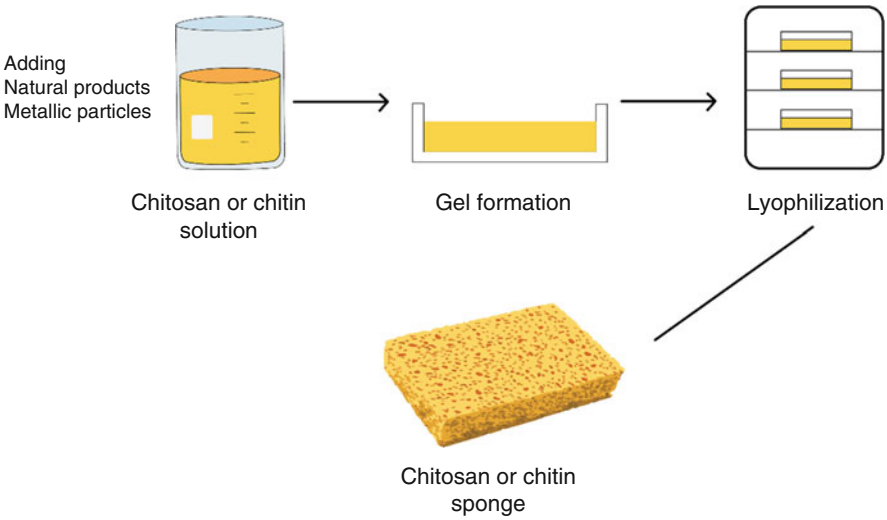

Fig. 6 Schematic illustration of chitin and chitosan sponge fabrication procedures

or improve the mechanical properties of chitosan. On the other hand, there are few studies on the fabrication of an asymmetric wettable chitosan-based sponge. A wound dressing with an asymmetric surface wettability is of great interest in wound healing. The hydrophobic surface could effectively prevent the external contamination (water, blood, and bacteria) to the dressing, while the hydrophilic surface could preserve the comfortable, moist environment to promote wound healing. One side of chitosan sponge can be modified with a thin layer of stearic acid to provide the asymmetric chitosan sponge [77].

For a chitin-based sponge, several studies have reported the use of modified chitin. Chitin was modified in form of quaternized chitin, where partial deacetylation occurred during the quaternization in a NaOH/urea solvent system. Glutaraldehyde was then used as a crosslinker of the quaternized chitin and the sponge was obtained after lyophilization [78]. The fabrication of chitin and chitosan sponges is illustrated in Fig. 6.

3.5 Hydrocolloids

Hydrocolloid dressings absorb the wound fluid and change into a jelly-like materials. Hydrocolloid dressings consist of two different structures. The inner hydrocolloid are particles that can absorb exudate to form a hydrated gel over the wound, creating a moist environment that promotes healing and protects the new tissue. The outer matrix (film, foam, or gel) not only protects the wound from bacterial contamination, and foreign debris, but it also maintains a moist environment and helps prevent

shearing [79–81]. Classical hydrocolloids always contain a gel forming agent, such as carboxymethyl cellulose (CMC), pectin, gelatin, or sodium CMC [81, 82].

Only a few fabrications of hydrocolloid wound dressing have been generated from chitin or chitosan. Tripolyphosphate, as a crosslinking agent, was added to the chitosan solution in order to obtain chitosan microbeads dispersed in the chitin solution and later transformed into a chitin hydrogel by gradual coagulation [83]. A calcium alginate hydrocolloid with chitin, chitosan, and fucoidan was reported. The dry powders of alginates, chitin, chitosan, and fucoidan were mixed and crushed. The mixed powders were then spread on filter paper and sprayed with distilled water to partially dissolve the powder into a paste. The paste on the supporter was immersed in calcium chloride solution to generate the calcium alginate hydrocolloid, which was then crosslinked with ethylene glycol diglycidyl ether [84]. Figure 7 presents the chitin and chitosan hydrocolloid dressing and its application over the wound to absorb the wound exudate as well as protect the wound from bacterial infection.

4 Commercially Available Chitin and Chitosan Wound Dressings

The ordered regeneration of wounded tissues requires the fabrication of chitin and chitosan mostly in the forms of nonwoven, fibrils, hydrocolloid, films, and sponges. With the advantages of chitin and chitosan that can be fabricated in various forms and with the variety of fabrication methods mentioned above (Sect. 3), introducing wound dressing materials based on chitin, chitosan, and their derivatives are well

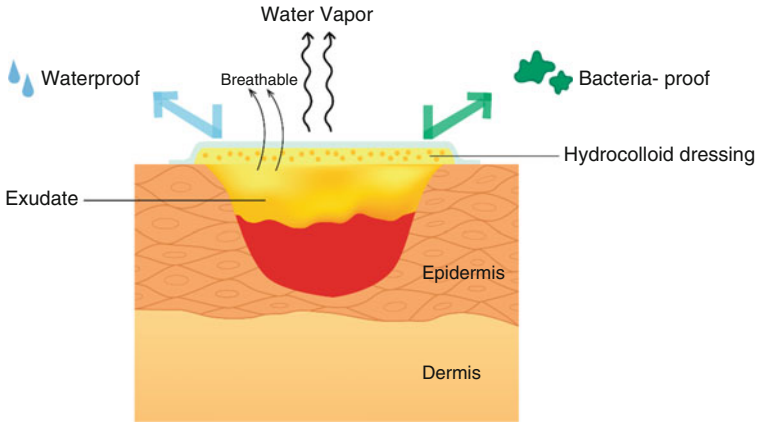

Fig. 7 Schematic illustration of the application of a chitin or chitosan hydrocolloid dressing to absorb the wound exudate and protect the wound from bacterial infection

known on the market and are produced and commercialized in many forms. The commercially available chitin- and chitosan-based wound dressings and their functional usages related to wound healing are summarized in Table 2.

5 Physical and Chemical Modification of Chitin and Chitosan for Wound Dressing in Specific Purposes

The worthwhile intrinsic properties of chitin and chitosan, including their biodegradability, biocompatibility, non-toxicity, hemostaticity, antimicrobial activity, anti-inflammatory, and antioxidant properties, make them appropriate for use as wound dressings. However, in order to develop chitin- and chitosan-based wound dressings for more specific purposes, they can be modified by means of physical or chemical modifications to add new functions, features, and to enhance some certain properties. Modification may be achieved through both covalent and noncovalent means.

There is considerable interest in providing chitin and chitosan with desired functionalities that have not been chemically modified. One investigated approach is property modification via physical modification. Physical or physicochemical modification is primarily considered because it does not require a complicated chemical reaction. Appropriate active substances are added for the specific purpose, enhancing the physicochemical properties, and might only absorb or disperse within the chitin or chitosan matrix without covalent bond formation.

Furthermore, chitosan has chemically active multiple functional groups, including the amino ($–NH_2$), and primary and secondary hydroxyl (–OH) groups, whereas chitin has the chemically active functional groups of primary and secondary hydroxyl groups. Thus, it is feasible to perform chemical modification to improve their physical and chemical properties, while their biodegradability, biocompatibility, and non-toxicity should be maintained. Furthermore, the clear definition of the positions of functional groups in their repeating units is crucial to exploit regioselectivity in the chemical modification reactions in order to perform precise and well-controlled structural modifications of chitin and chitosan derivatives.

The most favorable way for chemical modification of chitin is *O*-substitution, in which the reaction occurs at hydroxyl groups (–OH), since the acetamido group ($–NHCOCH_3$) of chitin is not chemically active unless partially deacetylated into amino groups. Whilst, the most favorable way for chemical modification of chitosan is *N*-substitution, in which the reaction occurs at the amino group ($–NH_2$) of chitosan, *O*-substitution can be performed similar to chitin. Since the amino groups have a higher reactivity than hydroxyl groups, the *O*-substitution of chitosan alone requires the protection and deprotection of the primary amino groups. The individual *N*- or *O*-substituted derivatives of chitin and chitosan provide new functional groups, giving different bulk and chemical properties belonging to well-designed molecular structure.

Table 2 Commercially available chitin- and chitosan-based wound dressings and their functional uses related to wound healing

Based materials	Trademark (company)	Forms	Functions related to wound healing process	Ref.
Chitin	Beschitin (Unitika)	Chitin nonwoven	1. Treat traumatic and surgical wounds 2. Favor early GTF, no retractive scar formation	[85]
	Chitipack S (Eisai Co. Ltd.)	Foam, sponge	1. Treat traumatic and surgical wounds 2. Favor early GTF	[85–87]
	Chitipack P (Eisai Co. Ltd.)	Chitin hydrocolloid in poly(ethylene terephthalate)	1. Treat large skin defects and those difficult to suture 2. Favor early GTF	[85, 87]
	Syvek-Patch (Marine Polymer Technologies)	Fibrils, fibrous material	1. Control bleeding at vascular access sites 2. Accelerate hemostatic process	[85, 86, 88]
Chitosan	HemCon® Bandage (HemCon Medical Technologies Inc.)	Sponge	1. Accelerate hemostatic process 2. Support antimicrobial activity 3. Use in emergency and civilian	[85, 86, 89, 90]
	TraumaStat (Ore-Medix)	Sponge	1. Treat traumatic wounds 2. Accelerate hemostatic process	[85, 86, 91]
	Chitopack C (Eisai Co. Ltd.)	Cotton-like chitosan nonwoven	1. Repair body tissue completely 2. Rebuild normal subcutaneous tissue 3. Regenerate skin regularly	[85, 87]
	Tegasorb and Tegaderm (3 M)	Chitosan hydrocolloid and film	Suitable for leg ulcers, sacral, and chronic wounds	[85, 87]

Therefore, both physical and chemical modification are the appropriate approaches to enhance the functional properties of chitin- and chitosan-based wound dressings in specific purposes.

6 Chitin and Chitosan-Based Functional Wound Dressings

Wound dressing should remove excess exudates, keep the wound interface moist, allow gaseous exchange, and act as a barrier to microorganisms. It should also fulfill the fundamental concerns of being non-toxic, non-allergenic, non-adherent, and easily removed without trauma. Beyond these fundamental requirements, functional wound dressings should be designed to provide certain other functional properties suitable for different wound types. In addition to the conducting ability of materials to promote and allow the migration and proliferation of epithelial, fibroblast, and endothelial cells, the synthesis of ECM components is also required in wound repair and skin regeneration [92]. Therefore, insight into the details of developing chitin- and chitosan-based wound dressings through their physical and chemical modification to acquire functional applications, such as the treatment hemorrhage (hemostatic wound dressings), infection (antimicrobial wound dressings), burns (wound dressings for burns), and chronic ulcers (diabetic wound dressings), are mentioned in this chapter.

6.1 Hemostatic (Blood Coagulant) Wound Dressings

Hemorrhage is a major mortality plan from traumatic injuries, which frequently happens in accidents, battlefields, and operation rooms [93]. Hemostasis is the first phase of wound healing and effective hemostasis is mandatory to stem life-threatening bleeding. Hemostatic agents derived from chitosan have been widely studied for their fast absorbing and localized hemostatic effects. Chitosan is also well known for its antimicrobial properties, where its protection against a wide range of bacteria enhances its appeal as a bandage. Several chitosan wound dressings have been developed for the rapid and effective control of bleeding. Some of them have been commercialized, such as ChitoFlex®, CELOX™, QuikClot®, and HemCon® [94].

Chitosan dressings were prepared by dissolving chitosan in dilute aqueous acetic acid, placing into a mold, lyophilized, and then neutralization with NaOH [95]. The obtained chitosan dressing presented a fast absorption rate of human whole blood in less than 5 s, owing to its homogeneous and penetrating porous structure. However, it showed a slow clotting rate when tested with human whole blood containing a normal (250,000 platelets/mL) platelet number.

Chitosan electrospun mats have been prepared to enlarge the porosity, and they showed a higher rate of blood clotting than chitosan sponges [96]. This result indicated that the thrombogenic activity of chitosan was enhanced by increasing the pore size and porosity [96]. The addition of polyphosphate to chitosan led to a more potent hemostat [97]. Polyphosphate can ionically interact with chitosan to form polyelectrolyte complexes. Both components activated coagulation by a different mechanism. The protonated amino groups of chitosan attracted negatively

charged residues on red blood cell membranes, causing strong hemagglutination [98]. Chitosan also adsorbed fibrinogen and plasma proteins, enhancing platelet aggregation [99]. In contrast, polyphosphate specifically shortened both the time lag for initial thrombin generation and the time to peak thrombin generation [100].

Although numerous studies have reported the hemostatic property of chitosan, its insolubility and weak antibacterial activity are still limitations. Surface modification of chitosan, such as carboxymethylation and quaternization, has been investigated to improve its water solubility and antibacterial activity. Accordingly, nonwoven of carboxymethyl chitosan (CMCS), *N*-succinyl chitosan (NSCS), and *N,N,N*-trimethyl chitosan (TMCS) were prepared to examine the hemostatic property. It was found that the nonwoven CMCS, NSCS, and TMCS all showed a better hemostatic property than nonwoven chitosan [101]. However, nonwoven TMCS had a lower blood absorption than the others, which might be due to the quaternary ammonium cationic group leading to hemolysis [101]. The negative charges on the surface of NSCS and CMCS could accelerate blood coagulation by activation of the intrinsic coagulation pathway along with the hemostatic mechanism of chitosan [101].

Superabsorbent polymers (SAPs) derived from chitosan and its derivatives are promising hemostatic materials with a good absorption capability and potential hemostatic ability [102]. The SAP formed from carboxymethyl chitosan grafted poly(acrylic acid) (CMCTS-g-PAA) was prepared by graft copolymerization of acrylic acid on the CMCTS [103]. The porous structure of CMCTS-g-PAA was obtained by precipitation of the hydrogel in ethanol, and was found to be non-toxic, with a high swelling capacity and with a good hemostatic performance in the treatment of the hemorrhage model in rabbits [104]. This excellent hemostatic property results from the synergistic effect of the protonated amino groups of chitosan and the strong swelling capacity of the porous materials.

During the hemostasis and wound healing, bacterial infection is also a serious issue. If there is no infection, the wound healing proceeds more smoothly. However, infection may cause a series of bacteremia reactions and greatly threaten human life. Therefore, antimicrobial wound dressings have been designed to protect wounds from infection for better wound healing.

6.2 Antimicrobial Wound Dressings

It is well known that one of the main problems in wound care treatment is wound infection. Bacterial wound infection delays the wound healing process and possibly gives rise to life-threatening complications [105]. Generally, a large amount of fluid loss and bacterial infection would lead to serious consequences in severe burns or extensive skin loss. Widely spread opinions among wound care practitioners are that Gram-positive bacteria, such as *Staphylococcus aureus* (*S. aureus*), and Gram-negative bacteria, like *Pseudomonas aeruginosa* (*P. aeruginosa*), are the primary causes of delayed healing and infection in both acute and chronic wounds [106]. Wound dressings not only stop blood loss, but also protect the wound from

bacterial infection and accelerate the wound healing process. In addition, the wound exudates between the wound and the dressing can also lead to an infection. Therefore, the application of antibacterial wound dressings with a broad activity spectrum and high bactericidal activity is one of the effective approaches for treating infected wounds.

Chitin itself does not exhibit any marked antibacterial activity [107], while chitosan exhibits antibacterial activity when the amino groups are protonated in an acidic condition, but not in a neutral (physiological) condition [108]. At a neutral pH, the antibacterial activity of chitosan increased as the MW decreased [108]. It was found that a low solubility and negative zeta potential values were determined for chitosan with a MW of more than 29.2 kDa, which may explain the loss of its antibacterial activity at pH 7.0 [108]. Therefore, the most simple physical modification of chitin and chitosan wound dressings for antibacterial activity was by the incorporation of antibacterial agents, like inorganic metal NPs and organic bioactive substances. In addition, some chemical modifications of chitin and chitosan have been developed to improve the antibacterial properties of wound dressings.

6.2.1 Physical Modification of Chitin and Chitosan for Antimicrobial Wound Dressings

Incorporation of AgNPs or Ag^+

Many research groups have been focused on incorporation of various NPs in chitin and chitosan matrices, including those of Ag, zinc oxide (ZnO), and gold [109]. Among those metal and metal oxide NPs, AgNPs are the most widely used in wound dressings and wound healing, since they exhibit significant antimicrobial activities over a wide spectrum of pathogenic and drug-resistant strains [110], as well as an anti-inflammatory activity [111]. Structurally, AgNPs are clusters of silver atoms with particle sizes ranging in diameter from 1 to 100 nm, and so provide a high surface area. They have been prepared by the reduction of silver nitrate into AgNPs using sodium citrate as the reducing agent [112]. The minimum inhibitory concentration and minimum bactericidal concentration of AgNPs are in the range of 1.56 to 6.25 μg/mL and 12.5 μg/mL, respectively, against a broad spectrum of microorganisms, including *Escherichia coli* (*E. coli*), *P. aeruginosa*, *Salmonella abony*, *Salmonella typhimurium*, *Klebsiella aerogenes*, *Proteus vulgaris*, *S. aureus*, *Bacillus subtilis*, and *Staphylococcus epidermidis* [113]. The addition of AgNPs at concentrations of 0.001, 0.003, and 0.006% (w/w) into chitin hydrogels was evaluated for their antibacterial activity against *E. coli* and *S. aureus*, whole blood clotting, and cell viability on vero (epithelial) cells [112]. Owing to the physical absorption of AgNPs within the chitin hydrogels, the AgNPs were released from the chitin hydrogels and exhibited antibacterial activity against *E. coli* and *S. aureus.* The bacterial inhibition zone increased with increasing concentrations of AgNPs. The Gram-negative bacteria (*E. coli*) were more susceptible to the AgNPs than the

Gram-positive bacteria (*S. aureus*), which might be due to the limited penetration ability of AgNPs through the thick peptidoglycan wall of Gram-positive bacteria.

The bactericidal mechanism is certainly due to the released Ag^+ ions that act as reservoirs for the Ag^+ bactericidal agent. The Ag^+ ions are known to exert several antibacterial mechanisms. One is the interaction with phosphorus and sulfur groups of the bacterial cell wall and plasma membrane proteins [114, 115]. The interaction between Ag^+ and the membrane leads to dysfunction of the proteins, causing bacterial death. The Ag^+ ions can also bind to negatively charged components of the bacterial membrane, creating holes, leading to cytoplasmic leakage, and causing cell death. Once inside the cell, the Ag^+ ions can also disturb the bacterial electron transport [116]. In addition, the bactericidal effect of silver is achieved through cell membrane disruption, followed by protein dysfunction and breakage of DNA strands, resulting in an increased level of intracellular reactive oxygen species (ROS) [116]. The mode of action of Ag^+ is similar to that of AgNPs, but with stronger antibacterial efficiency [117].

In addition, chitin hydrogels with a high concentration of AgNPs shorten the blood clotting time [113, 118], while remaining non-toxic to vero cells. Indeed, low doses of AgNPs were reported to be non-toxic both in vitro and in vivo [113]. However, in contrast, the addition of AgNPs into a chitin/silk fibroin scaffold at 0.001, 0.01, and 0.1% (w/w) revealed cytotoxicity to HFF2 (fibroblast) cells, where the higher the AgNP concentration, the lower the HFF2 cell viability, especially at 0.1% (w/w) AgNPs [118].

Hybridization of Chitosan and ZnO

The addition of AgNPs at high concentrations causes a change in color and induces cell cytotoxicity, which seriously limits their applications. An alternative way to reduce the cytotoxicity but enhance the antibacterial activity is the hybridization of chitosan with other metal oxides, such as ZnO. A 0.3% (w/v) chitosan/2 mM ZnO hybrid was developed and coated on cotton fabric by sonochemical coating to a ZnO/chitosan mass ratio of around 0.086 [119]. The physicochemical properties of the individual composite were probably due to the formation of the Zn^{2+}/chitosan complex within the hybrid coatings, following the dissolution of ZnO in the chitosan/acetic acid solution. Therefore, two possible models for hybridization of metal ions with chitosan were the pendant model, where an ion was bound to only one amino group of chitosan, and the bridge model, where an ion was bound to several amino and hydroxyl groups of one or bridging more chitosan molecules [119]. The fabrics coated with the chitosan/ZnO hybrid were tested for their antibacterial activity with a 60 min contact time and exhibited a reduction in the bacterial viability of around 98% and 99% against *S. aureus* and *E. coli*, respectively, compared to 61 and 31% for ZnO and chitosan individually, respectively, against *S. aureus* and 78 and 99% against *E. coli*, respectively.

Chitosan and ZnO have been widely reported as efficient antibacterial agents. The mode of antibacterial action of ZnO involves the oxide dissolution to Zn^{2+} and the

association of Zn^{2+} with oxidative stress in bacteria cells and generation of ROS, which subsequently oxidized the cell contents and cause cell death [120]. The mode of antimicrobial activity by chitosan has been reported to be as follows: (1) interaction with negatively charged components in microbial membranes to alter the cell permeability and (2) binding to the DNA of bacterial cells to inhibit protein synthesis [121]. The hybrid complexes of chitosan with metal ions exhibited several-fold enhanced antibacterial properties compared to the individual components.

The cytotoxicity of chitosan, ZnO, and chitosan/ZnO hybrid was determined using an indirect contact method with fibroblasts for 24 h. The fibroblast cell viability following exposure to the ZnO-coated fabric dropped to less than 5%, whilst the chitosan and the chitosan/ZnO hybrid coating did not induce considerable cytotoxicity. Therefore, the synergistic effects of chitosan and ZnO NPs resulted in an enhanced antibacterial efficiency compared to the individual chitosan or ZnO coatings, as well as avoiding the risk of adverse effects on human health [119].

6.2.2 Chemical Modification of Chitin and Chitosan for Antimicrobial Wound Dressings

Currently, the usage of broad spectrum antibiotics is generally regarded as the most effective solution to such infections. Nevertheless, the overuse of antibiotics frequently leads to the evolution and spread of multi-drug-resistant bacteria. Therefore, new antibacterial agents from natural polymeric materials are worth developing. The amino groups of chitosan are protonated (positively charged) under an acidic condition, giving chitosan an interesting inherent antibacterial property. The antibacterial activity of chitosan is greater than chitin because it has a greater number of exposed protonated amino groups that can electrostatically interact with negatively charged proteins, lipids, and carbohydrates on the surface of bacterial cells, resulting in the inhibition of bacterial growth. Owing to their own comparatively low antibacterial activities at physiological pH (unprotonated state), several studies have proposed the synthesis of new chitin and chitosan derivatives for antibacterial agents. In particular, chitin derivatives can be produced by *O*-modification, while chitosan derivatives can be produced by *O*-modification, *N*-modification, or *N,O*-modification as mentioned above (Sect. 5). Thus, chitin, chitosan, and their derivatives have become one of the desirable materials for fabrication of antibacterial wound dressings. Several research works have focused on developing new derivatives of chitin and chitosan, especially quaternary ammonium derivatives, and to fabricate them as hydrogels or apply them as a textile finishing with an outstanding inherent antibacterial activity for wound therapy.

The modification of chitin and chitosan with quaternary ammonium groups enhances their water solubility and antibacterial properties, but only a limited amount of research has been focused on developing the quaternary derivatives of chitin compared to that for chitosan. Aminoethyl chitin [122] was synthesized by the heterogeneous reaction in alkaline condition of a mixed NaOH/isopropanol solvent with 2-AECH [122]. Amino ethyl chitin hydrogels have a good antibacterial activity

against *S. aureus*, where it is hypothesized that the antibacterial activity of chitin derivatives is related to the amino groups, and so the introduction of a multi-amino chitin derivative of poly(aminoethyl) chitin (PAEMC) was prepared via grafting aminoethyl moieties onto alkaline chitin chains with an excess amount of 2-AECH [123]. The PAEMC was synthesized in the form of polymerized amino ethyl monomers and exhibited a higher antimicrobial activity against Gram-positive bacteria than against Gram-negative ones [123]. The PAEMC exerted its antibacterial activity by a membrane damage mechanism.

Poly(amino ethyl) chitosan (PAEMCS) was also synthesized by the deacetylation of PAEMC [45], and its hydrogels were fabricated under the participation of dipotassium hydrogen phosphate. The resulting hydrogels showed a higher antibacterial activity against *E. coli* and *S. aureus* than that of chitosan, implying that the increased amount of amino groups improved the antibacterial activity. However, increasing the density of amino groups in PAEMCS showed a mild cytotoxicity to the L929 mouse fibroblast cell line and human umbilical vein endothelial cells (HUVECs). Similarly, PAEMCS also exhibited a higher antimicrobial activity against Gram-positive bacteria than Gram-negative ones [45].

Hydroxypropyl trimethylammonium chitin (HPTMAC) [124] was synthesized by a heterogeneous reaction in an alkaline condition using a NaOH/isopropanol solvent with 3-chloro-2-hydroxypropyltrimethylammonium chloride [124]. In addition, HPTMAC can also be synthesized by the homogeneous reaction in other green systems of a NaOH/urea aqueous solution [125] or a potassium hydroxide/urea aqueous solution [126] with 2,3-epoxypropyltrimethylammonium chloride. The obtained quaternary derivatives exhibited antibacterial activity. For HPTMAC, it exhibited excellent antimicrobial activities against *E. coli*, *S. aureus*, *Candida albicans*, and *Rhizopus oryzae* and revealed biocompatibility and significant accelerating consequences on the healing of uninfected, *E. coli*-infected, and *S. aureus*-infected wounds. Thus, it can be applied as a novel wound dressing for skin regeneration, particularly for infected wounds [126].

Moreover, chitin betainate was modified to enhance its antibacterial activity over a wide pH range [107]. It was prepared by the acylation of chitin with carboxymethyl trimethyl ammonium chloride (CMA). Chitin betainate at 10 mg/mL exhibited complete bactericidal activity against *E. coli* within 10 min, but a 45.2% and 78% reduction in *S. aureus* viability after 10 min and 24 h exposure, respectively, whilst chitin did not exhibit any significant antibacterial activity. The bactericidal activity was concentration-dependent against both bacteria, but it was more efficient against *E. coli* than *S. aureus* [107]. A chitin betainate hydrogel was prepared for antibacterial wound dressing purposes and had good water absorption by forming an interpenetrating network with PEG.

In order to investigate the structural effect of the quaternary ammonium chitin on the antibacterial activity and specificity against *E. coli* and *S. aureus*, chitin was modified with three different quaternary ammonium groups and spacers: (1) CMA to obtain CTCMA or chitin betainate as mentioned before, (2) 3-carboxypropyl trimethyl ammonium chloride to yield CTCPA, and (3) 3-carboxypropyl-*N*-dodecyl-*N,N*-dimethylammonium chloride to give CTDDMAB [127]. The

CTCMA consisted of an *N,N,N*-trimethyl substituent with a C1 (methyl) spacer; CTCPA consisted of an *N,N,N*-trimethyl substituent with a C3 (propyl) spacer; and CTDDMAB consisted of *N*-dodecyl-*N,N*-dimethyl substituent with a C3 spacer. Thus, CTCPA had the similar quaternary substituent as CTCMA but with a longer spacer (C3) between the chitin and quaternary substituent, whilst CTDDMAB had the same C3 spacer as CTCPA but with an *N*- long alkyl substituent.

The chemical structure of these quaternary ammonium chitin derivatives showed specificity and strongly influenced the antibacterial activity against *E. coli* and *S. aureus*. The outer cell wall of *E. coli*, which is rich in negatively charged lipopolysaccharide, is susceptible to the positively charged quaternary ammonium substituents of CTCMA and CTCPA, while the thick peptidoglycan cell wall of *S. aureus* functions as a barrier against penetration of polar substances. Accordingly, CTDDMAB with its longer propyl (C3) spacer or the *N*-long alkyl substituent in its structure enhanced the penetration and antibacterial activity against *S. aureus*. Therefore, the antibacterial activity of quaternary ammonium chitins against *E. coli* required only the positively charged quaternary ammonium substituents to contact with the outermost cell wall, while that against *S. aureus* required not only the length of the spacer, but also the *N*-long alkyl substituents.

Since chitosan itself exhibits antimicrobial activity without modification, but at comparatively low antibacterial activities at neutral or physiological conditions. Therefore, research on chemical modification of chitosan has focused on enhancing its antimicrobial activities. Quaternization of chitosan can be performed by two common ways, which are substitution of quaternary ammonium groups onto the chitosan backbone and substitution of those groups as the side chains. *N,N,N*-trimethyl chitosan (TMC), which has quaternary ammonium groups on the chitosan backbone, was synthesized by first treating chitosan with formic acid and formaldehyde, followed by methylation with methyl iodide [128]. The antibacterial activity of TMC was more effective than chitosan at pH 5.5, but less effective at pH 3.5. Because the lower pH represses the ionization of trimethylated amino groups of TMC [128]. For *N*-[(2-hydroxy-3-trimethyl ammonium) propyl] chitosan (HTCC), with quaternary ammonium groups as side chains, it was synthesized by coupling glycidyltrimethylammonium chloride to chitosan in water [129, 130]. Then, the HTCC was further chemically modified to obtain double bond containing derivatives of *O*-acrylamidomethyl-HTCC, which is chemically bound with cellulose or cotton fabrics, to make a promising antibacterial fabric. For *N,N,N*-trimethyl-*O*-(2-hydroxy-3-trimethylammonium propyl)chitosan (TMHTMAPC), with quaternary ammonium groups on both the backbone and *O*-side substituent, it was synthesized by chemical modification of TMC with 3-chloro-2-hydroxypropyl trimethyl ammonium chloride. The obtained TMHTMAPC exhibited a higher antibacterial activity than TMC [131], indicating that quaternization of the more flexible side chains enhanced the interaction between the positively charged chitosan derivatives and the negatively charged bacterial cell envelope [131]. Representative schemes for the quaternization of chitin/chitosan derivatives are summarized in Fig. 8.

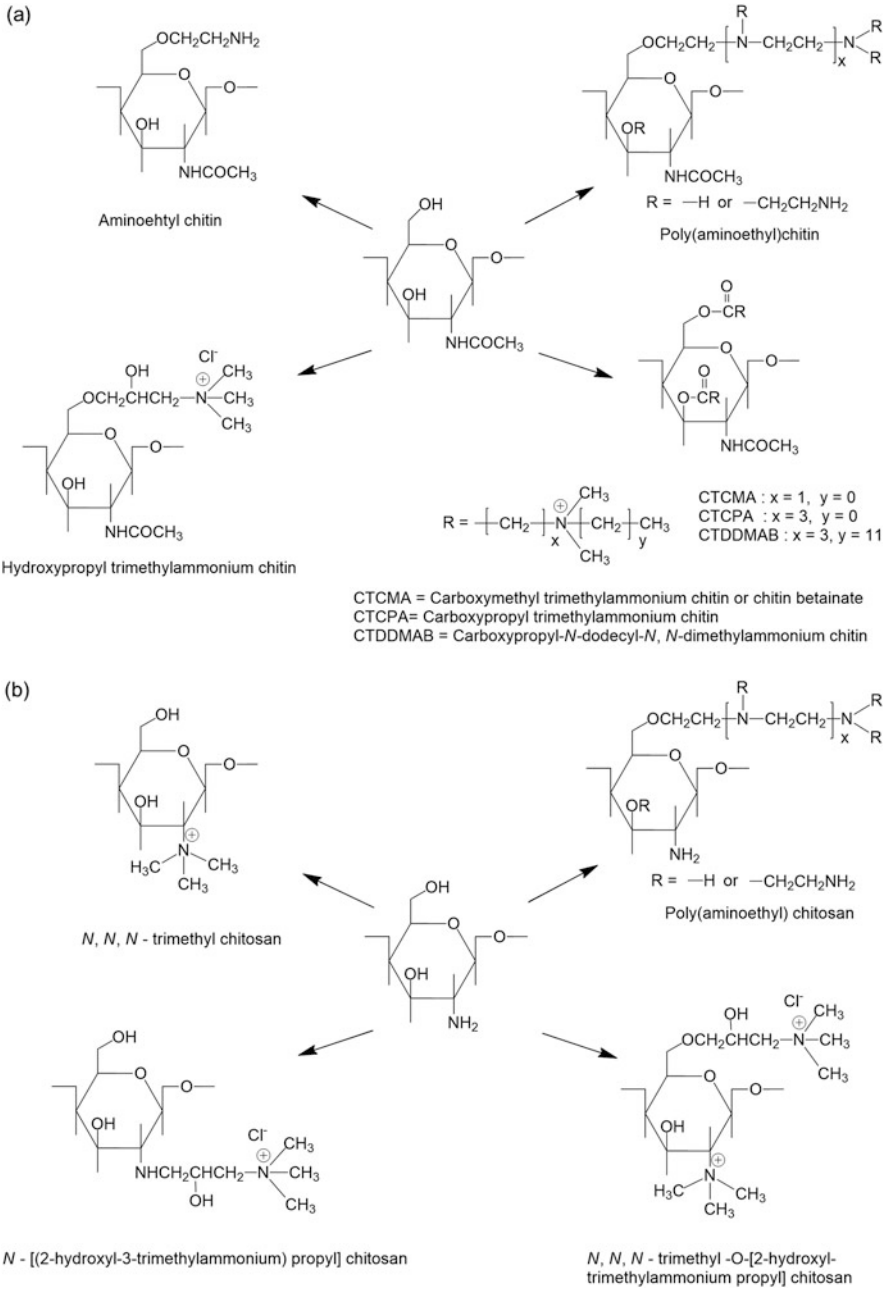

Fig. 8 Representative schemes for the quaternization of (**a**) chitin and (**b**) chitosan derivatives

Apart from the quaternary ammonium derivatives of chitin and chitosan, further information on the synthesis of chitin/chitosan derivatives and their molecular design for novel antimicrobial agents, mode of antimicrobial actions/mechanisms related to outermost membranes of microbes (bacteria, fungi, and viruses), and their compositions, as well as the effect of their MW and DA/DD on the antimicrobial activity has been presented elsewhere [132]. From the development of chitin and chitosan derivatives as antimicrobial agents, it is of great significance to fabricate them into antibacterial hydrogel wound dressings, taking advantage of their beneficial properties, including biodegradability, biocompatibility, non-toxicity, hemostatic, and antibacterial properties. Many molecularly designed chitin and chitosan derivatives appear to be promising candidates for antibacterial wound dressings.

6.3 Wound Dressings for Burn Wounds

The depth of a burn can be described as superficial, moderate partial thickness, deep partial thickness, or full thickness. A first-degree (superficial) burn is when the epidermis is injured with no significant structural damage, while a moderate second-degree (moderate partial-thickness) burn represents a superficial dermis injury. A deep second-degree (deep partial-thickness) burn is when the deep dermis is injured but the hair follicles and sweat glands remain intact, while a third-degree (full-thickness) burn is when the entire dermis is injured. Finally, a fourth-degree (full-thickness) burn is when the entire dermis is injured and it extends to the fat, muscle, and bone [133].

A superficial burn of the skin heals in a short time without a scar by epidermal resurfacing through the regeneration and migration of keratinocytes. A moderate second-degree burn heals within 1–2 weeks by epithelialization, but the skin pigmentation, somehow, changes. In contrast, a deep second-degree burn wound may not heal within 3–4 weeks by epithelialization, and it may form scars. Third- and fourth-degree burn wound healing requires tissue granulation followed by epithelialization. It often undergoes early surgical intervention [133].

Healing of a burn wound is one of the most complex healing process, causing severe discomfort and other complications. For treatment of burns of a high surface area, the patient might face a high risk of lethality and disability during the healing process. Patients are highly vulnerable to invasive microbial infections until complete re-epithelialization or recovery of the wound area has occurred. Consequently, burn wound sepsis is a major cause of mortality among these patients. Topical application of antibacterial ointment is the first choice of treatment in order to provide a moist environment together with antibacterial agents. Since chitosan itself possesses antimicrobial activity, it is particularly useful in wound treatment. The MW and DD of chitosan are structural parameters that influence its physicochemical, mechanical, and biological properties, such as its susceptibility to biodegradation by lysozyme and wound healing properties [134].

The effect of the MW and DD of chitosan on the wound healing process has been evaluated by topically applying three samples of chitosan gels with a different MW and DD on burn wounds in rats [135]. The gels were prepared at a concentration of 2% (w/v) in dilute aqueous acetic acid as CH-H (MW of 200,0000; DD of 92%), CH-M (MW of 750,000; DD of 75%), and CH-L (MW of 70,000; DD of 63%). The high MW (CH-H) gel resulted in the formation of significantly more epithelial tissue and wound contraction (around 80% within 8 days) than the wounds treated with the CH-M or CH-L gels, while the control wounds (no gel treatment) healed very slowly with a wound contraction of only around 40% after 12 days. It is evident that chitosan with a high DD and MW led to an accelerated wound healing and induced GTF or re-epithelialization in the early stages of wound healing [135]. Moreover, the incorporation of EGF at 10 μg/mL into a 2% (w/w) chitosan gel was reported to fasten the epithelialization rate and to stimulate GTF and tissue formation [136]. As known, EGF acts by binding to the EGF receptor–tyrosine kinase, thereby initiating a series of events that ultimately regulates cell proliferation [137]. Although present in small amounts, EGF exerts a powerful influence on the process of wound repair.

Wound dressings usually lead to rapid wound closure by preventing wound sepsis and excessive fluid loss through the open wounds. Therefore, suitable wound dressings should effectively prevent subsequent microbial invasion of the burn wounds as well as absorb excessive exudate. Chitosan acetate bandage (HemCon®), which is normally used as a hemostatic dressing, was applied to third-degree burns in mice infected with fatal doses of two invasive bacterial species (*P. aeruginosa* and *P. mirabilis*) [138]. The survival rates within 4 weeks of infection with *P. aeruginosa* and *P. mirabilis* were 73.3% and 66.7%, respectively, in mice treated with chitosan acetate bandages, whilst they were 13.3% and 0%, respectively, in the untreated (control) groups. The chitosan acetate bandage effectively controlled the growth of bacteria in the burn and prevented the development of systemic sepsis that led to fatality. Therefore, a chitosan acetate bandage could act as an effective topical antimicrobial dressing for infected burns. Furthermore, when *N*-carboxymethyl chitosan (NCMC), a water soluble chitosan derivative, was applied on deep second-degree burn wounds, the healing time of the wound was reduced to 25 days compared to 35 days for the untreated control group [139]. On day 25, a number of fibroblasts in the dermis could be observed in the NCMC treated groups, revealing that GTF was accelerated by the application of the NCMC. The GTF was required for the permanent closure of the wound because it filled the wound defect and prepared a route for epithelialization.

6.4 Wound Dressings for Diabetic Ulcers

Diabetes mellitus (DM), commonly known as diabetes, is a group of metabolic disorders characterized by a high blood sugar level over a long period of time (chronic hyperglycemia) caused by a low insulin level. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding

properly to the produced insulin. The progression of wound healing normally undergoes the four phases of hemostasis, inflammation, proliferation, and remodeling, as mentioned above (Sect. 2). However, wound healing in diabetics is complicated. A failure of completely healed diabetic foot ulcers (DFU) [140] is a consequence of chronic inflammation, peripheral neuropathy, impaired vascular function, or impaired angiogenesis [140, 141]. Moreover, fibroblasts fail to produce ECM proteins, while keratinocytes do not form an epithelium. The DFU is associated with a high risk of limb loss as a consequence of amputation and leads to a reduced survival [142]. To reduce the risk, much attention had been paid to the design and development of wound dressings that are suitable for diabetics. This chapter mentioned in detail on chitin- and chitosan-based wound dressings that have been developed through physical or chemical modification to promote the wound healing process for diabetic ulcers, which are categorized by those impaired consequences.

6.4.1 Wound Dressings Involved in Chronic Inflammation

A DFU is mostly at risk of infection due to the long existence of extensive sloughs, bacteria, and biofilms, which results in a delayed inflammatory phase. Therefore, wound dressings are often incorporated with antibacterial agents to promote their antibacterial activity, even though some polymeric materials, such as chitosan, may already have an antibacterial property. Several physical and chemical modifications of chitin and chitosan wound dressings involved in reducing chronic inflammation are mentioned in this section.

Physical Modification of Chitin and Chitosan Wound Dressings Involved in Chronic Inflammation

Incorporation of AgNPs or Ag^+

Both Ag^+ and AgNPs have been known as an antibacterial agent for a long time. Their toxicity to human cells is significantly lower than that to bacteria, which is the most favorable feature of silver [143], giving it a high availability for various applications, including wound dressing. In diabetes induced rabbits, the AgNP-loaded chitosan-PEG hydrogels were found to exhibit an excellent antibacterial activity against both Gram-positive (*S. aureus*, *B. subtilis*, and *B. pumilus*) and Gram-negative bacteria (*P. aeruginosa* and *E. coli*) [144]. Moreover, the AgNP-loaded chitosan-PEG hydrogels also offered a better wound healing capacity with a greater anti-inflammatory response and accelerated the re-epithelialization and collagen deposition compared to that with the blank hydrogel without AgNPs [144]. The wound healing potential of the AgNP-incorporated chitosan-PEG hydrogel was even faster than the blank hydrogel without AgNPs, indicating that the

combination of AgNPs and chitosan hydrogel significantly and synergistically enhanced wound healing in diabetes induced rabbits.

In addition to the anti-inflammatory function, the incorporation of growth factors into the AgNP-chitosan gel, such as EGF, can enhance keratinocyte and fibroblast proliferation, GTF, and ECM protein synthesis, and so result in promoting the efficiency of wound repair for diabetics. However, the antimicrobial activity of AgNPs may be considerably diminished after binding with proteins, such as growth factors [145]. Such unfavorable conditions likely occur even when Ag^+ and proteins are immobilized in the same matrix, whereby the usability of Ag^+ in the EGF-embedded hydrogel is seriously hindered. An alternative way is to encapsulate EGF into chitosan NPs and then disperse these into a AgNP-incorporated chitosan-PVA hydrogel, i.e. AgNPs and EGF-encapsulated chitosan hydrocolloid in chitosan-PVA hydrogel (SNP_ECHG) [145]. The SNP_ECHG was incorporated with different amounts of AgNPs (reduced from Ag^+ concentrations of 6, 12, 24, and 48 mM) and different concentrations of EGF (0.6, 6, and 60 μg/mL). Owing to the release of Ag^+, the SNP_ECHG exhibited antimicrobial activity against *S. aureus* and *S. epidermidis* when the hydrogel contained more than 24 mM Ag^+, whilst the cytotoxicity of the hydrogels to both fibroblasts and keratinocytes increased in a AgNP concentration-dependent manner. Therefore, for an effective antimicrobial activity and minimal cytotoxicity, the optimal Ag^+ dose in the hydrogel was 24 mM, while the optimal dose of EGF for promoting cell growth was around 60 μg/mL [145]. Furthermore, SNP_ECHG revealed a clear efficiency for diabetic wound healing, which was ascribed to its antimicrobial and cell growth promoting activities compared to treatment with AgNPs alone, EGF alone, or without silver and EGF [145].

Incorporation of Curcumin (Cur)

In addition to AgNPs, Cur, the main bioactive substance of turmeric, has been evaluated for its anti-inflammatory and antioxidant potential [146]. It is well known that macrophages play an important role in regulating the inflammatory response as well as in removing dead cells and cell debris from the wound. The delayed wound healing in diabetes is attributed to the prolonged inflammation, which is always induced by a large amount of inflammatory mediators secreted by macrophages. Curcumin-loaded chitosan NPs (Cur-CS-NPs) could effectively inhibit macrophage-mediated inflammation and reduce the release of inflammatory factors from the RAW264.7 mouse mononuclear macrophage leukemia cell line and attenuated local inflammation in the wound site of diabetic rats, thereby promoting the wound healing process to shift from the inflammatory phase to the proliferation and remodeling phases [147]. Furthermore, Cur-CS-NPs enhanced angiogenesis by promoting proliferation and migration of HUVECs as well as to the diabetic wound site [147]. Moreover, Cur-CS-NPs accelerated the wound closure rates, revealing that it significantly promoted wound contraction and accelerated wound healing.

Chemical Modification of Chitin and Chitosan Wound Dressings Involved in Chronic Inflammation

The addition of antimicrobial substances is straightforward to the specific purposes of developing anti-inflammatory wound dressings. However, as mentioned before, dysfunctional macrophages can induce chronic inflammation and impair tissue regeneration in diabetic wounds. Therefore, improving macrophage behaviors and functions may enhance the therapeutic effects of diabetic wound healing [148]. The incorporation of exogenous cytokines to improve macrophage behaviors is, however, subject to the instability of cytokines, their high cost, and uncertain dosage. To improve the macrophage response, the design of a functional biomaterial without the addition of cytokines is of interesting in diabetic wound healing.

Sulfated chitosan (SCS), a heparin-like substance, was crosslinked with collagen type I (Col I/SCS) and evaluated for its activity on cultured peritoneal macrophage (PM) in terms of the inflammatory response [148]. Macrophages polarize their functional phenotypes from pro-inflammatory (M1) to anti-inflammatory (M2) and provide different functions. Pro-inflammation macrophages (M1) remove pathogens and tissue debris and secrete pro-inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β), ROS, and proteases, while the anti-inflammation macrophages (M2) secrete growth factors (e.g., FGF, EGF, and VEGF) and anti-inflammatory cytokines (e.g. IL-4 and IL-10) to resolve inflammation and stimulate tissue regeneration. It was found that Col I/SCS hydrogel reduced the expression of IL-6 and increased the expression of IL-4, IL-10, and TGF-β1 in macrophages, i.e. a Col I/SCS hydrogel promoted the secretion of anti-inflammatory cytokines and reduced the secretion of pro-inflammatory cytokines in macrophages [148], implying it could improve the shift of wound healing phases. Furthermore, the Col I/SCS hydrogel promoted the transdifferentiation of macrophages into fibroblasts, which enhanced collagen deposition and accelerated wound contraction in diabetic wounds.

6.4.2 Wound Dressings Involved in Neuroactive Substances

Incorporation of Neuropeptides

It is evident that peripheral nerves and cutaneous neurobiology contribute to diabetic wound healing [149], where a loss of peripheral sensory and autonomic nerves along with diminished neuropeptide production precedes the clinical symptoms of neuropathy [149]. Peripheral sensory neuropathy is considered to be a major contributor to the increased risk of foot amputations. Therefore, neuropeptides are an important link that directly connects neuropathy to wound healing. Neurotensin (NT) are bioactive neuropeptides that are widely distributed in the brain and in several peripheral tissues. They are involved in the activation, growth, migration, and maturation of specific skin cells, such as keratinocytes, macrophages, and mast cells, and affect new vessel formation and enhance angiogenesis during wound healing [141]. However, local administration of neuropeptides has the problem of

their short half-life and loss of bioactivity in the wound environment. Therefore, an alternative way is to load NT into chitosan-based wound dressings for their sustained delivery [150].

A water soluble chitosan derivative of 5-methyl pyrrolidinone chitosan (MPC) was synthesized and used as an NT carrier in wound dressing. A different healing profile was observed different for the non-loaded and NT-loaded MPC treated wounds in both the control (normal) and diabetic mice. Interestingly, wound reduction was evidently observed at around 20 and 40% on day 1, and at around 50 and 70% on day 10 post-wounding with MPC and NT-loaded MPC treatment in diabetic mice, respectively, indicating that an NT-loaded MPC was significantly more effective than MCP treatment alone. Moreover, granulation tissue filled the wound bed quicker in the early phase of wound healing in diabetic mice treated with NT and/or MPC. These phenomena indicated that NT-loaded MPC wound dressing improved the early wound healing in diabetics. Histopathological observation of the wound on day 10 revealed specific re-epithelialization profiles from the bottom to the top with basal cells in the epidermis covering the scar in control mice, whilst that occurred over the granulation inflammatory tissue which was undergoing repair [150].

Incorporation of Melatonin

Melatonin (*N*-acetyl-5-methoxytryptamine; Mel), the main hormone released from the pineal gland release, possesses widespread neuroprotective, antioxidant, and anti-inflammatory properties [151]. Its neuroprotective property makes it beneficial for treatment in neurological diseases, such as stroke, Alzheimer's disease, and Parkinson's disease [152]. Its antioxidant and anti-inflammatory properties, together with its neuroprotective property, make Mel an interesting potential therapeutic agent for application in wound healing related to the peripheral neuropathy of diabetic wounds. However, Mel is highly susceptible to oxidation, and so encapsulation of Mel into polymeric NPs is used to prevent its degradation. Therefore, Mel was loaded in lecithin-chitosan NPs (Mel-NP) [153]. The volume of the formulations was calculated according to the individual daily weight and locally dropped on the wound of diabetic rats at a Mel dose of 1.2 mg/kg rats/day. The wounds of the Mel-NP and NP alone treated groups were almost closed on day 7 with significant wound reduction on day 14, compared to the free Mel treated groups. In addition, NP alone stimulated fibroblast proliferation, whilst Mel-NP accelerated collagen deposition and blood vessel proliferation [153].

Another study of Mel loaded in a chitosan hydrogel also supported that Mel-loaded hydrogels promoted angiogenesis in the early stage of wound healing and reduced inflammation in the late stage [154]. The Mel-loaded hydrogel also markedly increased the expression of collagen III, α-smooth muscle actin and TGF-β1 proteins and reduced collagen I expression. These results suggested that Mel-loaded hydrogel promoted GTF and accelerated wound healing by reducing inflammation and promoting angiogenesis and collagen deposition [154].

Diabetes or hyperglycemia increases the levels of pro-inflammatory cytokines, including IL-1β, IL-6, and TNF-α, and increases oxidative stress, which mainly cause an impaired wound healing. Excessive production of ROS results in damage to cellular membranes, lipids, proteins, and DNA as well as becoming harmful to wound healing [151]. Melatonin down-regulates a variety of pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α, by preventing the translocation of nuclear factor-kappa B to the nucleus and its binding to DNA, as well as reducing oxidative stress [155]. Moreover, the molecular mechanisms of Mel action on diabetic wound healing had been examined in high glucose (HG) cultured keratinocytes [151]. It was found that (1) Mel reduced the mRNA expression level and release of pro-inflammatory cytokine in HG cultured keratinocytes, (2) reduced HG-induced oxidative stress in keratinocytes, (3) inhibited HG-induced activation of nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing three inflammasome, and (4) enhanced proliferation and migration as well as reduced apoptosis of keratinocytes. Therefore, Mel contributes to diabetic wound healing, including via inflammation suppression, oxidative stress attenuation, proliferation, migration promotion, and apoptosis inhibition.

6.4.3 Wound Dressings Involved in Angiogenesis

Incorporation of Bioactive Substances

Angiogenesis, the main step in the proliferative phase of wound healing, involves the formation of new blood vessels into the wound site and provides nutrition for wound healing [156]. Wound dressings can be used to deliver bioactive agents to wound sites for promoting epithelialization and treatment of severe injuries. To this purpose of promoting angiogenesis and wound healing, several research works have been reported to incorporate bioactive substances into the chitin or chitosan matrix. The analysis of non-healing acute or chronic wounds in vivo and in vitro demonstrated that those wounds were de-regulated for various growth factors, such as PDGF, VEGF, and basic (b)FGF [157]. Therefore, using exogenous growth factors and cytokines suggests a potential therapy for non-healing wounds, such as DFUs, pressure ulcers, and chronic venous leg ulcers.

Basic FGF, released by platelets, is a potent modulator for fibroblasts and the proliferation and angiogenesis of vascular endothelial cells [157]. The application of bFGF solution onto the wound, however, cannot accelerate wound healing because the bFGF is rapidly washed off at the wound site by the exudate. The incorporation of bFGF into chitosan-based wound dressings is an alternative way to stabilize and control the release of growth factors [158]. A bFGF was mixed with hydroxypropyl chitosan (HPCH) acetate, and cast as 1×1 cm^2 films with total bFGF concentrations of 0.6, 2, and 6 μg/film. The bFGF-HPCH films with different bFGF concentrations and HPCH film were placed onto full-thickness wounds excised on the back of diabetic mice. Normal saline was used in the control group. The wound sizes in the HPCH film and control groups were similar, indicating no significant acceleration of

impaired wound healing by the application of the HPCH film alone [158]. On the other hand, bFGF-HPCH films accelerated the wound healing in a bFGF dose-dependent manner. The wound sizes in the bFGF-HPCH film groups (2 μg and 6 μg/film) were significantly smaller than those in the three other groups, which indicated that the bFGF-HPCH film provided a sustained release of bFGF and accelerated wound healing in genetically diabetic mice [158].

However, the growth factors absorbed in a chitosan matrix are diffusible in vivo. The bFGF mostly caused a burst release with a low long-term accumulation at the wound sites. In order to maintain the bFGF concentration in the therapeutic range, a high dose administration is required. However, a high concentration of bFGF will lead to a vascular tumor and cancer angiogenesis [159]. To this end, bFGF immobilized onto the chitin binding domain (ChtBD) was made and named ChtBD-bFGF [160]. The ChtBD was derived from the chitinase of *Bacillus circulans* WL-12 and the ChtBD-bFGF or bFGF were absorbed onto the surface of chitin films by immersion. The amount of ChtBD-bFGF absorbed on the surface of the chitin film was determined by immunofluorescence and found to be 3.02-fold higher than that of bFGF. On the other hand, the ChtBD-bFGF showed a sustained release manner from the chitin film, whilst bFGF without ChtBD immobilization had a high burst release. The chitin films bound with ChtBD-bFGF or bFGF were subsequently evaluated for their ability in wound healing in terms of cell proliferation and promoting vascularity. It was found that the number of fibroblast cells induced by ChtBD-bFGF was twofold higher than by bFGF with a regular cell morphology, and an orderly and parallel actin filament. Furthermore, the endothelial cell-specific protein marker (CD31) was used to evaluate neovascularization at the implanted site. No obvious neovascularization was observed at the implanted site of the bFGF chitin film or chitin film alone, but was highly observed at the implanted site of the ChtBD-bFGF chitin film, indicating the promotion of angiogenesis [160].

The impaired wound healing of diabetic wounds is attributed to the low levels of endogenous growth factors, including VEGF, that normally stimulate multiple phases of wound healing. Besides, chronic diabetic wounds exhibit high levels of matrix metalloproteinases and give rise to the degradation of growth factors [161]. Therefore, attention had been paid to the incorporation of VEGF-loaded NPs to a chitosan-hyaluronic acid composite sponge, which served as a growth factor releasing wound dressing [162]. The released VEGF was sufficient to enhance tube formation in endothelial cells (HUVECs) in vitro, whilst the chitosan-hyaluronic acid sponge did not show any such property. The mechanism by which VEGF induced angiogenesis involves activation of integrins, which is essential for endothelial cell proliferation [162].

Moreover, as multiple proteins are involved in the wound healing process, it might be insufficient to use a single growth factor to accelerate wound closure in diabetic ulcers. While VEGF induces endothelial cell proliferation and migration, bFGF is a potent mitogen for fibroblasts and keratinocytes, and is involved in the proliferative phase of wound repair. It has been demonstrated that combined gene transfers of VEGF and FGF improves the reparative processes in the wounded skin of diabetic mice better than the single-agent treatment [163].

Clinical treatment of applying mixed growth factors from an autologous platelet-rich plasma gel (PRP) combined with a porous chitosan-alginate membrane to venous chronic ulcers has been reported [164]. Various proteins necessary for tissue repair and healing process are secreted by three types of granules (alpha, delta, and lambda) located inside the platelets. Alpha-granules are the most abundant platelet granule, and their degranulation releases several growth factors, such as PDGF, PDEGF, VEGF, TGF-β, EGF, IGF, and other bioactive substances, such as fibrinogen, fibronectin, osteocalcin, vitronectin, IL-1, and thrombospondin-1 [34]. The combination of various growth factors within the PRP gel and chitosan-alginate membrane gave the effective results of GTF and angiogenesis [164]. One of the most favorable results was the pain reduction in wounds from the early treatment, which was probably due to the acceleration of wound healing that resulted in a decreased analgesic intake by the patients. Owing to its high swelling capability, the exudate released was well absorbed into porous membrane and contributed significantly to the reduction in the intensity of a bad smell [164].

7 Conclusion

Chitin and chitosan both have a high potential for wound healing applications because of their prominent properties, such as exudates absorbability, stimulating hemostasis, and accelerating tissue regeneration. They can be fabricated into various forms, such as hydrogels, membranes, fibrous mats, sponges, and hydrocolloids, making chitin and chitosan promising materials for wound dressings and being commercially available. Beyond the fundamental requirements of non-toxic, non-allergenic, and non-adherent, many functional wound dressings of chitin and chitosan have been designed to provide certain other functional properties suitable for different wound types.

In order to design effective chitin and chitosan wound dressings, this chapter provided an insight information into the wound healing process and factors effecting wound healing, the fabrication methods of chitin and chitosan wound dressings, commercially available chitin- and chitosan-based wound dressings as well as the physical and chemical modification of chitin and chitosan to make more functional wound dressings, such as hemostatic, antimicrobial, burn, and diabetic ulcer wound dressings.

References

1. Epstein FH, Singer AJ, Clark RA (1999) Cutaneous wound healing. N Engl J Med 341:738–746
2. Korting HC, Schöllmann C, White RJ (2011) Management of minor acute cutaneous wounds: importance of wound healing in a moist environment. J Eur Acad Dematol Venereol 25:130–137

3. Robson MC, Steed DL, Franz MG (2001) Wound healing: biologic features and approaches to maximize healing trajectories. Curr Probl Surg 38:72–140
4. Lazurus GS, Cooper DM, Knighton DR, Margolis DJ, Pecoraro RE, Rodeheaver G, Robson MC (1994) Definitions and guidelines for assessment of wounds and evaluation of healing. Arch Dermatol 130:489–493
5. Velnar T, Bailey T, Smrkolj V (2009) The wound healing process: an overview of the cellular and molecular mechanisms. J Int Med Res 37:1528–1542
6. Dreifke MB, Jayasuriya AA, Jayasuriya AC (2015) Current wound healing procedures and potential care. Mater Sci Eng C 48:651–662
7. Hinrichs WL, Lommen EL, Wildevuur CR, Feijen J (1992) Fabrication and characterization of an asymmetric polyurethane membrane for use as a wound dressing. J Appl Biomater 3:287–303
8. Akita S, Akino K, Imaizumi T, Tanaka K, Anraku K, Yano H, Hirano A (2006) A polyurethane dressing is beneficial for split-thickness skin-graft donor wound healing. Burns 32:447–451
9. Joseph B, Augustine R, Kalarikkal N, Thomas S, Seantier B, Grohens Y (2019) Recent advances in electrospun polycaprolactone based scaffolds for wound healing and skin bioengineering applications. Mater Today Commun 19:319–335
10. Chen H, Huang J, Yu J, Liu S, Gu P (2011) Electrospun chitosan-graft-poly (ε-caprolactone)/poly (ε-caprolactone) cationic nanofibrous mats as potential scaffolds for skin tissue engineering. Int J Biol Macromol 48:13–19
11. Gomaa SF, Madkour TM, Moghannem S, El-Sherbiny IM (2017) New polylactic acid/cellulose acetate-based antimicrobial interactive single dose nanofibrous wound dressing mats. Int J Biol Macromol 105:1148–1160
12. Echeverría C, Muñoz-Bonilla A, Cuervo-Rodríguez R, López D, Fernández-García M (2019) Antibacterial PLA fibers containing Thiazolium groups as wound dressing materials. ACS Appl Bio Mater 2:4714–4719
13. Chen SL, Fu SH, Liao SF, Liu SP, Lin SZ, Wang YC (2018) A PEG-based hydrogel for effective wound care management. Cell Transplant 27:275–284
14. Shingel KI, Di Stabile L, Marty JP, Faure MP (2006) Inflammatory inert poly(ethylene glycol)–protein wound dressing improves healing responses in partial- and full-thickness wounds. Int Wound J 3:332–342
15. Xu R, Luo G, Xia H, He W, Zhao J, Liu B, Tan J, Zhou J, Liu D, Wang Y, Yao Z, Zhan R, Yang S, Wu J (2015) Novel bilayer wound dressing composed of silicone rubber with particular micropores enhanced wound re-epithelialization and contraction. Biomaterials 40:1–11
16. Pinese C, Jebors S, Stoebner PE, Humblot V, Verdie P, Causse L, Garric X, Taillades H, Martinez J, Mehdi A, Subra G (2017) Bioactive peptides grafted silicone dressings: a simple and specific method. Mater Today Chem 4:73–83
17. Jayakumar R, Prabaharan M, Sudheesh Kumar PT, Nair SV, Tamura H (2011) Biomaterials based on chitin and chitosan in wound dressing applications. Biotechnol Adv 29:322–337
18. Moeini A, Pedram P, Makvandi P, Malinconico M, Gomez d'Ayala G (2020) Wound healing and antimicrobial effect of active secondary metabolites in chitosan-based wound dressings: a review. Carbohydr Polym 233:115839
19. Cunha CS, Castro PJ, Sousa SC, Pullar RC, Tobaldi DM, Piccirillo C, Pintado MM (2020) Films of chitosan and natural modified hydroxyapatite as effective UV-protecting, biocompatible and antibacterial wound dressings. Int J Biol Macromol 159:1177–1185
20. Graça MFP, Miguel SP, Cabral CSD, Correia IJ (2020) Hyaluronic acid – based wound dressings: a review. Carbohydr Polym 241:116364
21. Duan Y, Lia K, Wang H, Wu T, Zhao Y, Li H, Tang H, Yang W (2020) Preparation and evaluation of curcumin grafted hyaluronic acid modified pullulan polymers as a functional wound dressing material. Carbohydr Polym 238:116195

22. Gaspar-Pintiliescu A, Stanciuc A-M, Craciunescu O (2019) Natural composite dressings based on collagen, gelatin and plant bioactive compounds for wound healing: a review. Int J Biol Macromol 138:854–865
23. Ying H, Zhou J, Wang M, Su D, Ma Q, Lv G, Chen J (2019) In situ formed collagen-hyaluronic acid hydrogel as biomimetic dressing for promoting spontaneous wound healing. Mater Sci Eng C 101:487–498
24. Varaprasad K, Jayaramudu T, Kanikireddy V, Toro C, Sadiku ER (2020) Alginate-based composite materials for wound dressing application: a mini review. Carbohydr Polym 236:116025
25. Zhang M, Zhao X (2020) Alginate hydrogel dressings for advanced wound management. Int J Biol Macromol 162:1414–1428
26. Radhakumary C, Antonty M, Sreenivasan K (2011) Drug loaded thermoresponsive and cytocompatible chitosan based hydrogel as a potential wound dressing. Carbohydr Polym 83:705–713
27. Rinaudo M (2006) Chitin and chitosan: properties and applications. Prog Polym Sci 31:603–632
28. Singh DK, Ray AR (2000) Biomedical applications of chitin, chitosan and their derivatives. J Macromol Sci Rev Macromol Chem Phys C 40:69–83
29. Aiba S (1992) Studies on chitosan: 4. Lysozymic hydrolysis of partially N-acetylated chitosans. Int J Biol Macromol 14:225–228
30. Pangburn S, Trescony P, Heller J (1982) Lysozyme degradation of partially deacetylated chitin, its films and hydrogels. Biomaterials 3:105–108
31. Paul W, Sharma CP (2004) Chitin and alginates wound dressings: a short review. Trends Biomater Artif Organs 18:18–23
32. Guo S, DiPietro LA (2010) Factors affecting wound healing. J Dent Res 89:219–229
33. Bergmeier W, Hynes RO (2012) Extracellular matrix proteins in hemostasis and thrombosis. Cold Spring Harb Perspect Biol 4:a005132
34. Lubkowska A, Dolegowska B, Banfi G (2012) Growth factor content in PRP and their applicability in medicine. J Biol Regul Homeost Agents 26(2 Suppl 1):3S–22S
35. Hart J (2002) Inflammation. 1: its role in the healing of acute wounds. J Wound Care 11:205–209
36. Mosser DM, Edwards JP (2008) Exploring the full spectrum of macrophage activation. Nat Rev Immunol 8:958–969
37. Singh S, Young A, McNaught C (2017) The physiology of wound healing. Surgery 35:473–477
38. Gonzalez AC, Costa TG, Andrade ZA, Medrado ARAP (2016) Wound healing – a literature review. An Bras Dermatol 91:614–620
39. Ogawa R, Akaishi S (2016) Endothelial dysfunction may play a key role in keloid and hypertrophic scar pathogenesis – keloids and hypertrophic scars may be vascular disorders. Med Hypotheses 96:51–60
40. Muzzareli RRA (1985) Chitin. In: Mark HF, Bikales NM, Overberger CG, Menges G, Kroschwitz JI (eds) Encyclopedia of polymer science and engineering, vol 3. Wiley, New York, pp 430–440
41. Li H, Cheng F, Wei X, Yi X, Tang S, Wang Z, Zhang YS, He J, Huang Y (2021) Injectable, self-healing, antibacterial, and hemostatic N,O-carboxymethyl chitosan/oxidized chondroitin sulfate composite hydrogel for wound dressing. Mater Sci Eng C 118:111324
42. Balitaan JNI, Hsiao C-D, Yeh J-M, Santiago KS (2020) Santiago innovation inspired by nature: biocompatible self-healing injectable hydrogel based on modified-β-chitin for wound healing. Int J Biol Macromol 162:723–736
43. Tanodekaew S, Prasitsilp M, Swasdison S, Thavornyutikarn B, Pothsree T, Pateepasen R (2004) Preparation of acrylic grafted chitin for wound dressing application. Biomaterials 25:1453–1460

44. Ma M, Zhong Y, Jiang X (2020) Thermosensitive and pH-responsive tannin-containing hydroxypropyl chitin hydrogel with long-lasting antibacterial activity for wound healing. Carbohydr Polym 236:116096
45. Zhang Y, Dang Q, Liu C, Yan J, Cha D, Liang S, Li X, Fan B (2017) Synthetic, characterization, and evaluation of poly(aminoethyl) modified chitosan and its hydrogel used as antibacterial wound dressing. Int J Biol Macromol 102:457–467
46. Shahzadi L, Bashir M, Tehseen S, Zehra M, Mehmood A, Chaudhry AA, Rehman I, Yar M (2020) Thyroxine impregnated chitosan-based dressing stimulate angiogenesis and support fast wounds healing in rats: potential clinical candidates. Int J Biol Macromol 160:296–306
47. Cifuentes A, Gómez-Gil V, Ortega MA, Asúnsolo Á, Coca S, Romná JS, Álvarez-Mon M, Buján J, García-Honduvilla N (2020) Chitosan hydrogels functionalized with either unfractionated heparin or bemirin improve diabetic wound healing. Biomed Pharmacother 129:110498
48. Masood N, Ahmed R, Tariq M, Ahmed Z, Masoud MS, Ali I, Ashar R, Andleeb A, Hasan A (2019) Silver nanoparticle impregnated chitosan-PEG hydrogel enhances wound healing in diabetes induced rabbits. Int J Pharm 559:23–26
49. Yang J, Chen Y, Zhao L, Feng Z, Peng K, Wei A, Wang Y, Tong Z, Chen B (2020) Preparation of a chitosan/carboxymethyl chitosan/AgNPs polyelectrolyte composites physical hydrogel with self-healing ability, antibacterial properties, and good biosafety simultaneously, and its application as a wound dressing. Compos B Eng 197:108139
50. Zhang Y, Jiang M, Zhang Y, Cao Q, Wang X, Han Y, Sun G, Li Y, Zhou J (2019) Novel lignin-chitosan-PVA-composite hydrogel for wound dressing. Mater Sci Eng C 104:110002
51. Bukzem AL, Signini R, dos Santos DM, Lião LM, Ascheri DPR (2016) Optimization of carboxymethyl chitosan synthesis using response surface methodology and desirability function. Int J Biol Macromol 85:615–624
52. Wang D, Zhang N, Meng G, He J, Wu F (2020) The effect of form of carboxymethyl-chitosan dressings on biological properties in wound healing. Colloids Surf B Biointerfaces 194:111191
53. Singh R, Singh D (2014) Chitin membranes containing silver nanoparticles for wound dressing application. Int Wound J 11:264–268
54. Singh R, Chacharkar MP, Mathur AK (2008) Chitin membrane for wound dressing application – preparation, characterization and toxicological evaluation. Int Wound J 5:665–673
55. Chakravarty J, Semerdzhiev D, Silby MW, Ferreira T, Brigham CJ (2019) Properties of solvent-cast chitin membranes and exploration of potential applications. Materialia 8:100452
56. Miguel SP, Moreica AF, Correia IJ (2019) Chitosan based-asymmetric membranes for wound healing: a review. Int J Biol Macromol 127:460–475
57. Behera SS, Das U, Kumar A, Bissoyi A, Singh AK (2017) Chitosan/TiO_2 composite membrane improves proliferation and survival of L929 fibroblasts cells: application in wound dressing and skin regeneration. Int J Biol Macromol 98:329–340
58. Kenawy E, Omer AM, Tamer TM, Elmeligy MA, Eldin MSM (2019) Fabrication of biodegradable gelatin/chitosan/cinnamaldehyde crosslinked membranes for antibacterial wound dressing application. Int J Biol Macromol 139:440–448
59. Mi F-L, Wu Y-B, Shyu S-S, Chao A-C, Lai J-Y, Su C-C (2003) Asymmetric chitosan membranes prepared by dry/wet phase separation: a new type of wound dressing for controlled antibacterial release. J Membr Sci 212:237–254
60. Mi F-L, Shyu S-S, Wu Y-B, Lee S-T, Shyong J-Y, Huang R-N (2001) Fabrication and characterization of a sponge-like asymmetric chitosan membrane as a wound dressing. Biomaterials 22:165–173
61. Morgado PI, Lisboa PF, Ribeiro MP, Miguel SP, Simões PC, Correia IJ, Aguiar-Ricardo A (2014) Poly (vinyl alcohol)/chitosan asymmetrical membranes: highly controlled morphology toward the ideal wound dressing. J Membr Sci 469:262–271

62. Yang JM, Yang SJ, Lin HT, Wu T-H, Chen H-J (2008) Chitosan containing PU/poly (NIPPAm) thermosensitive membrane for wound dressing. Mater Sci Eng C 28:150–156
63. Lin W-C, Lien C-C, Yeh H-J, Yu C-M, Hsu S-H (2013) Bacterial cellulose and bacterial cellulose-chitosan membranes for wound dressing applications. Carbohydr Polym 94:603–611
64. Ignatova M, Starbova K, Markova N, Manolova N, Rashkov I (2006) Electrospun nano-fiber mats with antibacterial properties from quanternised chitosan and poly (vinyl alcohol). Carbohydr Res 341:2098–2107
65. Trinca RB, Wastin CB, Silva JAF, Moraes ÂM (2017) Electrospun multilayer chitosan scaffolds as potential wound dressings for skin lesions. Eur Polym J 88:161–170
66. Naseri N, Algan C, Jacobs V, John M, Oksman M, Mathew AP (2014) Electrospun chitosan-based nanocomposite mats reinforce with chitin nanocrystals for wound dressing. Carbohydr Polym 109:7–15
67. Chen J-P, Chang G-Y, Chen J-K (2008) Electrospun collagen/chitosan nanofibrous membrane as wound dressing. Colloids Surf A Physiochem Eng Aspects 313–314:183–188
68. Zou P, Lee W-H, Gao Z, Qin D, Wang Y, Liu J, Sun T, Gao Y (2020) Wound dressing from polyvinyl alcohol/chitosan electrospun fiber membrane loaded with OH-CATH30 nanoparticles. Carbohydr Polym 232:115786
69. Ignatova M, Manolova N, Rashkov I (2007) Novel antibacterial fibers of quaternized chitosan and poly (vinyl pyrrolidone) prepared by electrospinning. Eur Polym J 43:1112–1122
70. Hao Y, Zhao W, Zhang L, Zeng X, Sun Z, Zhang D, Shen P, Li Z, Han Y, Li P, Zhou Q (2020) Bio-multifunctional alginate/chitosan/fucoidan sponges with enhances angiogenesis and hair follicle regeneration for promoting full-thickness wound healing. Mater Des 193:108863
71. Berretta J, Bumgardner JD, Jennings JA (2017) Lyophilized chitosan sponges. Chitosan Based Biomater 1:239–253
72. Anbazhagan S, Thangavelu KP (2018) Application of tetracycline hydrochloride loaded-fungal chitosan and *Aloe vera* extract based composite sponges for wound dressing. J Adv Res 14:63–71
73. Sathiyaseelan A, Shajahan A, Kalaichelvan PT, Kaviyarasan V (2017) Fungal chitosan based nanocomposites sponges – an alternative medicine for wound dressing. Int J Biol Macromol 104:1905–1915
74. Phaechamud T, Yodkhum K, Charoenteeraboon J, Tabata Y (2015) Chitosan-aluminum monostearate composite sponge dressing containing asiaticoside for wound healing and angiogenesis promotion in chronic wound. Mater Sci Eng C 50:210–225
75. Shao W, Wu J, Wang S, Huang M, Liu X, Zhang R (2017) Construction of silver sulfadiazine loaded chitosan composite sponges as potential wound dressings. Carbohydr Polym 157:1963–1970
76. Chen C, Liu L, Huang T, Wang Q, Fang Y (2013) Bubble template fabrication of chitosan/poly (vinyl alcohol) sponges for wound dressing application. Int J Biol Macromol 62:188–193
77. Xia G, Zhai D, Sun Y, Hou L, Guo X, Wang L, Li Z, Wang F (2020) Preparation of a novel asymmetric wettable chitosan-based sponge and its role in promotion chronic wound healing. Carbohydr Polym 227:115296
78. Fang Y, Xu Y, Wang Z, Zhou W, Yan L, Fan X, Liu H (2020) 3D porous chitin sponge with high absorbency, rapid shape recovery, and excellent antibacterial activities for noncompressible wound. Chem Eng J 388:124169
79. Barnes HR (1993) Wound care: fact and fiction about hydrocolloid dressings. J Gerontol Nurs 19:23–26
80. Dumville JC, Deshpande S, O'Meara S, Speak K (2012) Hydrocolloid dressings for healing diabetic foot ulcer (review). Cochrane Collab 2:1–52
81. Ousey K, Cook L, Young T, Fowler A (2012) Hydrocolloids in practice made easy. Wounds 8 (1):1–6
82. Schoukens G (2019) Bioactive dressings to promote wound healing. Adv Textiles Wound Care 2:135–167

83. Hiranpattanakul P, Jongjitpissamai T, Aungwerojanawit S, Tachaboonyakiat W (2018) Fabrication of a chitin/chitosan hydrocolloid wound dressing and evaluation of its bioactive properties. Res Chem Intermed 44:4913–4928
84. Yanagibayashi S, Kishimoto S, Ishihara M, Murakami K, Aoki H, Takikawa M, Fujita M, Sekido M, Kiyosawa T (2012) Novel hydrocolloid-sheet as wound dressing to stimulate healing-impaired wound healing in diabetic *db/db* mice. Biomed Mater Eng 22:301–310
85. Muzzarelli RAA (2009) Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydr Polym 76:167–182
86. Francesko A, Tzanov T (2011) Chitin, chitosan and derivatives for wound healing and tissue engineering. In: Nyanhongo GS, Steiner W, Gübitz GM (eds) Biofunctionalization of polymers and their applications: advances in biochemical engineering biotechnology, vol 125. Springer, Heidelberg, pp 1–28
87. Liu H, Wang C, Li C, Qin Y, Wang Z, Yang F, Li Z, Wang J (2018) A functional chitosan-based hydrogel as a wound dressing and drug delivery system in the treatment of wound healing. RSC Adv 8:7533–7449
88. Fischer TH, Connolly R, Thatte HS, Schwaitzberg SS (2004) Comparison of structural and hemostatic properties of the poly-N-acetyl glucosamine Syvek Patch with products containing chitosan. Microsc Res Tech 63:168–174
89. Burkatovskaya M, Tegos GP, Swietlik E, Demidova TN, Castano AP, Hamblin MR (2006) Use of chitosan bandage to prevent fatal infections developing from highly contaminated wounds in mice. Biomaterials 27:4157–4164
90. Brown MA, Daya MR, Worley JA (2009) Experience with chitosan dressings in a civilian EMS system. J Emerg Med 37:1–7
91. Arnaud F, Teranishi K, Okada T, Parreño-Sacdalan D, Hupalo D, McNamee G, Carr W, Burris D, McCarron R (2011) Comparison of combat gauze and TraumaStat in two severe groin injury models. J Surg Res 169:92–98
92. Balakrishnan B, Mohanty M, Umshanakar PR, Jayakrishnan A (2005) Evaluation of an *in situ* forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials 26:6335–6342
93. Yang X, Liu W, Li N, Wang M, Liang B, Ullah I, Neve AL, Feng Y, Chen H, Shi C (2017) Design and development of polysaccharide hemostatic materials and their hemostatic mechanism. Biomater Sci 5:2357–2368
94. Devlin JJ, Kircher S, Kozen BG, Littlejohn LF, Johnson AS (2011) Comparison of ChitoFlex®, CELOX™, and QuikClot® in control of hemorrhage. J Emerg Med 41:237–245
95. Kang P-L, Jen Chang S, Manousakas I, Lee CW, Yao C-H, Lin F-H, Kuo SM (2011) Development and assessment of hemostasis chitosan dressings. Carbohydr Polym 85:565–570
96. Gu BK, Park SJ, Kim MS, Kang CM, Kim J-I, Kim C-H (2013) Fabrication of sonicated chitosan nanofiber mat with enlarged porosity for use as hemostatic materials. Carbohydr Polym 97:65–73
97. Ong S-Y, Wu J, Moochhala SM, Tan M-H, Lu J (2008) Development of a chitosan-based wound dressing with improved hemostatic and antimicrobial properties. Biomaterials 29:4323–4332
98. Yang J, Tian F, Wang Z, Wang Q, Zeng YJ, Chen SQ (2008) Effect of chitosan molecular weight and deacetylation degree on hemostasis. J Biomed Mater Res B Appl Biomater 84:131–137
99. Chou TC, Fu E, Wu CJ, Yeh JH (2003) Chitosan enhances platelet adhesion and aggregation. Biochem Biophys Res Commun 302:480–483
100. Smith SA, Mutch NJ, Baskar D, Rohloff P, Docampo R, Morrissey JH (2006) Polyphosphate modulates blood coagulation and fibrinolysis. Proc Natl Acad Sci U S A 103:903–908
101. Yan D, Hu S, Zhou Z, Zeenat S, Cheng F, Li Y, Feng C, Cheng X, Chen X (2018) Different chemical groups modification on the surface of chitosan nonwoven dressing and the hemostatic properties. Int J Biol Macromol 107:463–469

102. Hattori H, Amano Y, Nogami Y, Takase B, Ishihara M (2010) Hemostasis for severe hemorrhage with photocrosslinkable chitosan hydrogel and calcium alginate. Ann Biomed Eng 38:3724–3732
103. Chen Y, Tan HM (2006) Crosslinked carboxymethylchitosan-g-poly(acrylic acid) copolymer as a novel superabsorbent polymer. Carbohydr Res 341:887–896
104. Chen Y, Zhang Y, Wang F, Meng W, Yang X, Li P, Jiang J, Tan H, Zheng Y (2016) Preparation of porous carboxymethyl chitosan grafted poly(acrylic acid) superabsorbent by solvent precipitation and its application as a hemostatic wound dressing. Mater Sci Eng C 63:18–29
105. Wang Y, Beekman J, Hew J, Jackson S, Issler-Fisher AC, Parungao R, Lajevardi SS, Li Z, Maitz P (2018) Burn injury: challenges and advances in burn wound healing, infection, pain and scarring. Adv Drug Deliv Rev 123:3–17
106. Bowler PG, Duerden BI, Armstrong DG (2001) Wound microbiology and associated approaches to wound management. Clin Microbiol Rev 14:244–269
107. Tachaboonyakiat W, Sukpaiboon E, Pinyakong O (2014) Development of an antibacterial chitin betainate wound dressing. Polym J 46:505–510
108. Chang S-H, Lin H-T V, Wu G-J, Tsai GJ (2015) pH effects on solubility, zeta potential, and correlation between antibacterial activity and molecular weight of chitosan. Carbohydr Polym 134:74–81
109. Mohandas A, Deepthi S, Biswas R, Jayakumar R (2018) Chitosan based metallic nanocomposite scaffolds as antimicrobial wound dressings. Bioact Mater 3:267–277
110. Rahimi M, Noruzi EB, Sheykhsaran E, Ebadi B, Kariminezhad Z, Molaparast M, Mehrabani MG, Mehramouz B, Yousefi M, Ahmadi R, Yousefi B, Ganbarov K, Kamounah FS, Shafiei-Irannejad V, Kafil HS (2020) Carbohydrate polymer-based silver nanocomposites: recent progress in the antimicrobial wound dressing. Carhohydr Polym 231:115696
111. Hebeish A, El-Rafie MH, EL-Sheikh MA, Seleem AA, El-Naggar ME (2014) Antimicrobial wound dressing and anti-inflammatory efficacy of silver nanoparticles. Int J Biol Macromol 65:509–515
112. Sudheesh Kumar PT, Abhilash S, Manzoor K, Nair SV, Tamura H, Jayakumar R (2010) Preparation and characterization of novel β-chitin/nanosilver composite scaffolds for wound dressing applications. Carbohydr Polym 80:761–767
113. Jain J, Arora S, Rajwade JM, Omray P, Khandelwal S, Paknikar KM (2009) Silver nanoparticles in therapeutics: development of an antimicrobial gel formulation for topical use. Mol Pharm 6:1388–1401
114. Yamanaka M, Hara K, Kudo J (2005) Bactericidal actions of a silver ion solution on *Escherichia coli*, studied by energy-filtering transmission electron microscopy and proteomic analysis. Appl Environ Microbiol 71:7589–7593
115. Priyadarshini S, Gopinath V, Meera Priyadharsshini N, MubarakAli D, Velusamy P (2013) Synthesis of anisotropic silver nanoparticles using novel strain, *Bacillus flexus* and its biomedical application. Colloids Surf B Biointerfaces 102:232–237
116. Jung WK, Koo HC, Kim KW, Shin S, Kim SH, Park YH (2008) Antibacterial activity and mechanism of action of the silver ion in *Staphylococcus aureus* and *Escherichia coli*. Appl Environ Microbiol 74:2171–2178
117. Li W-R, Sun T-L, Zhou S-L, Ma Y-K, Shi Q-S, Xie X-B, Huang X-M (2017) A comparative analysis of antibacterial activity, dynamics, and effects of silver ions and silver nanoparticles against four bacterial strains. Int Biodeter Biodegr 123:304–310
118. Mehrabani MG, Karimian R, Mehramouz B, Rahimi M, Kafil HS (2018) Preparation of biocompatible and biodegradable silk fibroin/chitin/silver nanoparticles 3D scaffolds as a bandage for antimicrobial wound dressing. Int J Biol Macromol 114:961–971
119. Petkova P, Francesko A, Fernandes MM, Mendoza E, Perelshtein I, Gedanken A, Tzanov T (2014) Sonochemical coating of textiles with hybrid ZnO/chitosan antimicrobial nanoparticles. Appl Mater Interfaces 6:1164–1172

120. Applerot G, Lipovsky A, Dror R, Perkas N, Nitzan Y, Lubart R, Gedanken A (2009) Enhanced antibacterial activity of nanocrystalline ZnO due to increased ROS-mediated cell injury. Adv Funct Mater 19:842–852
121. Tao Y, Qian L-H, Xie J (2011) Effect of chitosan on membrane permeability and cell morphology of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Carbohydr Polym 86:969–974
122. He G, Wang Z, Zheng H, Yin Y, Xiong X, Lin R (2012) Preparation, characterization and properties of aminoethyl chitin hydrogels. Carbohydr Polym 90:1614–1619
123. Liang S, Dang Q, Liu C, Zhang Y, Wang Y, Zhu W, Chang G, Sun H, Cha D, Fan B (2018) Characterization and antibacterial mechanism of poly(aminoethyl) modified chitin synthesized via a facile one-step pathway. Carbohydr Polym 195:275–287
124. Chen Q, Wu Y, Pu Y, Zheng Z, Shi C, Huang X (2010) Synthesis and characterization of quaternized β-chitin. Carbohydr Res 345:1609–1612
125. Ding F, Shi X, Li X, Cai J, Duan B, Du Y (2012) Homogeneous synthesis and characterization of quaternized chitin in NaOH/urea aqueous solution. Carbohydr Polym 87:422–426
126. Xu H, Fang Z, Tian W, Wang Y, Ye Q, Zhang L, Cai J (2018) Green fabrication of amphiphilic quaternized β-chitin derivatives with excellent biocompatibility and antibacterial activities for wound healing. Adv Mater 30:1801100
127. Morkaew T, Pinyakong O, Tachaboonyakiat W (2017) Structural effect of quaternary ammonium chitin derivatives on their bactericidal activity and specificity. Int J Biol Macromol 101:719–728
128. Xu T, Xin M, Li M, Huang H, Zhou S (2010) Synthesis, characteristic and antibacterial activity of N,N,N-trimethyl chitosan and its carboxymethyl derivatives. Carbohydr Polym 81:931–936
129. Kim JY, Lee JK, Lee TS, Park WH (2003) Synthesis of chitooligosaccharide derivative with quaternary ammonium group and its antimicrobial activity against *Streptococcus mutans*. Int J Biol Macromol 32:23–27
130. Lim S-H, Hudson SM (2004) Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. Carbohydr Res 339:313–319
131. Xu T, Xin M, Li M, Huang H, Zhou S, Liu J (2011) Synthesis, characterization, and antibacterial activity of *N,O*-quaternary ammonium chitosan. Carbohydr Res 346:2445–2450
132. Tachaboonyakiat W (2017) Antimicrobial applications of chitosan. In: Jennings JA, Bumgardner JD (eds) Chitosan based biomaterials, vol 2. Woodhead Publishing, Cambridge, pp 245–274
133. Panasci K (2014) Burns and wounds In: Paz JC, West MP (eds) Acute care handbook for physical therapists. 5th edn. W.B. Saunders, St. Louis, pp 283–311
134. Cho Y-W, Cho Y-N, Chung S-H, Yoo G, Ko S-W (1999) Water-soluble chitin as a wound healing accelerator. Biomaterials 20:2139–2145
135. Alsarra IA (2009) Chitosan topical gel formulation in the management of burn wounds. Int J Biol Macromol 45:16–21
136. Alemdaroğlu C, Değim Z, Celebi N, Zor F, Oztürk S, Erdoğan D (2006) An investigation on burn wound healing in rats with chitosan gel formulation containing epidermal growth factor. Burns 32:319–327
137. Wenczak BA, Lynch JB, Nanney LB (1992) Epidermal growth factor receptor distribution in burn wounds. Implications for growth factor-mediated repair. J Clin Invest 90:2392–2401
138. Dai T, Tegos GP, Burkatovskaya M, Castano AP, Hamblin MR (2009) Chitosan acetate bandage as a topical antimicrobial dressing for infected burns. Antimicrob Agents Chemother 53:393–400
139. Chang J, Liu W, Han B, Peng S, He B, Gu Z (2013) Investigation of the skin repair and healing mechanism of *N*-carboxymethyl chitosan in second-degree burn wounds. Wound Repair Regen 21:113–121
140. Moura LIF, Dias AMA, Carvalho E, de Sousa HC (2013) Recent advances on the development of wound dressings for diabetic foot ulcer treatment – a review. Acta Biomater 9:7093–7114

141. da Silva L, Carvalho E, Cruz MT (2010) Role of neuropeptides in skin inflammation and its involvement in diabetic wound healing. Expert Opin Biol Ther 10:1427–1439
142. Morbach S, Furchert H, Gröblinghoff U, Hoffmeier H, Kersten K, Klauke GT, Klemp U, Roden T, Icks A, Haastert B, Rümenapf G, Abbas ZG, Bharara M, Armstrong DG (2012) Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade. Diabetes Care 35:2021–2027
143. Clement JL, Jarrett PS (1994) Antibacterial silver. Metal-Based Drugs 1:467–482
144. Masood N, Ahmed R, Tariq M, Ahmed Z, Masoud MS, Ali I, Asghar R, Andleeb A, Hasan A (2019) Silver nanoparticle impregnated chitosan-PEG hydrogel enhances wound healing in diabetes induced rabbits. Int J Pharm 559:23–36
145. Lee Y-H, Hong Y-L, Wu T-L (2021) Novel silver and nanoparticle-encapsulated growth factor co-loaded chitosan composite hydrogel with sustained antimicrobility and promoted biological properties for diabetic wound healing. Mater Sci Eng C 118:111385
146. Meng B, Li J, Cao H (2013) Antioxidant and antiinflammatory activities of curcumin on diabetes mellitus and its complications. Curr Pharm Des 19:2101–2113
147. Li F, Shi Y, Liang J, Zhao L (2019) Curcumin-loaded chitosan nanoparticles promote diabetic wound healing via attenuating inflammation in a diabetic rat model. J Biomater Appl 34:476–486
148. Shen T, Dai K, Yu Y, Wang J, Liu C (2020) Sulfated chitosan rescues dysfunctional macrophages and accelerates wound healing in diabetic mice. Acta Biomater 117:192–203
149. Lindberger M, Schröder HD, Schultzberg M, Kristensson K, Persson A, Ostman J, Link H (1989) Nerve fibre studies in skin biopsies in peripheral neuropathies. I Immunohistochemical analysis of neuropeptides in diabetes mellitus. J Neurol Sci 93:289–296
150. Moura LIF, Dias AMA, Leal EC, Carvalho L, de Sousa HC, Carvalho E (2014) Chitosan-based dressings loaded with neurotensin – an efficient strategy to improve early diabetic wound healing. Acta Biomater 10:843–857
151. Song R, Ren L, Ma H, Hu R, Gao H, Wang L, Chen X, Zhao Z, Liu J (2016) Melatonin promotes diabetic wound healing in vitro by regulating keratinocyte activity. Am J Transl Res 8:4682–4693
152. Alghamdi BS (2018) The neuroprotective role of melatonin in neurological disorders. J Neurosci Res 96:1136–1149
153. Correa VLR, Martins JA, de Souza TR, de Castro Nunes Rincon G, Miguel MP, de Menezes LB, Amaral AC (2020) Melatonin loaded lecithin-chitosan nanoparticles improved the wound healing in diabetic rats. Int J Biol Macromol 162:1465–1475
154. Chen K, Tong C, Yang J, Cong P, Liu Y, Shi X, Liu X, Zhang J, Zou R, Xiao K, Ni Y, Xu L, Hou M, Jin H, Liu Y (2021) Injectable melatonin-loaded carboxymethyl chitosan (CMCS)-based hydrogel accelerates wound healing by reducing inflammation and promoting angiogenesis and collagen deposition. J Mater Sci Technol 63:236–245
155. Reiter RJ, Tan DX, Osuna C, Gitto E (2000) Actions of melatonin in the reduction of oxidative stress: a review. J Biomed Sci 7:444–458
156. Folkman J, Shing Y (1992) Angiogenesis. J Biol Chem 267:10931–10934
157. Barrientos S, Brem H, Stojadinovic O, Tomic-Canic M (2014) Clinical application of growth factors and cytokines in wound healing. Wound Repair Regen 22:569–578
158. Mizuno K, Yamamura K, Yano K, Osada T, Saeki S, Takimoto N, Sakurai T, Nimura Y (2003) Effect of chitosan film containing basic fibroblast growth factor on wound healing in genetically diabetic mice. J Biomed Mater Res 64A:177–181
159. Giavazzi R, Sennino B, Coltrini D, Garofalo A, Dossi R, Ronca R, Tosatti MP, Presta M (2003) Distinct role of fibroblast growth factor-2 and vascular endothelial growth factor on tumor growth and angiogenesis. Am J Pathol 162:1913–1926
160. Wang Y, Fu C, Wu Z, Chen L, Chen X, Wei Y, Zhang P (2017) A chitin film containing basic fibroblast growth factor with a chitin-binding domain as wound dressings. Carbohydr Polym 174:723–730

161. Trengove NJ, Stacey MC, MacAuley S, Bennett N, Gibson J, Burslem F, Murphy G, Schultz G (1999) Analysis of the acute and chronic wound environments: the role of proteases and their inhibitors. Wound Repair Regen 7:442–452
162. Mohandas A, Anisha BS, Chennazhi KP, Jayakumar R (2015) Chitosan–hyaluronic acid/VEGF loaded fibrin nanoparticles composite sponges for enhancing angiogenesis in wounds. Colloids Surf B Biointerfaces 127:105–113
163. Jazwa A, Kucharzewska P, Leja J, Zagorska A, Sierpniowska A, Stepniewski J, Kozakowska M, Taha H, Ochiya T, Derlacz R, Vahakangas E, Yla-Herttuala S, Jozkowicz A, Dulak J (2010) Combined vascular endothelial growth factor-A and fibroblast growth factor 4 gene transfer improves wound healing in diabetic mice. Genet Vaccines Ther 8:6
164. Muñoz AL, Merchán WH, Resende Pires AL, Moraes ÂM, Gómez LA (2019) Biostimulation of venous chronic ulcers with platelet-rich plasmariaa gel and biocompatible membranes of chitosan and alginate: a pilot study. Wound Med 26:100161