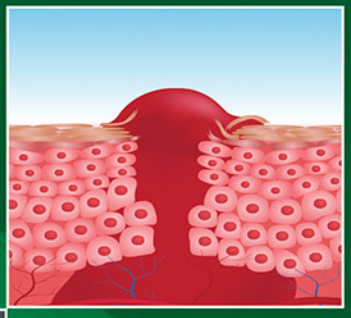
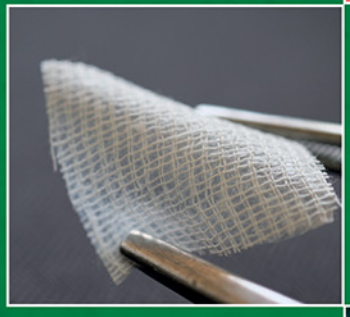
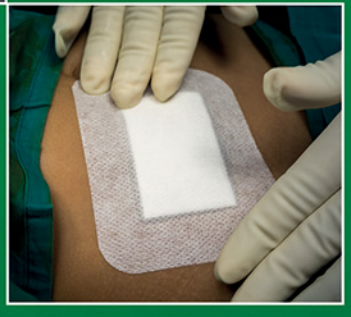
NATURAL POLYMERS IN WOUND HEALING AND REPAIR

FROM BASIC CONCEPTS TO EMERGING TRENDS







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Natural Polymers in Wound Healing and Repair

From Basic Concepts to Emerging Trends

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Preface

The complexities in the wound management are a growing challenge faced worldwide. Since the wound healing is a very complex process and requires a comprehensive treatment strategy, the book aiming to bring these at a place could play a significant role for the researchers and professionals. This book provides the readers an insight into the natural wound healing processes, describes the state-of-the-art tissue-engineered scaffolds for wound healing and repair, and comprehensively covers various biopolymers and bioactive agents being employed for the same. This book covers both fundamental and advanced aspects in wound healing strategies and the mechanism behind so as to attract the readers at the beginner's as well as the advanced level. We greatly acknowledge all the contributors for their wonderful efforts in compiling the best of the dedicated topics in a timely manner. We are also indebted to the reviewers for their precious time and critical suggestions. We take this opportunity to extend our thanks to Leticia Lima, Sara Greco, Edward Payne, Nalini Thangavelu, Praveen Anand S., and the entire editorial team from Elsevier Inc. and Woodhead Publishing Ltd. for their invaluable efforts in the successful publication of this book. Finally, we deeply believe that applications of natural polymers for wound healing and tissue repair would remain as favorite strategies, and this book is an attempt to compile the latest trends in the field. However, we appreciate any constructive comments or suggestions from peers and experts in academics and industries to enhance the quality of the work as well as to further expand the scope of the field in the future.

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Naresh Kasoju
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Introduction

This book provides up-to-date coverage of natural polymer–based biomaterials in wound healing and repair, covering sources, processing, and properties. It describes cellular and molecular events in wound healing and introduces conventional and advanced methodologies for wound management. It also offers a comprehensive understanding of state-of-the-art and emerging concepts in wound healing, including drug-eluting matrices, cell-laden systems, and personalized biofabrication strategies. This edited book consists of a total of 18 chapters written by a combination of young scientists as well as well-established experts in the field, including those from the industry. Chapter 1 introduces the basic principles and models being utilized for developing the wound care systems. Chapter 2 describes the wound types and characterization methodologies for developing ideal wound care dressings. This is followed by discussion on the treatment strategies like engineering designs of transdermal treatment patches in Chapter 3, and controlled drug delivery systems in Chapter 4. The fabrication methods for the artificial skin are elaborated in Chapter 5, with special emphasis on the different physicochemical functionalization strategies for the polymers; Chapter 6 discusses various polysaccharide- and protein-based biopolymers, with Chapter 7 entailing the different electrospun systems based on the natural biopolymers and Chapter 8 on 3D printing of skin tissue equivalents. Chapter 8 also discusses the bioink formulation methods required in the printing process. Furthermore, Chapter 9 reviews the blood-derived additives and their role in wound healing. Chapter 10 discusses on the role of plant-based antimicrobial and bioactive agents. Chapter 11 discusses on the extent of wound healing achieved by incorporating curcumin. Collagen; silk fibroin and silk sericin; chitin and chitosan; alginate; and eggshell membrane—based strategies, their various forms employed, and molecular mechanisms behind effective wound healing are discussed in detail in Chapters 12–16, respectively. Finally, decellularized extracellular matrices–based strategies in wound healing and skin tissue engineering are described in Chapter 17. Beyond the topics and contents covered in the previous chapters, in Chapter 18, the editors discuss and conclude about the latest advances achieved for wound healing through the promising technology intervention. With such an amazing mix of topics, it is anticipated that this book would attract a wide spectrum of readers from different levels across multiple disciplines.

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CHAPTER

Chitin- and chitosan-based strategies in wound healing

14

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Chapter Outline

14.1 Introduction

Skin is the largest and the most external organ of the human body, being exposed to different external agents that may cause structural and functional damage. When such damage or any disorder affects the structural integrity and functions of the skin, a wound arises [1]. This situation can occur as a consequence of different events, such as surgery, trauma, burns, chemicals, or physical forces [2,3]. Wounds can be classified based on the healing time as acute or chronic wounds. Acute wounds can heal in 8–12 weeks,

*Authors Cátia S.D. Cabral and Mariana F.P. Graça contributed equally to this chapter.

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through the normal wound healing process. In turn, chronic wounds remain in the inflammatory phase and consequently, last several months or years, which increases the dressings renewal frequency and treatment costs, as well as reduces the patient's quality of life [2,4].

To assist the wound healing process, different types of conventional dressings (gauzes, absorbent cotton, bandages) have been used in clinical practice. Despite their benefits, these conventional dressings do not provide a moist environment and in some cases adhere to the wound bed, thus inducing a new lesion and pain to patient when removed [4]. Furthermore, none of the substitutes developed until now are capable of re-establishing all the native features of skin [5]. To overcome these drawbacks, researchers from the tissue engineering area have been developing novel wound dressings (hydrogels, films, sponges, membranes) that aim to reproduce the structure and composition of the native skin, while supporting the wound healing process. Ideally, a wound dressing should: (1) provide a moist environment at the injured site, (2) stimulate cell proliferation and migration, (3) improve angiogenesis, (4) protect the wound against microorganisms' invasion, (5) allow the exchange of gases and fluids, and (6) be biocompatible, biodegradable, and cost-effective [3,5,6].

In order to achieve the desired properties, researchers are using biodegradable and natural polymers (e.g., collagen, hyaluronic acid, chitin, alginate, and elastin) to produce new types of dressings [7]. Some of these natural polymers have also the particularity of activating/directing specific cellular responses that can improve the healing process as well as prevent possible antibacterial infections [8]. Among them, chitin is one of the most abundant natural marine biopolymers [1]. Chitin and its derivatives, such as chitosan, have been widely explored for the development of wound dressings or even in the production of artificial skin and skin substitutes. Besides that, these polymers are already used in the clinic as wound dressings, approved by Food and Drug Administration [9–12]. Particularly, this natural polymer presents notable biodegradability and biocompatibility, antibacterial and anti-inflammatory properties, which prompts its application in the development of wound dressings [13–19]. For example, Jung et al. analyzed the effect of chitin-based electrospun fibers in the wound healing process [13]. These nanofibers based on chitin (1) inhibited the inflammatory response, (2) decreased the exudate secretion and the wound area, (3) increased the deposition of collagen, and (4) prompted a more uniform surface thickness of the epithelial layer and a more consistent orientation of the connective tissue [13]. In addition, chitin presents some derivatives such as chitosan, which results from the deacetylation of chitin, and are also promising for developing wound dressings. Chitosan is one of the most used biopolymers in the production of wound dressings due to its biodegradability, biocompatibility, and processability into different forms (gels, foams, and membranes) [1,20–25]. In addition, this biopolymer also shows antimicrobial activity as well as capacity to accelerate the wound healing process [1].

Therefore, this chapter aims to provide an overview of the properties of chitin and chitosan as well as the use of these biopolymers in the assembly of dressings (films, nanofibrous membranes, hydrogels, and sponges) for wound healing applications.

14.2 Chitin

Chitin [or poly(β-(1→4)-*N*-acetyl-D-glucosamine)] is the second most abundant polysaccharide on earth, after cellulose [26,27]. Chitin, derived from the Greek word "chiton" that means envelop or

tunic, was isolated from mushrooms for the first time in 1811 by Henri Braconnot [27]. This polymer occurs in nature as a crystalline microfibrillar structure clustered with six polypeptide chains of a protein structure and it is produced by many living organisms. In fact, chitin is the major structural component in the exoskeletons of molluscs, crustaceans, and insects as well as in the cell walls of fungi, providing protection and structural integrity to these organisms [28]. Chitin presents a white and semitransparent aspect, and it is a nitrogenous polysaccharide. Moreover, this material is also inelastic, insoluble in water and common organic solvents, and displays a low chemical reactivity. Due to its insolubility, chitin was disregarded for a long time when compared to other polysaccharides [27,29]. Nowadays, chitin is extracted at a large scale, mostly from the abundant waste of shellfish (e.g., crab, shrimp, and lobster shells) processing industries. This biodegradable and biocompatible polymer can also be deacetylated, yielding chitosan [27].

14.2.1 Chemical structure and solubility

The chemical structure of chitin $(C_8H_{13}O_5N)_n$ is similar to that of cellulose, differing only on an acetamide group ($-NHCOCH_3$) at the C2 position (see Fig. 14.1) [30]. Chitin is a high molecular weight (HMW) linear polysaccharide composed of *N*-acetyl-2-amido-2-deoxy-D-glucose units linked by β(1,4) bonds, with a molecular weight between 1×10^6 and 2.5×10^6 Da, and a degree of acetylation $\geq 50\%$ [26,31,32].

Chitin can be found in three different crystalline allomorphs (α, β, and γ), depending on its source and the orientation of the microfibrils [27,33]. *N*-Acetyl glycosyl moiety is a common crystallographic unit in all of these three different polymorphisms, differing only in its β-(1−4)-2-acetamido-2-deoxy-β-D-glucosamine units, due to the polymerization of monosaccharides. α-Chitin is the most abundant form found in nature, namely in various arthropod species and crustaceans or other aquatic invertebrates especially those with a hard outer shell. This isomorph of chitin is formed by antiparallel chains of *N*-acetylglucosamine, which confers a robust and stable structure due to stronger hydrogen bonding [27]. β-Chitin is obtained mostly from squid pen and, contrarily

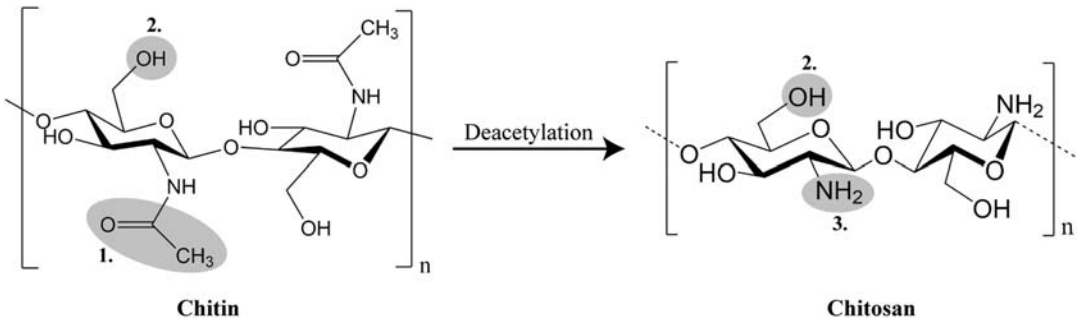

FIGURE 14.1

Representation of the main molecular structure of chitin and chitosan. Chitin can be modified in the (1) acetyl group that results in its deacetylated form, that is, chitosan, and (2) hydroxyl group (−OH). Furthermore, chitosan can also be modified in its hydroxyl group and/or (3) amino group ($-NH_2$).

to α-chitin, has a weaker molecular interaction due to the parallel arrangement of its chains. Moreover, β-chitin is less crystalline than α-chitin, whereby it can be dissolved in formic acid and presents moderate swelling in water [27,33]. Meanwhile, γ-chitin has an arrangement of parallel and antiparallel chains, formed by two parallel strands and a third chain of chitin in antiparallel. γ-Chitin is commonly found in mushrooms and cocoons of insects. Both γ-chitin and β-chitin can be irreversible converted into α-chitin through acid and alkali treatments. Except for the β-chitin that is present in centric diatoms, the remaining chitin forms are found cross-linked to other structural components. The chitin present in fungi is covalently linked to the β(1→3)-glucan moieties in cell walls, whereas in insects and invertebrates, chitin is bonded to specific proteins through covalent and noncovalent interactions [27,34]. Chitin is a positively charged polysaccharide and a higher number of amino groups ($-NH_2$) can be obtained by increasing its degree of deacetylation. Moreover, the presence of acetyl, amino, and hydroxyl groups in the chitin chain tends to generate intra- and intermolecular hydrogen bonds, prompting the aggregation of chitin, which in turn affects its solubility. Chitin is extremely hydrophobic due to its highly expanded hydrogen-bonded semicrystalline structure, making it insoluble in water and most common organic solvents. Generally, for biomedical applications, hexafluoroisopropanol, hexafluoroacetone, methane sulfonic acid, formic, dichloroacetic, trichloroacetic acids or even fluoroalcohols, chloroalcohols are the most used solvents to dissolve chitin [27,28].

However, many of these solvents are toxic, corrosive, degradative, or mutagenic, and therefore can compromise the application of chitin in medical applications. The choice of chitin solvent depends on several parameters such as the polymer concentration, solution pH, presence of solubilizing chemical entities, temperature, degree of acetylation, molecular weight, solution viscosity, type of application, and also on the source of chitin [33].

14.2.2 Chitin isolation procedures

Crustacean shells obtained from crab, shrimp, and crayfish are the most commonly used sources of chitin for commercial purposes [30,35]. In crustacean shells, chitin is enclosed in a complex network composed of minerals (i.e., calcium carbonate and phosphate), proteins, and a small fraction of lipids and pigments [30]. Therefore, the extraction of chitin requires the removal of the two major components of the shell, proteins, and inorganic calcium carbonate, by deproteinization and demineralization processes, respectively [35]. Despite the several methodologies reported for obtaining pure chitin, there is no standard extraction protocol. In general, the different extraction protocols can be classified as chemical or biological (i.e., based on enzymes or microorganisms), as briefly described in the following topics [26,36].

14.2.2.1 Chemical extraction of chitin

Chemical extraction of chitin involves the use of strong acids and bases to dissolve calcium carbonates and proteins, respectively. This conventional process includes three main steps (1) deproteinization, for the removal of residual proteins by chemical or enzymatic hydrolysis, (2) demineralization, to remove mineral salts by acid treatments, and (3) treatments for removal of lipids and pigments [32].

14.2.2.1.1 Chemical deproteinization

The deproteinization treatment requires the disruption of chemical bonds between chitin and proteins [26,36]. In the deproteinization step, the removal of protein content is carried out by alkaline treatments. For this purpose, a wide range of alkaline solutions can be used such as those based on sodium hydroxide, sodium carbonate, sodium hydrogen carbonate, calcium hydroxide, sodium sulfite, sodium hydrogen sulfite, calcium hydrogen sulfite, trisodium phosphate, and sodium sulfide [26]. Among all, sodium hydroxide solution is the most used, being applied at concentrations ranging from 0.125 to 5.0 M, with a temperature up to 160°C, from few minutes to a few days [37]. The efficiency of deproteinization is dependent on the concentration of the alkali, solid-to-solvent ratio, duration, and temperature of the treatment [37].

14.2.2.1.2 Chemical demineralization

Demineralization allows the removal of minerals from crustacean shells, specifically, calcium carbonate. This step is carried out by acidic treatment, applying acids such as hydrochloric acid, nitric acid, sulfuric acid, or acetic acid [26]. Hydrochloric acid is the most used acid for the production of pure chitin on an industrial scale [36]. During the demineralization process, it occurs the decomposition of calcium carbonate into the water-soluble calcium salt as well as the release of carbon dioxide. Other mineral deposits are also removed by similar reactions, yielding soluble salts in the presence of the acid. Then, filtration and washing steps can be performed to recover the chitin in the solid phase and remove the water-soluble salts. Similar to the deproteinization process, the efficiency of the demineralization treatment also varies according to the crustacean shells' mineralization degree, the extraction process, particles' size, acid concentration, solvent ratio, and temperature [26,36].

14.2.2.1.3 Decolorization

Decolorization (also called as bleaching step) is applied to remove the natural pigments remaining after deproteinization and demineralization processes [35]. The blenching step allows the obtention of colorless pure chitin. For such, acetone, chloroform, alcohol solutions, hypochlorite, hydrogen peroxide, phosphorus pentoxide solutions, potassium permanganate, a mixture of oxalic acid, and sulfuric acid are used [37].

14.2.2.2 Biological extraction

In the chemical extraction, environmental concerns may arise due to the use of hazardous chemicals. Moreover, the use of high temperatures for extended periods can compromise the chitin's structure and thus affect the chitin's physicochemical properties. Therefore, in recent years, the interest in green extraction methods based on the use of enzymes or microorganisms has been increasing [26,36]. Biological extraction methods, when compared to chemical extraction processes, are less aggressive but lead to the obtention of a chitin with a lower degree of purity [26].

Enzymatic extraction requires the use of proteases normally derived from plants, animals, and microbial sources [36]. In crustacean shells, several proteins can be removed through the action of proteolytic enzymes such as alcalase, pepsin, papain, pancreatine, devolvase, and trypsin [28,36]. This step allows the isolation of chitin, while minimizing its deacetylation and depolymerization. Besides purified proteases, crude proteases can also be used in chitin's extraction, thus reducing the

costs and increasing the deproteinization efficiency due to the presence of coexisting proteases. Crude proteases can be derived from fish viscera and bacteria, being bacterial proteases the most explored ones [36,38]. Usually, an additional sodium hydroxide treatment can be applied to the enzymatic extraction method to reduce the protein content (5%−10% of proteins) that remains after the isolation of chitin [38].

To reduce the costs associated to the enzymatic process, the crustacean shells' deproteinization by fermentation has been the most used biological method. Microbial fermentation uses the waste of marine products, namely shrimp shells, prawn shells, crawfish to recover chitin [36,38,39]. Similar to the enzymatic extraction, the use of microorganism-based extraction approaches is a more environmental-friendly process than the above presented chemical methods. However, the microorganism-based extraction approaches are longer processes with poorer accessibility of proteases, which results in a higher content of protein contaminants. To improve that, it is possible to use simultaneous or successive fermentation steps, for example, two-step fermentations or co-fermentations [36].

14.3 Chitosan

Chitosan, the deacetylated form of chitin, is a modified natural linear polysaccharide composed of (1−4)-2-acetamido-2-deoxy-β-D-glucan (*N*-acetyl D-glucosamine) and (1−4)-2-amino-2-deoxy-β-D-glucan (D-glucosamine) units (Fig. 14.1) [35]. Chitosan was first discovered by Rouget in 1859 upon heating chitin in an alkaline solution [40]. This polymer is normally obtained by removing the acetyl groups ($-CH_3-CO$) from chitin, in order to obtain a polymer that is soluble in commonly diluted acids. In fact, chitosan and chitin only differ in the acetyl content, that is, if the acetylation degree is $\leq$50%, the polymer becomes chitosan [27].

Chitosan presents a semicrystalline and rigid fiber-like aspect, formed by inter- and intramolecular hydrogen bonding due to the amino and hydroxyl groups [41]. Owing to its chemical structure with abundant primary amine groups, chitosan presents enhanced biological and physicochemical properties when compared to chitin. Besides its good biocompatibility, biodegradability, and antimicrobial activity, the abundant presence of amino groups in the chitosan structure allows it to be protonated, providing greater solubility and improved biological and hemolytic profiles [35,42].

14.3.1 Chemical structure and solubility

Similarly to chitin, chitosan also presents three different crystal isomorphs (α, β, and γ). Due to its parallel and inversed arranged chains, α-chitosan has a higher crystallinity and intermolecular force, being also the most common isomorph of chitosan [35,43].

The properties conferred by chitosan are dependent on several factors, such as degree of deacetylation, molecular weight, and crystallinity. Chitosan can present different degrees of deacetylation, which is dependent on the amino groups distribution along the polymeric chain, being calculated through the determination of the percentage of D-glucosamine units present in chitosan. Commercially available chitosan generally has a deacetylation degree of 70%−95% [44]. The

degree of deacetylation confers to chitosan an important feature, that is, its polycationic nature in acidic media [28].

Moreover, the commercially available chitosan can be classified according to its molecular weight as: (1) chitosan oligosaccharides (COS, <5 kDa), (2) low molecular weight chitosan (LMW, 50−190 kDa), (3) medium molecular weight chitosan (MMW, 190−310 kDa), and (4) HMW chitosan (>310 kDa) [45−47]. HMW chitosan forms solutions with a higher viscosity and presents a higher thermal stability than the LMW [48]. Wound dressings prepared with HMW chitosan can display an increased mechanical strength when compared to its equivalents assembled using LMW chitosan [49,50]. Tangsadthakun and colleagues evaluated the influence of the molecular weight in collagen/chitosan scaffolds and observed that HMW chitosan enhanced the compressive modulus of scaffolds, comparatively to LMW chitosan scaffolds [49]. Also, Qin and Wang noticed that increased molecular weight of chitosan provided improved tensile strength in chitosan-based foams [51]. Besides that, HMW chitosan-based wound dressings also exhibit enhanced biological properties, namely cell attachment and proliferation [52]. However, chitosan with HMW presents a long molecular chain, which leads to the formation of tightly packed wound dressings with a lower air permeability [53]. In turn, the MMW chitosan can form solutions with suitable viscosity conferring enhanced mechanical strength to the polymeric matrix of the wound dressing [54]. LMW chitosan has enhanced antibacterial, antioxidant, and antifungal properties, as well as higher mucoadhesiveness. However, the lower viscosity of LMW chitosan solutions facilitates the degradation of these materials when compared to HMW chitosan [46]. COS are the degraded products of chitosan and generally exhibit a degree of deacetylation higher than 90% and a degree of polymerization of 2−20 units in a segment [31,55]. When compared to chitosan with a higher molecular weight, COS present improved antimicrobial properties against various bacteria and fungi [55,56]. Moreover, these COS also exhibit excellent anti-inflammatory and antioxidant properties [55−57].

The solubility of chitosan is intrinsically correlated with the protonation of its amino groups. In this way, chitosan's solubility depends on its molecular weight, degree of deacetylation, and pH. Regarding the influence of the molecular weight, chitosan with a HMW displays a lower solubility, probably due to intra- and intermolecular hydrogen bonds [58]. Generally, COS are soluble in water, partially soluble in methanol and dimethyl sulfoxide, and insoluble in ethanol, propanol, butanol, acetone, and ethyl acetate [56]. The degree of deacetylation also influences this property, that is, the greater the degree of deacetylation, the higher degree of protonation of amino groups in the molecular chain of chitosan and, consequently, the easier it is to dissolve the chitosan [41,59]. The amino groups of chitosan present different charges depending on the pH. So, the chitosan is positively charged at low pH due to the protonation of the amino groups, making it a water-soluble cationic polyelectrolyte. On the other hand, at pH >6−6.5, the amino groups of chitosan are deprotonated, whereby chitosan becomes an insoluble polymer due to the loss of charge [33,43,59].

Nevertheless, chitosan is soluble in common inorganic and organic acids, namely in acetic acid, formic acid, hydrochloric acid, L-glutamic acid, lactic acid, and succinic acids, and can also be soluble in diluted acidic solutions [33,43,59].

14.3.2 Chitosan production

Chitosan is not extensively present in the environment but can be easily obtained from the partial deacetylation of chitin [27,42,59,60]. The deacetylation of chitin to obtain chitosan can be performed through chemical hydrolysis, under acidic or alkaline conditions, or by enzymatic

hydrolysis (e.g., using chitin deacetylase) [43]. Furthermore, chitosan molecular weight can differ depending on its production method. For example, commercially available COS are generally obtained by chemical or enzymatic hydrolysis [61]. Moreover, COS and LMW chitosan are frequently obtained by enzymatic degradation from HMW chitosan [61,62]. These commonly used methods are discussed in further detail in the following subsections.

14.3.2.1 Chemical deacetylation method

At an industrial level, the chemical deacetylation of chitin is the most used method to obtain chitosan due to its low cost, scalability, and high efficiency [36,43]. The chitin chemical deacetylation can be achieved through the utilization of both acids and alkalis [36]. Nonetheless, the glycosidic bonds of chitin are very susceptible to acids, and therefore alkaline solutions (e.g., sodium hydroxide, potassium hydroxide) are more suitable and frequently used [36,59].

Chitosan obtention is generally performed by incubate the chitin in a hot concentrated solution of sodium hydroxide for few hours. This method allows the obtention of regular distribution of residual *N*-acetyl-D-glucosamine and D-glucosamine and a degree of deacetylation above 85% [36,63].

Furthermore, the chitosan deacetylation degree can be adjusted by modifying the alkali solution concentration, the reaction time, the reaction temperature, and the ratio of chitin/sodium hydroxide solution. These parameters can have impact on the obtained chitosan, namely the degree of acetylation, distribution of acetyl groups along the chains, molecular weight, and solution viscosity [43].

14.3.2.2 Enzymatic deacetylation method

Despite its reproducibility, the chemical method presents some limitations such as environmental pollution, large waste of alkaline and acidic solutions, and energy consumption. As alternative, enzymatic methods have been employed. These methods explore the use of chitin deacetylases, being a simple and ecofriendly approach. This enzymatic deacetylation guarantees a controlled and nondegradable process, being mostly used to prepare COS with excellent solubility in water [28,60].

Chitin deacetylases catalyze the hydrolysis of *N*-acetamido bounds of *N*-acetyl-D-glucosamine residues present in chitin. The proteolytic enzymes used to achieve this enzymatic treatment are mainly derived from plant, microbial, and animal sources, namely chitinase, chitosanase, glycanases, proteases, or lipases [28,31].

14.3.3 Chitosan modifications and functionalization

To expand chitosan applicability and to improve its physical and chemical features, several chemical modifications have been employed [31]. Chitosan structure contains reactive amino ($-NH_2$) and hydroxyl ($-OH$) groups that can be easily modified (Fig. 14.1). In fact, chemical modifications can occur on amino, hydroxyl, or both amino and hydroxyl groups to form *N*-modified, *O*-modified, or *N*,*O*-modified chitosan derivatives [28,43]. The following subsections report the main modifications used to improve the solubility and antibacterial ability of chitosan.

14.3.3.1 Chemical modifications to improve the solubility of chitosan

One modification that has been employed to improve chitosan solubility comprises the introduction of carboxylic groups into the main chain of chitosan [43,64,65]. Lv and collaborators synthetized carboxymethyl chitosan (CMC) by re-suspending chitosan in an isopropyl alcohol solution followed by the addition of sodium hydroxide and a monochloroacetic acid/isopropanol solution [66]. After that, the solution was filtrated and the solid product was resuspended in methanol and neutralized with glacial acetic acid. Finally, the CMC was recovered after washing with ethanol (80%, v/v) and freeze-dried [66]. Moreover, CMC is not only soluble in water but also may provide improved physicochemical and biological properties, such as its hemostatic and antibacterial properties, as demonstrated by He et al. [67].

Alternatively, the chitosan alkylation can be accomplished through reaction with halogenated hydrocarbon or sulfate under basic conditions [43,65]. The introduction of an alkyl group into the chitosan chain weakens the intramolecular hydrogen bonds, which improves the chitosan water solubility [43,64]. Similarly, chitosan acylation, which can be achieved using organic acids, also disrupts intramolecular and intermolecular hydrogen bonds, thus decreasing the chitosan crystallinity and improving its solubility [43,64]. Moreover, hydroxyalkylation may also improve the water solubility of chitosan and these chitosan-derivatives are usually obtained by reacting the chitosan with epoxides, such as ethylene oxide, propylene oxide, and butylene oxide [43].

14.3.3.2 Chemical modifications to improve the antibacterial activity of chitosan

The chitosan antibacterial activity is closely related to the presence of amino groups ($-NH_2$) in its chemical structure. However, its antimicrobial activity is still not fully understood and has been mainly attributed to three different mechanisms [39,60,68]. One of the most described mechanisms purposes alterations on the permeability of the bacteria cell walls due to the interaction between the chitosan's positively charged groups and the negatively charged peptidoglycans present at the bacterial cell wall [39,68]. Furthermore, the chitosan may also act as a chelating agent, interacting with essential trace metals and oligo-elements that are essential for bacterial growth, and therefore mediates the production of toxins and inhibits microbial growth [39,68]. Finally, it is also purposed that the chitosan can form a polymeric envelope around bacteria, which inhibits nutrients absorption and intercellular exchanges [68].

Therefore, researchers have been focused on increasing the number of positively charged groups in the chitosan backbone to improve its antibacterial activity. One of the strategies widely reported in the literature explores the introduction of quaternary ammonium groups on chitosan [43,64,65]. For example, Ren et al. developed nanofibers based on quaternized chitosan (QCS), poly(L-lactic acid), and poly(D-lactic acid)-grafted QCS (PLA/QCS-PDLA/QCS) [69]. QCS was synthesized by reacting the glycidyl-trimethylammonium chloride with the amino groups of chitosan. The authors observed that the increase in the QCS degree of substitution, from 14.3% (QCS1) to 33.5% (QCS2) and 44.9% (QCS3), resulted in a decrease in minimum inhibitory concentration from 1 mg/mL (QCS1) to 0.5 mg/mL (QCS2 and QCS3) against *Escherichia coli* (*E. coli*). Furthermore, the addition of QCS to the nanofibers improves their antibacterial activity (as can be observed in Fig. 14.2) [69].

Otherwise, the introduction of quaternary ammonium groups also increases the chitosan water solubility by increasing the ionic strength and weakening the hydrogen bonds. Moreover, quaternary

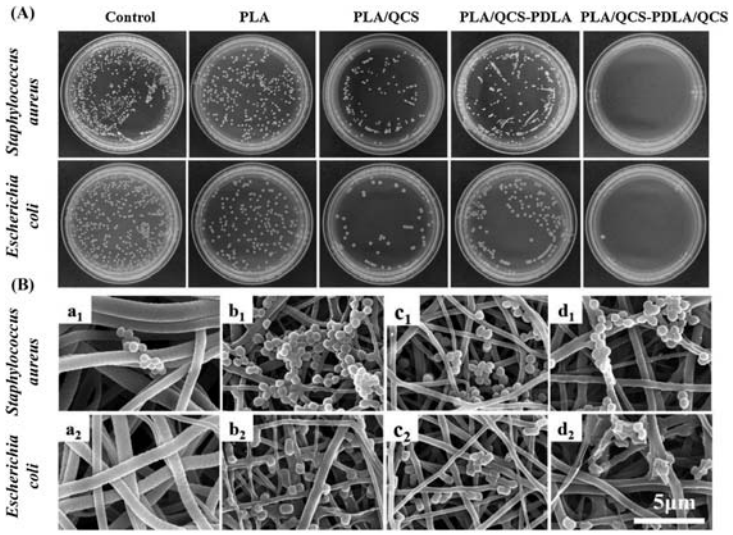

FIGURE 14.2

Colony images of *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) suspension being treated by PLA, PLA/QCS, PLA/QCS-PDLA, and PLA/QCS-PDLA/QCS membranes (A), SEM images (B) of pure PLA (a), PLA/QCS (b), PLA/QCS-PDLA (c), and PLA/QCS-PDLA/QCS (d) nanofibers after contact with *S. aureus* (a1–d1) and *E. coli* (a2–d2).

Reprinted from Ren Y, Huang L Wang Y, Mei, Fan R, He M, Wang C, Tong T, Chen H, Guo G. Stereocomplexed electrospun nanofibers containing poly (lactic acid) modified quaternized chitosan for wound healing. Carbohydr Polym 2020;247:116754. Copyright (2020) with permission from Elsevier.

ammonium chitosan also exhibits enhanced biocompatibility and biodegradability when compared to chitosan. Zhou and their collaborators synthesized *N,N,N*-trimethyl chitosan (TMC) fibers, through the methylation of chitosan fibers with iodomethane (with different amounts forming TMC1, TMC2, and TMC3) under alkaline conditions [70]. More specifically, alkyl groups were introduced in the amino moieties of chitosan fibers, producing TMC with different degrees of quaternization (19%, 25%, and 32% for TMC1, TMC2, and TMC3, respectively). The results demonstrated that the increase in the degree of quaternization enhanced the water absorption capacity of TMC fibers. In fact, the TMC1, TMC2, and TMC3 had water absorption values of 100%, 190%, and 285%, respectively, contrasting with the 20% of the chitosan fibers. Furthermore, the chitosan, TMC1, TMC2, and TMC3 fibers attained an *E. coli* inhibition of 52%, 63%, 85%, and 92%, respectively. Moreover, the three types of TMC fibers exhibited >99% of inhibition against *Staphylococcus aureus (S. aureus)*, which is highly superior to the 24% obtained with chitosan fibers [70].

Schiff base modification has also been employed in chitosan-biomedical applications to improve its antibacterial properties. Schiff base modification can be formed by reacting the chitosan with fatty aldehydes, aromatic aldehydes, or ketones in a neutral medium where the carbonyl groups of aldehyde or ketone can react with amino groups of chitosan [43,64]. On the other hand, thiolated chitosan derivatives presented enhanced antibacterial and hemostatic properties, swelling capacity,

and drug release ability. In addition, some authors have been functionalizing chitosan with different amino acids (such as thymine, adenine, arginine) also in order to improve its antibacterial activity [71–74].

14.4 Application of chitin and chitosan as wound dressings

After an injury occurs, the skin starts the wound healing process in order to restore as soon as possible its normal functions and integrity. Generally, superficial wounds do not require additional interventions but when the wound thickness and wound area are higher, the skin requires support for assisting the re-epithelization process and minimizing the formation of scars [60,75,76].

Thus, chitin and chitosan have been widely investigated over the years as biomaterials for enhancing the wound healing process due to their physicochemical and biological properties such as biocompatibility, biodegradability, antioxidant, and antibacterial features [31,60,77]. Such rendered the development of various wound dressings that are currently available in the clinic, such as nonwovens [Chitopack C (Eisai), Chitopack P (Eisai), ChitoSAM 100], hydrogels [BST-DermOn (BioSyntech)], and sponges [Chitopack S (Eisai), Vulnosorb (Tesla-Pharma)] [10,11,61].

Considering the base material, that is, chitin or chitosan, the higher number of amino groups available in the chitosan backbone can render to the wound dressing an enhanced biological performance. For example, the higher number of positively charged amino groups in the chitosan backbone renders to them a higher antibacterial potential (discussed in Section 14.3.2.2). Nevertheless, it is worth noticing that this will depend on the chitosan's degree of deacetylation, molecular weight, pH, and solubility [78,79]. Moreover, the chitosan's positively charged amino groups also enhance the interaction with cells in vitro, leading to an improved cell adhesion capacity [60]. Beyond these, the polycationic nature of chitosan also confers hemostatic activity to this polymer [31]. Chitosan presents a positive nature that can interact with the negative charge of the cells' membrane. In addition, amino groups of chitosan can be protonated in the presence of protons that are released in the inflammation area, conferring an anti-inflammatory effect [31,60].

Chitin and its derivates present susceptibility to enzymatic degradation, namely by lysozyme and *N*-acetylglucosoaminidase [27,30]. The products resulting from its degradation can stimulate macrophages, and increase collagen deposition, which accelerates the wound healing process. Moreover, the release of *N*-acetyl-β-D-glucosamine, a degradation product, can contribute for fibroblasts proliferation, collagen deposition, and hyaluronic acid synthesis at the wound site [27].

Despite all the efforts performed so far, the currently available wound dressings still have some limitations: (1) relatively high production costs, (2) inappropriate mechanical stability, (3) poor permeability to gases, (4) exudate accumulation, and (5) inadequate protection from bacterial infection [80]. To address these problems, novel wound dressings are currently being developed by researchers of the tissue engineering area (Fig. 14.3), namely chitin- and chitosan-based wound dressings.

14.4.1 Chitin- and chitosan-based films

Films are semipermeable dressings, which enable the transmission of gases, and are impermeable to bacteria, thus preventing bacterial contaminations [81]. Furthermore, due to their flexibility, the

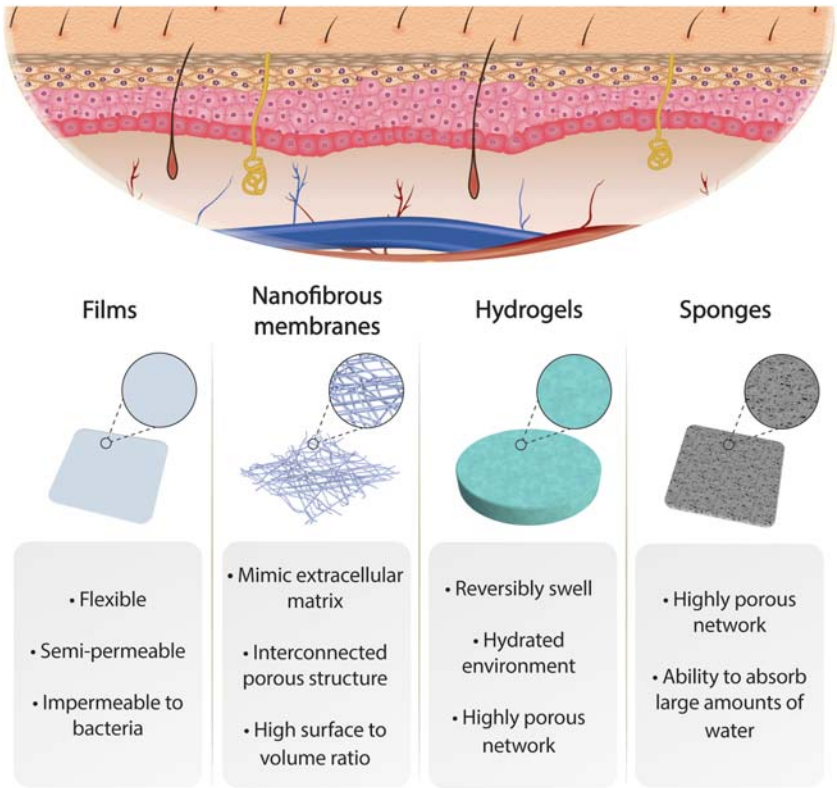

FIGURE 14.3

Illustration of the different types of wound dressings that have been produced using chitin/chitosan.

films can acquire the shape of the wound [82]. Despite the different advantages, the most common films need to be replaced during the healing process, which can induce damages to the wound bed, and its low absorptive capacity can also lead to an excess of exudate accumulation [81,83]. Taking this into account, some researchers have been producing some films based on chitin, chitosan, both, or even their combination with other polymers (as can be seen in Table 14.1) to improve their properties and the wound healing process [14,96,97].

Yusof and their collaborators developed different films based on chitin by a cold-press process [84]. In the in vivo studies, the wounds treated with the chitin film presented similar results to those treated with the commercial control (Opsite) in terms of wound closure and re-epithelization. In addition, no inflammatory cellular reaction was observed by the authors and increased collagen deposition was detected at the wound site. Moreover, the obtained wound healing results were better than those observed in the control group (wound covered with gauze), which support the potential of chitin films for wound healing applications [84].

The performance of chitin films can be improved through the incorporation of different natural compounds, growth factors, among others. For instance, Wang et al. introduced a chitin binding

Table 14.1 Chitin- and chitosan-based films for wound healing application.

Film composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Basic fibroblast growth factor-chitin binding domain/chitin (CTBD-bFGF/CT)	NA	NA	– After 3 days, CTBD-bFGF/CT film presented ≈225 3T3 cells per picture adhered to its surface, while bFGF/CT and chitin films only presented ≈112 and ≈59 cells per picture, respectively.	– CTBD-bFGF/CT film presented a higher neo-vascularization ability than the bFGF/CT film and chitin film (after 7 days of treatment).	[14]
Chitin	NA	NA	– Human skin fibroblast cells incubated with the chitin film presented a viability of 70%–80% (after 3 days).	– The wounds treated with Opsite or with the chitin film showed no inflammatory cellular reaction, increased collagen deposition and presented 70% of re-epithelization, contrasting with the 8% of the gauze-treated wound (after 7 days of treatment); – The wounds covered with Opsite or chitin film were completely closed after 14 days, while with gauze group	[84]

(*Continued*)

Table 14.1 Chitin- and chitosan-based films for wound healing application. ***Continued***

Film composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
				only closed after 21 days of treatment.	
Chitosan/poly(vinyl alcohol)/ polyvinylpyrrolidone (CS/PVA/PVP concentrations of 50/ 25/25 and 60/20/20 named by A3, B4, respectively)	DD = 90%	– A3 film presented 8×10^4 CFU/mL and 1.1×10^4 CFU/ mL, while the control showed 2×10^9 CFU/mL and 8×10^9 CFU/ mL, for *E. coli* and *S. aureus*, respectively; – B4 film presented 4.5×10^3 CFU/mL and 4×10^5 CFU/ mL, while the control 3×10^9 CFU/mL and 1×10^{10} CFU/ mL, for *E. coli* and *S. aureus*, respectively.	NA	NA	[85]
Chitosan/gelatin/ bacterial nanocellulose (CS/GEL/NC)	MW = 310−337 kDa	NA	NA	– The wounds treated with CS/GEL/NC film showed an area reduction of 95%, whereas the control group presented ≈78% of wound closure; – The wounds treated with CS/GEL/NC	[86]

				film showed ≈110 and ≈144 mm^3 volume of epidermis and dermis, respectively, while the control group showed ≈71 and ≈117 mm^3 (after 15 days of treatment).	
Chitosan/Fe-hydroxyapatite (CS/FeHAp)	MW = 190−310 kDa DD = 75%−85%	– CS/FeHAp and chitosan films decreased the *E. coli* viability from >6 to ≈4.0 log CFU/mL and 4.5 log CFU/mL (after 4 h), respectively; – CS/FeHAp film reduced the *MRSA* viability from ≈6.5 to ≈5 log CFU/mL (after 4 h), while the chitosan film reduced to ≈5.5 log CFU/mL; – CS/FeHAp film increased the *C. albicans* viability from ≈5 to ≈5.5 log CFU/mL (after 8 h) while chitosan film reduced the viability to 4.5 log CFU/mL.	– Keratinocyte cells incubated with CS/FeHAp film presented a viability of ≈100% (after 1 day).	NA	[87]
Chitosan	DD ≥ 75%	NA	– Dermal fibroblast and LPS-stimulated dermal fibroblasts cells incubated with chitosan film	NA	[88]

(*Continued*)

Table 14.1 Chitin- and chitosan-based films for wound healing application. *Continued*

Film composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
			presented a viability of 95.8% ± 7.1% and 96.0% ± 0.2%, respectively; – The in vitro wound closure of the dermal fibroblasts treated with the chitosan film was 28% ± 8% (after 1 day), while the control group showed a 22% ± 6% wound closure.		
Chitosan	MW = 190−310 kDa DD = 75%−85%	NA	NA	– The wounds treated with a chitosan film showed an area reduction of 40%, whereas the untreated group presented an ≈29% of wound closure; – The chitosan group showed reduced TNF-α, IL-1β and IL-10 expression comparatively with the untreated group (after 14 days of treatment);	[89]

				– The chitosan group showed a completely re-epithelized area with highly mature collagen fibers, no inflammatory infiltrate and no necrosis (after 15 days of treatment).	
Chitosan/ polyvinylpyrrolidone (CS/PVP)	DD = 79%	NA	– Fibroblast cells incubated with CS/ PVP film exhibited a viability of ≈100% (after 2 days).	– The wounds treated with chitosan film showed an area reduction of 94.91% ± 6.3%, whereas the gauze group showed a 90.95% ± 3.9% of wound closure (after 14 days of treatment).	[90]
Chitosan/konjac glucomannan (BIL)	DD = 83%	– The konjac glucomannan film displayed an inhibition area of ≈10 mm against *S. aureus* and *C. albicans*, ≈1 mm against *P. aeruginosa* and *E. coli*; – The chitosan film displayed an inhibition area of ≈10 mm against *S. aureus*, *P. aeruginosa*, *C. albicans*, and	– The Chinese hamster ovary cells incubated with the BIL film exhibited a viability of 118% ± 5% (after 1 day).	NA	[91]

(*Continued*)

Table 14.1 Chitin- and chitosan-based films for wound healing application. ***Continued***

Film composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
		$\approx$11 mm against *E. coli*.			
Chitosan	MW = 50−190 kDa DD = 85%	– Chitosan film decreased the *P. aeruginosa* and *S. aureus* viability from 10^6 to $\approx 8.8 \times 10^5$ CFU and from $\approx 9.3 \times 10^6$ to $\approx 8.5 \times 10^6$ CFU, respectively (after 1 day).	NA	– The wounds treated with chitosan film showed an area reduction of $\approx$87%, while the gauze-treated group presented $\approx$81% of wound closure (after 15 days of treatment); – The chitosan film-treated wound presented a re-epithelization of 85.58% ± 2.19%, 66.38 ± 16.15 microvessels, 139.00 ± 21.53 infiltrated inflammatory cells, 49.55 ± 7.19% of regions occupied with collagen and a granulation tissue area of 4.25 ± 0.55 mm^2, while the gauze group showed a re-epithelization of 70.66% ± 6.79%, 154.24 ± 28.36 microvessels, 441.13 ± 122.71 infiltrated inflammatory cells, 34.32% ± 8.88% of regions occupied	[92]

				with collagen and a granulation tissue area of 6.39 ± 1.58 mm^2.	
Alginate/chitosan-cerium and alginate/chitosan-calcium ions (ALG/Ce-CS and ALG/Ca-CS)	MW = 50−190 kDa DD = 75%−85%	– The ALG/Ce-CS and ALG/Ca-CS films exhibited higher antibacterial activity against *E. coli* and *S. aureus*, comparatively with the films without chitosan.	NA	NA	[93]
Chitosan/gelatin (CS/GEL)	LMW DD = 98%	– The CS/GEL film displayed an inhibition area of ≈5 mm and ≈4 mm, against *E. coli* and *S. aureus*, respectively.	– Fibroblast cells incubated with the film showed a viability of 89% (after 5 days); – The CS/GEL film was nonhemolytic (hemolysis ≈1.4%); – The CS/GEL film presented an antioxidant activity of ≈6%−25% and anti-inflammatory activity of ≈9%−36%.	NA	[94]
Chitosan	MW = 50−190 kDa DD = 85%	– The chitosan film showed an inhibition zone of 2 ± 0.2 cm and 1.24 ± 0.28 cm, against *E. coli* and *S. aureus*, respectively, after 1 day.	– Fibroblasts cells incubated with the film presented a viability of ≈73% (after 3 days).	NA	[95]

ALG/Ca-CS, Alginate/chitosan-calcium ions; ALG/Ce-CS, alginate/chitosan-cerium; bFGF/CT, basic fibroblast growth factor/chitin; BIL, chitosan/konjac glucomannan; C. albicans, Candida albicans; CFU, colony-forming unit; CS/FeHAp, chitosan/Fe-hydroxyapatite; CS/GEL/NC, chitosan/gelatin/bacterial nanocellulose; CS/GEL, chitosan/gelatin; CS/PVA/PVP, chitosan/poly(vinyl alcohol)/polyvinylpyrrolidone; CS/PVP, chitosan/polyvinylpyrrolidone; CTBD-bFGF/CT, basic fibroblast growth factor-chitin binding domain/chitin; DD, deacetylation degree; E. coli, Escherichia coli; IL-10, interleukin-10; IL-1β, interleukin-1beta; LMW, low molecular weight; LPS, lipopolysaccharide; MRSA, multiple-resistant *Staphylococcus aureus;* MW, molecular weight; NA, not applicable; P. aeruginosa, *Pseudomonas aeruginosa;* S. aureus, *Staphylococcus aureus;* TNF-α, tumor necrose factor-alpha.

domain (CTBD) into basic fibroblast growth factor (CTBD-bFGF) to decrease bFGF instability and diffusion [14]. Then, chitin films were produced via freezing-thaw and were incorporated with CTBD-bFGF (CTBD-bFGF@CT). After incubation for 3 days, a higher number of 3T3 cells were adhered to the surface of the CTBD-bFGF@CT films, comparatively to bFGF@CT films and chitin films. Furthermore, CTBD-bFGF@CT presented a higher capacity to induce angiogenesis in rat models, presenting higher number of new blood vessels after the 7 days of implantation [14].

Chitosan and its derivatives have also been used in the production of films due to their facility upon mild gelation to form these dressings [89,92]. Rodrigues Pereira et al. produced chitosan films incorporating *Mansoa hirsuta* fraction (CMHF) through the solvent casting method [89]. The wounds treated with blank chitosan films presented a wound contraction of 66% (after 10 days), which was improved to 100% with the incorporation of CMHF. Moreover, due to the presence of CMHF, the group treated with CS/CMHF presented a lower inflammatory response as well as higher collagen deposition, re-epithelization, and neovascularization [89]. Similarly, Taheri et al. produced chitosan/gelatin films through a casting method and these dressings were able to mediate a wound contraction of 93%−95%, contrasting with the 78.04% presented by the control group. Moreover, the incorporation of tannic acid (TA) and/or bacterial nanocellulose further improved the epithelialization and collagen deposition during the wound healing process [86].

Kim et al. produced chitosan films though casting and verified that these films had the capacity to decrease the *Pseudomonas aeruginosa* (*P. aeruginosa*) and *S. aureus* viability from 10^6 to $\approx 8.8 \times 10^5$ colony-forming unit (CFU) and from $\approx 9.3 \times 10^6$ to $\approx 8.5 \times 10^6$ CFU, respectively, after 1 day of films being in contact with bacteria [92]. After 15 days of treatment, the wounds covered with chitosan film showed a reduction in the area of $\approx 87\%$, while the gauze-treated group presented an $\approx 81\%$ of wound closure. Furthermore, the wounds treated with chitosan film presented $85.58\% \pm 2.19\%$ of re-epithelization, 66.38 ± 16.15 of microvessels, 139.00 ± 21.53 of infiltrated inflammatory cells, $49.55\% \pm 7.19\%$ of regions occupied with collagen and a granulation tissue area of 4.25 ± 0.55 mm^2. Comparatively, the gauze group showed $70.66\% \pm 6.79\%$ of re-epithelization, 154.24 ± 28.36 of microvessels, 441.13 ± 122.71 of infiltrated inflammatory cells, $34.32\% \pm 8.88\%$ of regions occupied with collagen and a granulation tissue area of 6.39 ± 1.58 mm^2. Moreover, the addition of S-nitrosoglutathione as a nitric oxide donor improved the antibacterial properties of the film ($\approx 6 \times 10^5$ CFU and $\approx 2.7 \times 10^6$ CFU for *P. aeruginosa* and *S. aureus* viability, respectively), and its wound healing capacity ($\approx 95\%$ of wound closure) [92].

14.4.2 Chitin- and chitosan-based nanofibrous membranes

Membranes can act as physical barriers that impair the penetration of microorganisms [68]. Among them, nanofibrous membranes are a promising technology due to their high surface-to-volume ratio and interconnected pores. These properties confer the capacity of allowing gas exchange and nutrient supply, while simultaneously supporting cell proliferation and migration [98]. Furthermore, this type of membranes can be produced by different techniques, such as self-assembly, phase separation, and electrospinning [99]. Different works where chitin- and chitosan-based membranes have been developed to improve the wound healing process are listed in Table 14.2.

Wu and their collaborators developed chitin fibers reinforced with bacterial cellulose nanocrystals (BCNC/CT) by a wet-spinning process followed by an additional coating with chitin (CT/BCNC/CT) [101]. After 10 days of treatment, the CT/BCNC/CT promoted the complete wound

Table 14.2 Chitin- and chitosan-based nanofibrous membranes for wound healing application.

Nanofibrous membrane composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Chitin-/chitosan-glucan (CTCSGC)	NA	– CTCSGC membrane displayed an inhibition area of 12, 8, 13, and 11 mm against *E. coli*, *K. pneumoniae*, *S. aureus*, and *B. subtilis*, respectively.	– The produced membrane (100−1000 mg/mL) did not exhibit any toxicity for the fibroblast (after 24, 48, and 72 h).	– The wound treated with CTCSGC membrane showed an area reduction of 95%, whereas the control presented 72% of wound closure (after 15 days of treatment).	[100]
Chitin/bacterial cellulose nanocrystals (CT/BCNC/CT)	NA	NA	NA	– CT/BCNC/CT membrane promoted the complete wound closure, without any signs of inflammatory lesions and increased the amount of collagen (after 10 days of treatment).	[101]
Dibutyryl chitin (DBC)/poly (lactic acid) (DBC/PLA)	NA	NA	– Keratinocytes cells incubated with DBC (25−100 μg/mL) showed spreading rates of ≈63%−86%, while in the control this value was ≈50% (after 1 day). – Keratinocyte cells incubated with DBC (25−100 μg/mL) presented a type I collagen synthesis of ≈107%−130%, while the control had 100% (after 1 day).	– The wounds treated with DBC/PLA membrane showed reduced moisture, increased expression of type I collagen and filaggrin, as well as reduced cellular infiltration and wounded area, comparatively with the nontreated control group.	[102]

(*Continued*)

Table 14.2 Chitin- and chitosan-based nanofibrous membranes for wound healing application. *Continued*

Nanofibrous membrane composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Chitin	MW = 920 kDa DD = 8%	NA	– Chitin nanofiber showed more cell attachment and spreading for both normal human keratinocytes and fibroblasts, comparatively with commercial chitin microfibers.	– The wounds treated with the chitin nanofiber presented penetration of connective tissue into the short fiber fragments, fibroblasts arranged linearly and no infiltration of inflammatory cells (after 28 days of treatment).	[103]
Poly(vinyl alcohol)/chitosan (PVA/CS)	MW = 60−120 kDa DD > 95%	– PVA/CS (≥3 mg/mL) displayed an inhibition capacity of ≈25%−86% and ≈39%−84% against *E. coli* and *S. aureus*, respectively.	– HaCaT cells incubated with PVA/CS membrane (≥0.25 mg/mL) exhibited a viability of ≈95%−109% (after 12 h).	– The wounds treated with PVA/CS membrane showed an area reduction of ≈95%, whereas untreated group presented ≈92% of wound closure.	[104]
Poly (ε-caprolactone)/ chitosan oligomers (COS, 2%−16%) (PeCL/COS)	MW = 270 kDa	– PeCL/COS membrane displayed an inhibition area of $8.7 \pm 0.2-13 \pm 0.1$ and $9 \pm 0.1-12.5 \pm 0.5$ mm, against *P. aeruginosa* and *S. aureus*, respectively.	– Fibroblast cells incubated with PeCL/COS membrane exhibited a viability of ≈65%−111% (after 1 day); – PeCL/COS membrane displayed a blood-clotting index of ≈37%−60%.	– The wounds treated with PeCL/COS membrane showed an area reduction of ≈62%−71%, whereas the control (without sample) presented ≈57% of wound closure; – PeCL/COS membrane with higher concentration of COS showed higher re-epithelization and reduced scar area.	[105]

Poly(vinyl alcohol)/chitosan (PVA/CS)	MW = 50−190 kDa	– PVA/CS membrane displayed an inhibition area of 14.1 ± 0.8, 15.8 ± 1.0, 13.0 ± 0.7, and 5.4 ± 0.5 mm, against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*, respectively; – PVA/CS membrane presented an *E. coli* and *S. aureus* viability reduction of 83.6% ± 3.0% and 75.5% ± 4.1%, respectively.	– PVA/CS membrane (12.5−200 μg/mL) presented an antioxidant activity of 9.5%−52%.	– The wounds treated with PVA/CS membrane showed an area reduction of ≈77%, whereas untreated group presented 52.3% ± 2.8% of wound contraction (after 12 days of treatment); – PVA/CS membrane treated wounds showed a significantly increased amount of collagen when compared to the untreated group.	[106]
Deacetylated and arginine-chitosan (CS-A)	MW = 310−375 kDa DD = 97.26% ± 0.02%	– CS-A membrane displayed an inhibition area of ≈48 and ≈30 mm against *E. coli* and *S. aureus*, respectively.	– Fibroblast cells incubated with CS-A membrane exhibited a viability of ≈102% (after 3 days).	– The wounds treated with CS-A membrane exhibited a wound size of ≈28%, whereas the control group showed ≈39% (after 21 days of treatment); – CS-A membrane treated group showed a higher re-epithelialization degree and tissue reorganization, when compared to the control group	[74]

(*Continued*)

Table 14.2 Chitin- and chitosan-based nanofibrous membranes for wound healing application. *Continued*

Nanofibrous membrane composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
				(after 21 days of treatment).	
Poly(L-lactic acid/poly (D-lactic acid) grafted quaternized chitosan (PLA/QCS-PDLA/QCS) (GTMAC: chitosan ratio 1:1, 2:1, and 3:1 corresponding to QCS1, QCS2 and, QCS3, respectively)	MW = 100 − 150 kDa DD > 90%	– With the increase of degree of substitution of QCS, the minimum inhibitory concentration against *E. coli* decreased (1 mg/mL for QCS1 and 0.5 mg/mL for QCS2 and QCS3); – PLA/QCS-PDLA/QCS electrospun membrane obtained an antibacterial activity of ≈98%, against *E. coli* and *S. aureus*.	– QCS (5 mg/mL) presented an antioxidant activity of ≈81%−100%.	– PLA/QCS-PDLA/QCS electrospun membrane showed higher regularity of connective tissue with more fibroblasts, higher collagen content, less inflammatory cells, more blood vessels, and a complete epithelium structure, comparatively to the control group (Tegaderm) (after 15 days of treatment); – The wounds treated with PLA/QCS-PDLA/QCS membrane showed a complete wound	[69]

				closure, while control group presented ≈88% of wound contraction.	
Polycaprolactone/ chitosan/ poly (ethylene oxide) (PCL/CS-PEO)	MW = 50−190 kDa DD = 75%−85%	NA	NA	– The wounds treated with PCL/CS-PEO membrane exhibited a wound size of ≈25%, whereas PCL group showed ≈48% (after 9 days of treatment); – PCL/CS-PEO membrane-treated group showed higher inflammatory infiltrate, expression of proliferating cell nuclear antigen (PCNA), and smooth muscle actin, comparatively with PCL group (after 3 days of treatment); – PCL/CS-PEO membrane-treated group exhibited a higher amount of inflammatory infiltrate, expression of tumor necrosis factor and PCNA, immunolabeling of monocyte chemoattractant protein-1, and deposition of collagen fibers, comparatively with PCL group (after 7 days of treatment);	[107]

(*Continued*)

Table 14.2 Chitin- and chitosan-based nanofibrous membranes for wound healing application. *Continued*

Nanofibrous membrane composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Poly (ε-caprolactone)/ chitosan (PeCL/ CS)	MW = 50−190 kDa DD = 75%−85%	– PeCL/CS membrane obtained an antibacterial activity of 59.15% and 77.18% against *MRSA* and *E. coli* (extended spectrum b lactamase), respectively (after 2 days).	– Fibroblast cells incubated with PeCL/CS nanofiber exhibited a viability of ≈150% (after 5 days).	– The wounds infected with *MRSA* and treated with PeCL/CS nanofiber presented $\approx 1.2 \times 10^3$ CFU, while the control had $\approx 1.7 \times 10^3$ (after 15 days of treatment); – The wounds treated with PeCL/CS nanofiber showed advanced healing, with newly synthesized fibrous tissue, presence of a well-organized granulation tissue, and good epithelialization function, while the control presented low collagen content, a necrotic epithelium, and the onset of granulation tissue formation (after 15 days of treatment).	[108]

B. subtilis, Bacillus subtilis; CFU, colony-forming unit; COS, chitosan oligomers; CS-A, deacetylated-arginine-chitosan; CT/BCNC/CT, chitin/bacterial cellulose nanocrystals; CTCSGC, chitin-/chitosan-glucan complex; DBC/PLA, dibutyryl Chitin/poly(lactic acid); DBC, dibutyryl chitin; DD, deacetylation degree; E. coli, Escherichia coli; GTMAC, glycidyltrimethylammonium chloride; K. pneumoniae, Klebsiella pneumoniae; MRSA, multiple-resistant *Staphylococcus aureus;* MW, molecular weight; NA, not applicable; P. aeruginosa, *Pseudomonas aeruginosa;* PCL/CS-PEO, polycaprolactone/chitosan/poly(ethylene oxide); PCL, polycaprolactone; PCNA, proliferating cell nuclear antigen; PeCL/CS, poly(ε-caprolactone)/chitosan; PeCL/COS, poly(ε-caprolactone)/chitosan oligomers*;* PLA/QCS-PDLA/QCS, poly (L-lactic acid/poly(D-lactic acid) grafted quaternized chitosan; PVA/CS, poly(vinyl alcohol)/chitosan; QCS, quaternized chitosan; S. aureus*, Staphylococcus aureus.*

closure, presenting a performance quite similar to that of the commercial control (polyamine). Furthermore, the wounds treated with CT/BCNC/CT did not present signs of inflammatory lesions and also showed an increased amount of collagen [101]. In another work, Ahmed et al. produced an electrospun nanofibrous membrane of chitosan and poly(vinyl alcohol) (PVA) and analyzed its performance in the wound healing process [106]. The wounds treated with chitosan/PVA membranes showed a faster wound healing, that is, $\approx 77\%$ of wound closure after 12 days, comparatively with the control group ($52.3\% \pm 2.8\%$). The incorporation of ZnO nanoparticles further enhanced this capacity to accelerate the wound healing process ($90.5\% \pm 1.7\%$ of wound closure) [106].

In another work, Jang and collaborators analyzed the effect of dibutyryl chitin/poly(lactic acid) membranes on wounds and observed reduced moisture, increased expression of type I collagen and filaggrin, as well as reduced cellular infiltration and wounded area, comparatively with the non-treated control group [102].

Chitosan has also been used to improve the properties of some membranes through its blend incorporation or coating. Ho et al. coated plasma treated polycaprolactone (PCL) electrospun membrane with COS to improve its potential as a wound dressing [105]. The COS-coated PCL membrane exhibited high antibacterial activity against *P. aeruginosa* and *S. aureus*. Furthermore, higher COS densities contributed to improve the hemostasis ability of the PCL membrane, facilitate the re-epithelization, reduce the scar area, and provide free-radical scavenging capacity [105].

The combination of both chitin and chitosan in membranes has also been pursued. For example, Abdel-Mohsen et al. isolated chitin/chitosan-glucan complex (CTCSGC) from *Schizophyllum commune* and used it for producing nonwoven microfiber mats by wet-dry-spinning technique [100]. The nonwoven microfibers exhibited strong antibacterial activity against *E. coli*, *Klebsiella pneumoniae*, *S. aureus*, and *Bacillus subtilis*. Moreover, these microfibers promoted a wound closure of 95% with a complete re-epithelization, while the control only had 72%, after 15 days of the surgery [100].

Furthermore, asymmetric membranes have also been used as a new approach due to their ability to mimic both epidermis and dermis layers of the native skin [109]. Zanchetta et al. produced an asymmetric electrospun membrane where the bottom layer was produced with PCL and the top layer was composed of chitosan and poly(ethylene oxide) (PCL/CS-PEO) [107]. After 9 days, the wounds treated with PCL/CS-PEO membranes exhibited a wound size of $\approx 25\%$, whereas PCL-treated group showed $\approx 48\%$. In addition, after 7 days of treatment, the PCL/CS-PEO membrane-treated group exhibited a higher amount of inflammatory cells infiltrate, expression of tumor necrosis factor, and proliferating cell nuclear antigen, such as immunolabeling of monocyte chemoattractant protein-1 and deposition of collagen fibers, comparatively to the PCL-treated group [107].

14.4.3 Chitin- and chitosan-based hydrogels

Hydrogels are highly hydrated 3D structures developed by noncovalent or covalent cross-linking bonds of hydrophilic polymers. Hydrogels can reversibly swell, and therefore can provide to the wound a moist environment while absorbing the exudates. The hydrated environment provided by the hydrogels supports cell growth and proliferation [110,111]. These biological processes are also supported by the hydrogels' similarity with the extracellular matrix. Moreover, hydrogels also display a highly porous network, which facilitates the ingrowth of new blood vessels, cellular

proliferation, as well as the diffusion of gases, nutrients, and waste products resulting from metabolic activity [112,113]. Furthermore, hydrogels are a suitable polymeric network to load cells, antibacterial agents, growth factors, and other molecules (e.g., anti-inflammatory agents), which can enhance the wound healing process [113].

However, chitin and chitosan polymers present some limitations that impair their applicability as hydrogels, such as poor water solubility, weak structural resistance, and fast biodegradation [114]. Focused on the enhancement of physicochemical properties, performance, and applicability of chitin- and chitosan-based hydrogels, the researchers have been developing different strategies to improve the hydrogels' features. These approaches include the production of chitin and chitosan derivatives (e.g., hydroxypropyl chitin, succinyl chitosan, oxidized chitosan) and/or combination with other polymers (e.g., natural polymers, such as collagen, gelatin, silk fibroin, and/or synthetic polymers like PVA or PCL), and therapeutic agents (e.g., growth factors, and anti-inflammatory and/or antibacterial agents). Examples of chitin- and chitosan-based hydrogels developed so far for wound healing applications are listed in Table 14.3.

To accelerate the wound healing process, Shou and collaborators produced chitin hydrogels and used it to encapsulate stem cells [118]. After 16 days of treatment, the wounds treated with the chitin hydrogels exhibited a wound reduction size of $\approx 80\%$, while the control group (without treatment) presented a wound closure of $\approx 59\%$. Moreover, wounds treated with chitin hydrogel revealed a vascularization of ≈ 6 vessels per high-power field, while the control group presented 3 ± 1.13 vessels per high-power field. Furthermore, the encapsulation of bone marrow mesenchymal stem cells into the chitin hydrogel improved the wound healing ability ($\approx 95\%$ of wound closure and a vascularization of 17.42 ± 1.31 vessels per high-power field) [118].

Yang et al. produced an injectable chitin hydrogel composed of adipic dihydrazidegrafted carboxyethyl chitin and dibenzaldehyde-terminated poly(ethylene glycol) (CECT-AHD/PEG-DA) [117]. The CECT-AHD/PEG-DA hydrogels demonstrated effective gelation after 30 min of in vivo injection, and their complete degradation was observed 32 days after injection. After 32 days of treatment, no inflammatory cells were observed in tissues around the hydrogel, demonstrating the suitable immunotolerance and biodegradability of injectable CECT-ADH/PEG-DA hydrogels [117].

The hydroxypropyl chitin (HPCH), TA, and ferric ion hydrogels (HPCH/TA/Fe) developed by Ma et al. exhibited a highly efficient antibacterial activity against *S. aureus* and *E. coli* bacteria ($\approx 0\%$ bacteria viability), contrasting with phosphate-buffered saline (PBS), HPCH, and HPCH/Fe groups ($> 100\%$ bacteria viability) [116]. The HPCH/TA/Fe hydrogel reduced the area of the wound defect to $\approx 3\%$ after 12 days, while the PBS and HPCH hydrogel-treated groups only presented a reduction in the wound area to $\approx 8\%$ and $\approx 25\%$, respectively. Such features confirmed the applicability of in situ injectable HPCH/TA/Fe hydrogel as a suitable antibacterial wound dressing [116].

Regarding the development of chitosan-based hydrogels, He et al. produced a collagen peptide-functionalized CMC and oxidized methacrylate sodium alginate hydrogel (CMC-COP/OMSA) [126]. CMC/OSA, CMC/OMSA, and CMC-COP/OMSA hydrogels exhibited excellent hemocompatibility, with hemolysis ratios of 1.5%−1.8%. Moreover, wounds treated with CMC-COP/OMSA hydrogel displayed a 96.6% reduction of their area, 7 days post-operative, and exhibited a granulated tissue of ≈ 488 and $\approx 450\ \mu m$ thicker than the normal saline and commercial groups, respectively [126].

Table 14.3 Chitin- and chitosan-based hydrogels for wound healing application.

Hydrogel composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Acrylamide-modified β-chitin (Am-β-CT)	MW = 15.755 kDa	NA	NA	– The wounds induced on Zebrafish vertebrate model treated with Am-β-CT hydrogel showed a wound contraction of 72%, while the control group only reached a wound contraction of 50% (22 days post-wounding).	[115]
Hydroxypropyl-modified chitin with and without tannic acid and ferric ion (HPCH/TA/Fe and HPCH, respectively)	NA	– HPCH/TA/Fe hydrogel decreased the *E. coli* and *S. aureus* viability to ≈0% (after 1 day), contrasting with the ≈200%−225% obtained with the HPCH hydrogel.	– Viability of NHDF cells incubated with HPCH hydrogel was ≈100% (after 24 h).	– Wounds treated with HPCH/TA/Fe hydrogel displayed a wound size of ≈3%, while the groups treated with HPCH hydrogel and PBS exhibited a wound size of ≈25% and ≈8%, respectively (after 12 days of treatment).	[116]
Adipic dihydrazidegrafted carboxyethyl chitin with and without dibenzaldehyde-terminated poly (ethylene glycol) (CECT-ADH/PEG-DA and CECT-ADH, respectively)	NA	NA	– BMSC incubated with CECT-ADH hydrogel exhibited a viability >90% (after 3 days).	– No inflammatory cells were observed in surrounding tissues of wounds treated with CECT-ADH/PEG-DA hydrogel (after 32 days of treatment).	[117]

(*Continued*)

Table 14.3 Chitin- and chitosan-based hydrogels for wound healing application. *Continued*

Hydrogel composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Chitin nanofiber-based hydrogel (CNF) encapsulating bone marrow mesenchymal stem cells (BMSC)	NA	NA	– BMSC encapsulated within the CNF hydrogel maintained a viability >86% (after 16 days).	– The wounds treated with CNF hydrogel presented an ≈80% area reduction, whereas the control group showed an ≈59% reduction in the wound area (after 16 days of treatment); – Moreover, wounds treated with BMSC-encapsulated CNF hydrogel revealed a vascularization of 17.42 ± 1.31 vessels per high-power field, higher than the 3.00 ± 1.13 vessels per high-power field obtained in the control group (after 16 days of treatment).	[118]
Amidated pectin/oxidized chitosan (AP/OC) (AP:OC ratios of 65:35, 70:30, and 80:20 named by AP-OC-65, AP-OC-70, AP-OC-80, respectively)	MW = 190−375 kDa	NA	– AP-OC-65 hydrogel (with higher content of oxidized chitosan) exhibited the best hemocompatibility profile (≈1.5% hemolysis), compared to hydrogel with lower content of oxidized chitosan (≈3% and ≈9% of hemolysis for AP-OC-70 and AP-OC-80, respectively).	NA	[119]

Poly(vinyl alcohol)/chitosan/gelatin/honey (honey concentrations of 0%, 5%, 10%, and 20% w/w, named as H-0, H-5, H-10, and H-20, respectively)	MW = 190−310 kDa DD = 75%−85%	– Increased honey content enhanced the antibacterial capacity against *P. aeruginosa*, inhibition area of 4 ± 0.3 mm for H-0, 6.5 ± 1 mm for H-5, 9.6 ± 1.3 for H-10, and 14 ± 1 mm for H-20.	– Viability of fibroblast cells incubated with H-0, H-5, H-10, and H-20 hydrogel were ≈60%, ≈80%, ≈95%, and ≈75%, respectively (after 14 days).	– After 12 days of in vivo implantation, the H-10 and H-20 chitosan-based hydrogel enabled an ≈95% wound contraction.	[120]
Methacrylated RGD glycol chitosan and methacrylated lysozyme (RL1)	MW ≈ 100 kDa	– The control hydrogel without lysozyme did not show antibacterial activity, while the RL1 hydrogel prevented the proliferation of *S. aureus* and *E. coli* strains.	– RL1 hydrogel presented an enhanced cell migration both at the hydrogel' surface and within the polymeric network; – The type I collagen expression related with fibroblasts incubated with RL1 was also upregulated, presenting an increased ratio of type I collagen to type III collagen, when compared to the control group.	NA	[121]
Poly(D,L-lactide)-poly(ethylene glycol)-poly(D,L-lactide)/nanoscaled bioactive glass/catechol-modified quaternized chitosan (PLEL-nBG-QCS-C1)	MW = 190−310 kDa DD = 75%−85%	– PLEL-nBG-QCS-C1 hydrogel inhibited the bacterial growth of *S. aureus* and *E. coli* by more than 95%, contrasting with the <10% of PLEL and PLEL-nBG hydrogel.	– L929 cells incubated with hydrogel exhibited a viability >85% (after 24 and 48 h).	– The wounds treated with PLEL-nBG-QCS-C1 showed an area reduction of ≈99%, whereas suture- and fibrin glue- treated models presented ≈66% of wound closure.	[122]

(*Continued*)

Table 14.3 Chitin- and chitosan-based hydrogels for wound healing application. *Continued*

Hydrogel composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Oxidized hyaluronic acid/succinyl chitosan composite (OHA/SCS)	DD $\geq 95\%$	NA	– 3T3 cells incubated with OHA/SCS hydrogel presented viability of $\approx 100\%$ (after 24 h).	– OHA/SCS hydrogel and gauze-treated groups presented a wound closure of $\approx 50\%$ and $\approx 44\%$, after 6 days of treatment. This increased to $\approx 80\%$ and $\approx 76\%$ for OHA/SCS and gauze control group after 12 days of treatment.	[123]
N-Carboxyethyl chitosan/adipic acid dihydrazide and hyaluronic acid-aldehyde (N-CS/HA-ALD), loaded with bone marrow mesenchymal stem cells (BMSCs)	NA	NA	– BMSC loaded in N-CS/HA-ALD hydrogel presented an increased secretion of TGF-β1, VEGF, and bFGF (2.71 ± 0.26, 22.17 ± 3.65, and 3.63 ± 0.96 ng/L, respectively, after 3 days).	– The area of the wounds treated with N-CS/HA-ALD hydrogel was $14.48\% \pm 3.35\%$, whereas the control group presented a wound area of $41.55\% \pm 2.67\%$ (15 days posttreatment).	[124]
Methacrylated chitosan/methacrylated silk fibroin (CSMA/SFMA)	MW = 50–190 kDa DD >75%	– The viability of *E. coli* and *S. aureus* bacteria in contact with CSMA/SFMA hydrogel was $\approx 10\%$ (after 24 h).	NA	– Wounds treated with CSMA/SFMA hydrogel presented a wound area of $\approx 10\%$, while the control group displayed a wound area of $\approx 35\%$ (after 14 days of treatment).	[125]

Collagen peptide-functionalized carboxymethyl chitosan/oxidized methacrylate sodium alginate (CMC/OMSA)	MW ≈200 kDa	NA	– L929 cells incubated with CMC/OMSA hydrogel displayed a survival >95% (after 15 days); – L929 cells incubated with CMC/OMSA hydrogel exhibited a hemolysis of 1.6%.	– Wounds treated with CMC/OMSA hydrogel displayed a wound closure of 91%, whereas normal saline and commercial dressing groups were 54.9% and 75%, respectively (7 days post-operative).	[126]
Gallic acid/hexanoyl glycol chitosan (GA/HGC) (Degree of gallylation of 5% and 10%, named by GA5/HGC37 and GA10/HGC37, respectively)	DD = 12.33% ± 0.47%	NA	– NIH 3T3 cells incubated with GA5/HGC37 and GA10/HGC37 hydrogel presented a viability of ≈95% (after 48 h).	– Wounds treated with GA10/HGC37 hydrogel presented a wound contraction of ≈100%, while the control group was ≈90%, (after 15 days of wound induction); – The granulation tissue in the GA10/HGC37 hydrogel group was ≈60 μm thicker than that observed in the control group (after 15 days of treatment).	[127]

Am-β-CT, Acrylamide-modified β-chitin; AP/OC, amidated pectin/oxidized chitosan; bFGF, basic fibroblast growth factor; BMSC, bone marrow mesenchymal stem cells; CECT-ADH/PEG-DA, adipic dihydrazidegrafted carboxyethyl chitin/dibenzaldehyde-terminated poly(ethylene glycol); CECT-ADH, adipic dihydrazidegrafted carboxyethyl chitin; CMC/OMSA, collagen peptide-functionalized carboxymethyl chitosan/oxidized methacrylate sodium alginate; CNF, chitin nanofiber-based hydrogel; CSMA/SFMA, methacrylated chitosan/methacrylated silk fibroin; E. coli, Escherichia coli; GA/HGC, gallic acid/hexanoyl glycol chitosan; HPCH/TA/Fe, hydroxypropyl-modified chitin/tannic acid/ferric ion; HPCH, hydroxypropyl-modified chitin; NA, not applicable; N-CS/HA-ALD, N-carboxyethyl chitosan/adipic acid dihydrazide and hyaluronic acid-aldehyde; NHDF, normal human dermal fibroblasts; OHA/SCS, oxidized hyaluronic acid/succinyl chitosan; PBS, phosphate buffered saline; PLEL-nBG-QCS-C1, poly(D,L-lactide)-poly(ethylene glycol)-poly(D,L-lactide)/nanoscaled bioactive glass/catechol-modified quaternized chitosan; RGD, arginine-glycine-aspartate; S. aureus, *Staphylococcus aureus;* TGF-β1, transforming growth factor beta 1; VEGF, vascular endothelial growth factor.

In another study, Zheng et al. produced an injectable hydrogel containing catechol-modified quaternized chitosan (QCS-C ranging from 0 to 3 wt.%), poly(D,L-lactide)-poly(ethylene glycol)-poly(D,L-lactide) (PLEL), and nanoscaled bioactive glass (nBG), to provide improved adhesiveness and angiogenesis ability [122]. The incorporation of 1 wt.% of QCS-C in PLEL-nBG-QCS1 hydrogel inhibited successfully the *S. aureus* and *E. coli* bacterial growth in more than 95%, while PLEL and PLEL-nBG hydrogels displayed an antibacterial capacity inferior to 10%. Such confirms the effective antibacterial activity of QCS added to the hydrogel. Moreover, PLEL-nBG-QCS-C1 hydrogel exhibited an $\approx$99% wound closure in the in vivo assays, whereas the untreated, suture- and fibrin glue-treated models presented a wound closure of $\approx$65.5%, after 10 days of treatment. The combination of QCS-C and nBG with PLEL hydrogel resulted in an injectable and thermosensitive hydrogel with great antibacterial and healing potential to act as a wound dressing [122].

Zhu and colleagues developed hydrogels based on oxidized hyaluronic acid and succinyl chitosan (OHA/SCS) [123]. Wounds treated with OHA/SCS hydrogels achieved a wound closure of $\approx$80%, comparatively to the gauze control group that presented a wound closure of 76%, after 12 days of treatment. In addition, the incorporation of insulin-loaded micelles and epidermal growth factor into the OHA/SCS hydrogels enhanced the wound healing capacity. They promoted a wound closure of $\approx$95%, after 12 days of treatment [123].

14.4.4 Chitin- and chitosan-based sponges

Sponges are foam-like 3D structures with the ability to absorb large amounts of water due to their highly porous and interconnected network, which sustain a moist environment at the wound site. Moreover, the sponges' swelling capacity allows them to absorb the wound exudate [45,111]. However, sponges are mechanically weak and may provoke skin's maceration. In general, chitin and chitosan sponges can be functionalized through chemical modifications or even combined with other polymers and/or therapeutic agents, to improve their mechanical, hydrophilic, hemostatic, and antibacterial properties. In this way, chitin and chitosan have been combined with other materials to produce sponges to be exploited for wound healing, as listed in Table 14.4 [45].

Jiang et al. developed sponges based on chitin and observed that they presented capacity to improve the wound healing process [128]. After 11 days of treatment, the wounds treated with the chitin sponges presented a 62.5% of wound closure, while the control group (without treatment) only attained 50.2%. In addition, the incorporation of corn stalk and silver nanoparticles further increased the wound healing ability (76.5% of wound closure was accomplish).

Gao et al. produced sponges, by combining quaternized chitin and cellulose nanofibers (CEL-NF) suspensions at concentrations of 2, 5, and 10 mg/mL (CEL-NF2, CEL-NF5, and CEL-NF10, respectively), enriched with rectorite (REC) to improve their antibacterial and hemostatic properties [129]. The CEL-NF sponges did not present an intrinsic antibacterial activity, while the incorporation of REC conferred to CEL-NF/QCT/REC sponges antibacterial efficacy against *S. aureus* and *E. coli*. Moreover, CEL-NF/QCT/REC sponges reduced the in vivo healing time to 12 days, which was shorter than that obtained in the gauze-treated group (15 days until complete healing). Also, CEL-NF/QCT/REC sponges improved the formation of new collagen at the wound site ($\approx$50%), compared to the gauze group ($\approx$45%), after 9 days of treatment [129].

Hu et al. conjugated hydroxybutyl-modified chitosan (HBC) with neutral chitosan to produce CS/HBC sponges, with improved water solubility and controlled temperature-sensitive properties

Table 14.4 Chitin- and chitosan-based sponges for wound healing application.

Sponge composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Chitin/corn stalk/ silver nanoparticles (CH/CRNS/AgNPs)	NA	– CH/CRNS/AgNPs displayed an inhibition area >3 mm against *E. coli* and *S. aureus*.	– L929 cells incubated with CH/CRNS/AgNPs sponge showed a viability >100% (after 24 h); – CH/CRNS/AgNPs suspension (1000 μg/mL) presented a hemolysis value ≤ 3%.	– Wounds treated with CH/CRNS/AgNPs sponge exhibited a wound closure of 76.5%, higher than the 50.2% reported for the control group (after 11 days of treatment).	[128]
Cellulose nanofibers/ quaternized chitin/ rectorite (CEL-NF/QCT/REC)	NA	– *In vitro* antibacterial evaluation demonstrated that CEL-NF/QCT/REC sponge presented antibacterial activity against *S. aureus* and *E. coli*, due to QCT presence, while BACNF sponge had no antibacterial activity.	– L929 cells incubated with CEL-NF/QCT/REC sponge exhibited a viability >80% (after 24 h); – CEL-NF/QCT/REC sponge presented a hemolysis value <2%.	– Wounds treated with CEL-NF/QCT/REC sponge healed after 12 days, while the gauze group achieved complete wound closure after 15 days; – Wounds treated with CEL-NF/QCT/REC sponge showed improved collagen formation (≈ 50%), comparatively to the gauze group (≈ 45%), after 9 days of treatment.	[129]
Quaternary chitin/ chitin nanofiber (QCNS)	NA	– QCNS displayed an antibacterial activity of ≈ 100% against *E. coli* and *S. aureus*.	– Fibroblasts incubated with QCNS sponge presented a viability of 94% (after 48 h); – QCNS suspensions (0.5−4 mg/mL) exhibited hemolysis values ranging from 0.75% ± 0.05% to 2.8% ± 0.12%.	– Wounds treated with QCNS sponge reduced the blood loss from 1.82 ± 0.12 to 0.21 ± 0.02 g, while the gauze group reduced the blood loss from 1.82 ± 0.12 to 1.53 ± 0.11 g.	[130]

(*Continued*)

Table 14.4 Chitin- and chitosan-based sponges for wound healing application. *Continued*

Sponge composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
Chitosan/alginate/hyaluronic acid (CAHS) (Chitosan: alginate ratios of 1:3, 2:3, and 3:3, named by CAHS1, CAHS2, and CAHS3, respectively)	MW = 800 kDa DD ≈ 75%	NA	– HUVEC incubated with CAHSs extractions displayed a viability >80% (after 48 h); – CAHS1, CAHS2, and CAHS3 presented a hemolysis of 4.8% ± 0.3%, 2.3% ± 0.2% and 0.9% ± 0.2%, respectively.	– Wounds treated with CAHS1, CAHS2, and CAHS3 sponge exhibited a wound closure of ≈90%, ≈100%, and ≈97%, respectively (after 21 days).	[131]
Alginate/carboxymethyl chitosan (AC)	NA	– AC sponge exhibited an antibacterial activity of ≈80% and ≈40% against *E. coli* and *S. aureus*, respectively.	NA	– Wounds treated with AC sponge exhibited a wound contraction of ≈92%, superior to the ≈88% of the control group (after 14 days of treatment).	[67]
Thymine-modified chitosan (TC) [Degree of substitution ranging from 0.23 (TC-1) to 0.62 (TC-4)]	DD ≥90%	– With the increase of the degree of substitution of TC (0−0.62), the MIC of all strains decreased (*E. coli* from 16 to 8 μg/mL, *S. aureus*, *C. albicans*, and *P. aeruginosa* from 32 to 8 μg/mL, and *A. baumannii* from 64 to 16 μg/mL); – No colonies were observed on agar plates with TC sponge, contrary to unmodified chitosan sponge that presented colonies on agar plates.	– L929 cells incubated with TC sponge and unmodified chitosan sponge presented a viability of ≈100% (after 24 h); – TC and unmodified chitosan sponge presented hemolysis values of 0.5%−0.9%.	– Wounds treated with TC-1 and TC-4 sponges improved the collagen content (42.5% ± 3.4% and 58.3% ± 4.0%, respectively), comparatively to unmodified chitosan sponge and gauze group (21.3% ± 3.7% and 29.7% ± 2.7%, respectively), after 6 days of treatment; – VEGF expression levels in the groups treated with chitosan, TC-1, and TC-4 sponges were 1.4, 1.8, and 2.4 times higher than the	[72]

				gauze group, respectively. CD31 expression levels induced by chitosan, TC-1, and TC-4 sponges were 1.3, 1.8, and 2.1 times higher than the gauze group, respectively.	
Adenine-modified chitosan (CS-A) [Degree of substitution of 0.21 (CS-A1) and 0.55 (CS-A2)]	DD $\geq 90\%$	– Increasing the degree of substitution of adenine in chitosan (0.21 and 0.55) resulted in increased MIC values of CS-A against *E. coli* and *S. aureus* (4−16 and 8−32 μg/mL, respectively).	– L929 cells incubated with CS-A1 and CS-A2 sponges exhibited a viability of $\approx 100\%$ and $\approx 115\%$, respectively (after 24 h).; – CS-A derivatives (with a concentration of 2 mg/mL) exhibited a hemolysis $<1.5\%$.	– Wounds treated with sponge with a higher degree of substitution (CS-A2) exhibited a wound area of $\approx 6\%$, whereas sponge with lower degree of substitution (CS-A1) displayed a wound area of $\approx 10\%$ (after 12 days of treatment); – Collagen deposition of wounds treated with CS-A1 and CS-A2 sponges was 24% and 28%, respectively (after 6 days of treatment).	[73]
Chitosan/cellulose (CS/Cel) (Chitosan concentrations of 0.5%, 1%, 1.5%, and 2% named by 5CS/Cel, 1.0CS/Cel, 1.5CS/Cel, and 2.0CS/Cel, respectively)	DD $\geq 90\%$	– The incorporation of chitosan in cellulose sponge and the increase of the chitosan's concentration improved the antibacterial activity of sponge against *E. coli*, *S. aureus*, and *P. aeruginosa*.	– HUVEC incubated with 0.5CS/Cel, 1.0CS/Cel, 1.5CS/Cel, and 2.0CS/Cel sponges presented a viability of 86.6%, 97.8%, 85.8%, and 82.2%, respectively (after 3 days); – 1.0CS/Cel sponge exhibited a most suitable hemocompatible profile (hemolysis of 3.56%), comparatively to 0.5CS/Cel, 1.5CS/Cel, and 2.0CS/Cel sponges	– 1.0CS/Cel sponge presented a lower hemostasis time of 67, 89, and 105 s for mouse tail amputation, rat liver trauma, and rat leg artery trauma, respectively, whereas these values increased to 168, 172, and 486 s, respectively, in the gauze-treated group.	[132]

(*Continued*)

Table 14.4 Chitin- and chitosan-based sponges for wound healing application. *Continued*

Sponge composition	Molecular weight (MW) and deacetylation degree (DD)	Antibacterial properties	*In vitro* biological properties	*In vivo* biological properties	Reference
			(hemolysis values >4.5%).		
Chitosan/hydroxybutyl chitosan (HBC) (CS:HBC ratios of 1:3, 1:2, and 1:1, corresponding to HC-1, HC-2, and HC-3, respectively)	MW ≈700 kDa DD >75%	– CS, HC-1, HC-2, and HC-3 sponge against *S. aureus* and *E. coli* showed a bacterial inhibition >99.9%.	– HUVEC and L929 cells incubated with sponge displayed a cell viability of 110%–120% (after 48 h); – HC-1 sponge exhibited a hemolysis value <1%, while HC-2, HC-3, CS, and HBC sponges showed a hemolysis of ≈2%.	– The wounds treated with HBC sponge achieved a wound closure >90%, while the gauze control group showed a wound closure of ≈80% (after 12 days of treatment).	[133]
Poly(vinyl alcohol)/chitosan (PVA/CS)	MW = 100–150 kDa DD = 85%–95%	NA	– Fibroblasts displayed a viability of ≈100% when incubated with PVA/CS sponge, whereas PVA sponge led to a viability of ≈80% (after 24 h).	– Wounds treated with PVA/CS sponge showed a wound area of ≈10%, compared to wound areas of gauze and PVA groups that were ≈20% and ≈30%, respectively (after 10 days of treatment); – PVA/CS sponge exhibited an enhanced hemostatic time (2–3 min), contrasting to PVA sponge (>6 min).	[134]

A. baumannii, Acinetobacter baumannii; AC, alginate/carboxymethyl chitosan; C. albicans, Candida albicans; CAHS, chitosan/alginate/hyaluronic acid; CEL-NF/QCT/REC, cellulose nanofibers/quaternized chitin/rectorite; CH/CS/AgNPs, chitin/corn stalk/silver nanoparticles; CS/Cel, chitosan/cellulose; CS, chitosan; CS-A, adenine-modified chitosan; E. coli, Escherichiacoli; HBC, chitosan/hydroxybutyl chitosan; HUVEC, human umbilical vein endothelial cells; MIC, minimum inhibitory concentration; NA, not applicable; P. aeruginosa, *Pseudomonas aeruginosa;* PVA/CS, poly(vinyl alcohol)/chitosan; PVA, poly(vinyl alcohol); QCNS, quaternary chitin/chitin nanofiber; S. aureus, *Staphylococcus aureus;* TC, thymine-modified chitosan; VEGF, vascular endothelial growth factor.

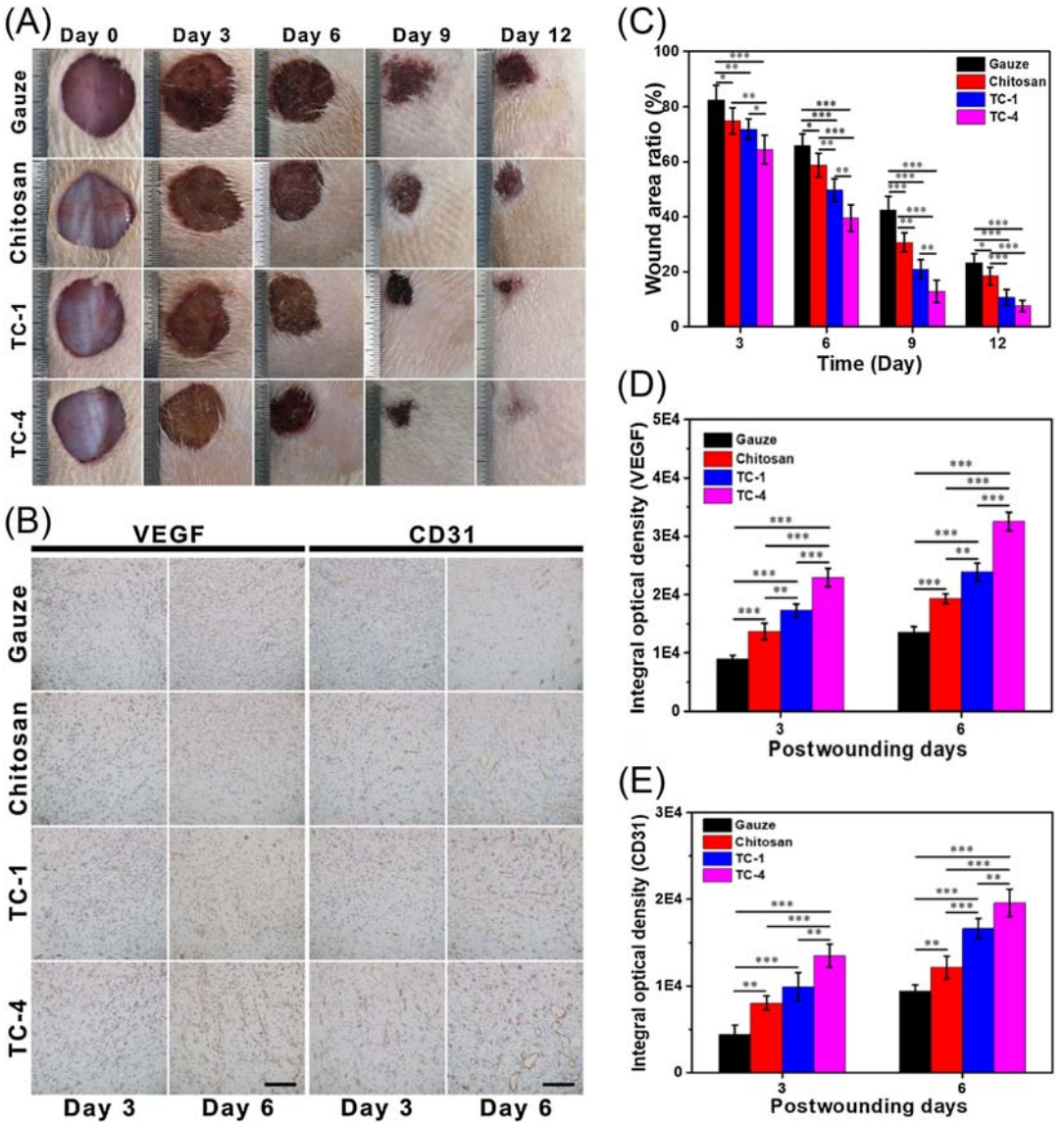

FIGURE 14.4

Representative images of the healing progress of the wounds covered with gauze, chitosan, and TC-1 and TC-4 sponges (A), and VEGF and CD31 staining of the wounds (B) (scale bar is 100 μm). Evaluation of the ratio of the wounds areas treated with gauze, chitosan, and TC-1 and TC-4 sponges (C). Immunohistochemistry analysis of neovascularization density by VEGF (D) and CD31 (E) staining. (Data represent the mean ± standard deviation, $*p<0.05$, $**p<0.01$, and $***p<0.001$).

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[133]. The researchers prepared different weight ratios of CS:HBC solution (1:3 and 1:2), designated as HC-1 and HC-2 sponges, respectively. HC-1 sponges showed improved wound healing capacity, achieving a wound closure of $\approx 100\%$, while chitosan, HBC and HC-2 sponges showed $\approx 90\%$ of wound closure, and gauze control exhibited $\approx 80\%$ of wound closure, after 12 days of treatment. Moreover, the HC-1 sponge−treated group also showed the complete formation of the granulation layer and the presence of numerous blood capillaries [133].

In another study, Deng and collaborators produced a sponge based on thymine-modified chitosan (TC) [with degrees of substitution ranging from 0.23 (TC-1) to 0.62 (TC-4)] [72]. TC sponges demonstrated a hemolysis rate of 0.5%−1.5%, corroborating that the introduction of thymine groups did not affect the hemocompatibility of chitosan. Furthermore, after 12 days of treatment, the wounds treated with TC-4, TC-1, chitosan sponges, and gauze displayed a wound area of $\approx 7\%$, $10.6\% \pm 2.9\%$, $18.5\% \pm 3.2\%$, and $23.4\% \pm 3.3\%$, respectively. Moreover, in comparison to the gauze-treated group, the treatment with chitosan, TC-1 or TC-4 increased the expression of vascular endothelial growth factor (VEGF) (1.4, 1.8, and 2.4 times, respectively) and the CD31 levels (1.3, 1.8, and 2.1 times, respectively) (Fig. 14.4). Such results emphasize the ability of TC sponges to effectively promote wound healing [72].

In the same way, Deng et al. produced sponges composed of adenine-modified chitosan with degrees of substitution of 0.21 (CS-A1) and 0.55 (CS-A2) [73]. With the increase of the degree of substitution of adenine in chitosan from 0.21 to 0.55, the antibacterial efficacy of the sponges decreased, exhibiting minimum inhibitory concentration values from 4 to 16 μg/mL and 8 to 32 μg/mL, against *E. coli* and *S. aureus* strains, respectively. Contrasting, wounds treated with sponges with higher degree of substitution (CS-A2) displayed enhanced wound healing ability with a reduction of wound area to $\approx 6\%$, while sponge with lower degree of substitution (CS-A1) presented a wound area of $\approx 10\%$, after 12 days of treatment. Moreover, wounds treated with CS-A2 sponges also exhibited an improved collagen deposition (28%) when compared to CS-A1 sponges (24%), after 6 days of treatment [73].

14.5 Conclusion

In this chapter, chitin- and chitosan-based wound dressings as well as strategies to improve their applicability in the healing process were reviewed.

Chitin and chitosan are polymers that are characterized by exhibiting amino groups on their backbone that confer them intrinsic properties that are crucial for improving the healing process. Due to these features, several chitin- and chitosan-based wound dressings, namely nanofibrous membranes, films, hydrogels, and sponges, have been developed over the years. In general, these chitin- and chitosan-based dressings exhibit the desired features to be applied in the skin regeneration, such as (1) flexibility, (2) hydrophilicity, (3) biodegradability, (4) hemostatic capacity, (5) bioadhesiveness, (6) mucoadhesiveness, (7) antibacterial activity, and (8) biocompatibility. Furthermore, based on their therapeutic performance, several chitin- and chitosan-based wounds dressings have reached the market and clinical practice [e.g., Chitopack C (Eisai), BST-DermOn (BioSyntech), Vulnosorb (Tesla-Pharma)]. These commercial dressings have been applied in the treatment of leg ulcers, traumatic and chronic wounds, and surgical tissue defects. Nevertheless, the commercially available chitin- and chitosan-based wound dressings still present some limitations,

including high costs associated, inadequate mechanical resistance, poor permeability of gases, and/or uncontrollable pore size.

Considering these shortcomings, researchers have been focused on improving the performance of chitin- and chitosan-based wound dressings. In fact, the chemical modification of the chitin/chitosan [i.e., in reactive amino ($-NH_2$) and hydroxyl ($-OH$) groups] or the inclusion of other polymers (e.g., natural polymers, such as gelatin, cellulose, and/or synthetic polymers like PCL or PVA) or therapeutic agents (e.g., growth factors, and anti-inflammatory and/or antibacterial agents) in the dressings have revealed to be promising approaches to enhance the dressings' wound healing capacity.

Despite these recent technological advances, more research is still required for accomplishing the optimization of chitin- and chitosan-based dressings physicochemical properties. Such research work is fundamental to further propel the development of new wound dressings and subsequently promote their translation into the clinic. On the other hand, the incorporation of stem cells into the wound dressings is currently being performed in order to obtain a fully recovered skin, including appendages (e.g., hair follicles, shafts, and glands). Moreover, it is also crucial to develop dressings that ensure sufficient vascularization for allowing a proper cell metabolism. In the future, addressing these challenges through the combination of different strategies and/or techniques will pave the way for the development of an ideal wound dressing that can restore a fully functional skin.

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Acronyms

Basic fibroblast growth factor	(bFGF)
Carboxymethyl chitosan	(CMC)
Catechol-modified quaternized chitosan	(QCS-C)
Cellulose nanofibers	(CEL-NF)
Chitin binding domain	(CTBD)
Chitosan oligosaccharides	(COS)
Colony-forming unit	(CFU)
Escherichia coli	(*E. coli*)
High molecular weight	(HMW)
Hydroxybutyl-modified chitosan	(HBC)
Hydroxypropyl chitin	(HPCH)
Low molecular weight chitosan	(LMW)
***Mansoa hirsuta* fraction**	(CMHF)
Medium molecular weight	(MMW)
Nanoscaled bioactive glass	(nBG)
***N,N,N*-Trimethyl chitosan**	(TMC)
Phosphate-buffered saline	(PBS)
Polycaprolactone	(PCL)
Pseudomonas aeruginosa	(*P. aeruginosa)*
Quaternized chitosan	(QCS)
Rectorite	(REC)
Staphylococcus aureus	(*S. aureus*)
Tannic acid	(TA)