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Tailoring Strategy of Chitosan-based Hydrogel for Improving Wound Healing: A Systematic Review

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Abstract

Chitosan-based hydrogels are promising wound dressing materials due to their biocompatibility, biodegradability, and ability to mimic the extracellular matrix. They have been known to promote moisture retention, prevent infection, and support cell proliferation. To enhance their properties, recent strategies include chemical and physical crosslinking, incorporation of bioactive agents, nanomaterials, and stimuli-responsive systems. Clinical studies have demonstrated the effectiveness of chitosan-based dressings in accelerating healing and reducing treatment costs, though regulatory challenges remain. Emerging innovations, by tunable their properties such as smart hydrogels and soft robotic systems, offer precise drug delivery and adaptability to wound environments. This review highlights recent advances and prospects for chitosan hydrogels as a cutting-edge technology for wound dressings with a systematic review.

Keywords: Wound, Hydrogel, Tunable, Precise medicine, Natural polymer, Biodegradable, Biocompatible

Introduction

Wound is a tissue damage either internally or externally, which is caused by cuts, burns, incisions, or punctures, etc. Chronic wounds are a syndrome that affects approximately 4% of the world's population due to several pathologies [1]. Superficial wounds heal naturally; however, large and deep wounds need proper medication for accelerating the healing process and preventing infection [2]. Recently, wound care usually applies drugs, ointments, and wound dressing materials like gauze, collagen membrane, which have been applied at clinical levels [3]. Membrane, cotton gauze, and biosynthetic graft replacement during wound treatment cause healed tissue abrasion and induce scar formation [4]. Some of the present wound dressing materials have significant drawbacks, including poor antibacterial characteristics, poor mechanical performance, inability to protect the wound against infection by bacteria, and inability to deliver moisture to accelerate the wound healing process [5-9]. Researchers urgently need to develop an ideal wound dressing to heal wounds faster and protect them from infection.

Hydrogels made from biopolymers exhibit the majority of the characteristics that are lacking in some of the currently proposed wound dressings. Hydrogels are easy to apply and offer comfort during dressing changes, helping to reduce pain in the injured tissue. Hydrogel is a 3-dimensional (3D) network consisting of hydrophilic polymers retaining a large amount of water and undergoing degradation for a certain period [3,10,11]. The water uptake capacity of hydrogel overcomes the debridement problem and wound dryness, as well as provides humidity for better angiogenesis of wound healing [12]. Chitosan is one of the potential natural biodegradable polymers that have excellent biocompatibility, non-toxicity, ready availability, and non-comedogenicity [13]. In addition of chitosan hydrogel's ability to absorb the exudate of the wound, it also prevents bacterial infection, maintain

a moist environment of the wound, and conjugate biomolecules for promoting wound healing [13,14]. Due to the natural extracellular matrix (ECM) mimic, the hydrogel provides a cell adhesion site and then induces cell proliferation to enclose wound gaps [13].

There are abundant studies of chitosan-based hydrogels for wound treatment with excellent regeneration outcomes. To elevate the swelling behavior, mechanical integrity, and biological activity of chitosan hydrogels. The strategies include physical crosslinking (ionic interaction, hydrogen bonding), chemical crosslinking (using reagent such as glutaraldehyde or genipin), and radiation-induced gelation. These fabrication methods allow hydrogel qualities to be tailored to specific clinical needs, such as wound exudate management or long-term medication release. Recent innovations, such as the addition of nanoparticles, growth factors, and other natural polymers, have increased the adaptability and therapeutic efficacy of chitosan-based hydrogels [13,14]. As a result, the creation of well-designed chitosan hydrogel wound dressings is an important step toward generating improved, biocompatible, and efficient wound care solutions.

This review highlights some strategies to modify chitosan hydrogel as an advanced wound dressing material, focusing on enhancing its physicochemical, biological, and functional properties. These strategies include the administration of bioactive agents (e.g., antibiotics, growth factors, herbal extracts), the development of stimuli-responsive systems (e.g., pH-, temperature-, or enzyme-responsive hydrogels), and the incorporation of nanomaterials (e.g., silver nanoparticles, graphene oxide, or zinc oxide) to improve antibacterial activity, mechanical strength, and controlled drug release [10,15-18]. Furthermore, recent advances incorporating 3D bioprinting and electrospinning have enabled the fabrication of tunable chitosan hydrogel structures that more closely replicate the natural extracellular matrix and promote cell proliferation and tissue regeneration [19]. By analyzing these improvements, this work hopes to provide an adequate understanding of how functional alterations to chitosan-based hydrogels might transform them into next-generation wound dressings suited for clinical translation. Soft robotic hydrogels enable precise control of the bioactive release due to pH, temperature, light, and enzymes. For biomimicking the specific morphology of the wound, 3D printer-assisted hydrogel engineering is applicable to tailor the chitosan hydrogels. This review points out that tailored chitosan hydrogels are ideal wound dressings for better wound healing activity. Chitosan is a highly versatile biomaterial platform for wound healing applications due to its tunable structure, multifunctional properties, and capacity for bioactive molecule delivery.

Methodology

This systematic review was conducted following the PRISMA guidelines to ensure a transparent and reproducible research process. The objective was to collect, evaluate, and synthesize published studies on the modification and application of chitosan-based hydrogels for wound management, with a focus on their physicochemical properties, biological performance, and functional enhancements.

Literature search strategy

This article reviewed original research and reviewed articles that were published on Scopus and Google Scholar from the year of 2000 to 2025. The literature search was conducted from February to June 2025. The keyword combination to search for those articles was chitosan, wound healing, hydrogel, biofilm, crosslinking, crosslinker, ideal hydrogel, wound dressing, conjugating, antibacterial, biocompatibility, smart hydrogel, and responsive hydrogel. All full articles were written in English, then analyzed in detail to observe that all detailed information about chitosan hydrogel for wound healing from chitosan characteristics, chitosan hydrogel synthesis, molecules conjugated to chitosan hydrogel, chitosan hydrogel biocompatibility, and antibacterial hydrogel. Boolean operators (AND/OR) were used. To refine the search results, filters have been set up that display only peer-reviewed full-text papers written in English.

Inclusive and exclusive criteria

This review article focuses on chitosan-based hydrogels for wound healing. The inclusive criteria are: (1) Articles describing the biology of wound healing stages, wound dressing, and the ideal strategy for wound dressing. (2) Articles describing the synthesis, characterization, and modification of chitosan

hydrogels. (3) Articles discussing antibacterial activity, biocompatibility, smart/responsive hydrogel behavior, or drug/bioactive molecule conjugation. Moreover, the following exclusion criteria were applied: (1) Non-English publications. (2) Pre-print article. (3) The article is an open-access thesis from a certain university which was not a part of a research article(s). (4) Articles not available in full text. (5) Studies not involving chitosan or not related to wound healing applications.

Article selection process

Relevant titles and abstracts were then further investigated for their appropriateness with the article topics. For those categorized as appropriate, articles were included in the reviews. The figures used in this study were modified, adapted from other references, and directly used from some journals with citation. After screening Scopus and or Google Scholar and applying inclusive and exclusive criteria, we found that the title related to our study was 417 articles, both research and review articles. After cleaning the references for duplication, there are 311 articles remaining, and reduced to 213 after abstracts eligibility. After screening with inclusive criteria, there are 186 articles employed in this study. However, there are 6 articles added, and 2 articles retrieved in the revision stage. The final version of the article applies 186 articles as references in this study (**Figure 1**).

Data extraction and analysis

Relevant data were extracted from the selected articles, including: (1) Wound healing process and ideal characteristics of wound dressing. (2) Hydrogel preparation method (physical, chemical, or radiation-induced crosslinking). (3) Properties of the hydrogel (e.g., swelling ratio, porosity, degradation). (4) Functional modifications (e.g., addition of nanoparticles, drugs, or growth factors). (5) Biological evaluations of chitosan hydrogel (e.g., antimicrobial activity, cytocompatibility, *in vitro* and *in vivo* studies). (6) Information from the selected studies was synthesized qualitatively to compare trends, highlight innovations, and identify research gaps in the development of tailored chitosan hydrogels for wound care.

Figure visualization

Figures exhibited in this review were either adapted, modified, or directly used from sources with appropriate citation. All visualizations were prepared to illustrate mechanisms, fabrication processes, and performance evaluations of chitosan-based hydrogels. The final figure was the conclusion of all content in this article to summarize all the topics discussed in this article. The drawing figures were prepared in BioRender.

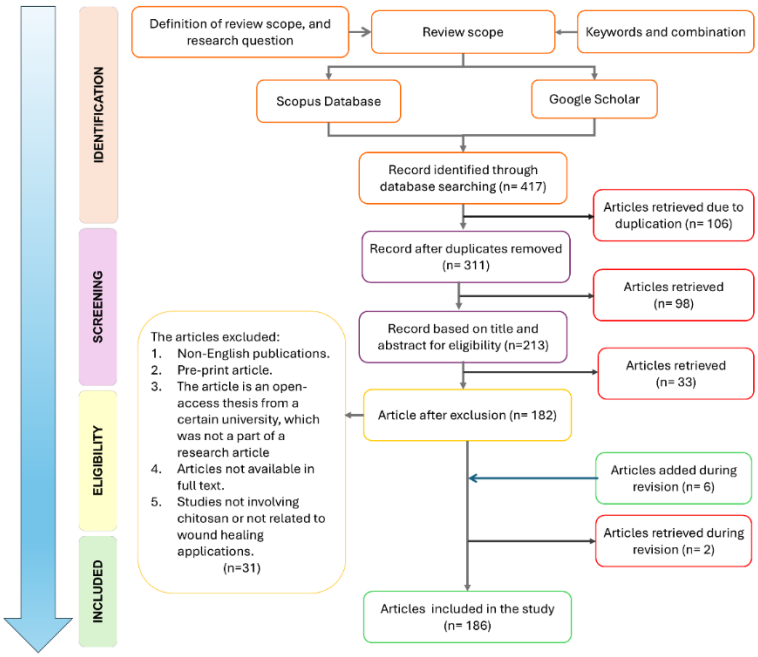

Figure 1 PRISMA methodology for this systematic review.

Biological process of wound healing stages

Wound healing is a dynamic and complex overlapping process in response to tissue damage. When the skin is injured, multiple cell types orchestrate at certain levels to heal the wounded area. These steps include hemostasis, inflammation, angiogenesis, proliferation, re-epithelialization, and re-modelling, which overlap with each other to repair the skin [20,21]. The first response to an injury was blood vessel constriction, which happened within minutes. In this process, platelets are attracted to the wound site to aggregate and form a platelet plug (**Figure 2(a)**). Platelets degranulate and then release growth factors and cytokines such as epidermal growth factor (EGF), insulin growth factor (IGF), and transforming growth factor (TGF-β) [20,21]. Thus, thrombin is released and catalyzes the coagulation cascade, which starts with fibrin activation to form a network as a prominent extracellular matrix (ECM) to avoid excessive bleeding. Moreover, platelets recruit leukocytes (neutrophils and macrophages) to the wound site and initiate the inflammation stage (**Figure 2(b)**) [22].

During the inflammation phase, neutrophils and macrophages phagocytose damaged epithelial cells, bacteria, and any debris. In the prompt inflammation stage, the complement cascade is activated and leads to the migration of neutrophil granulocytes to the wound site within 24 to 48 h by some chemoattractant such as TGF-β, complement components, and ECM protein. The secreted growth factors further induce fibroblast migration and proliferation at the wound site. Fibroblasts release an ECM network, mainly collagen, to replace the prominent ECM and continue to proliferate, forming granulation tissue (**Figure 2(c)**) [21,22]. Afterwards, new blood vessels are regenerated to provide oxygen and nutrition to the newly formed granulation tissue. Thus, epithelial cells shift to the wound edge and then cover the defect to form the basement layer, a process known as epithelialization (**Figure 2(d)**) [21,22].

In the hemostasis phase, blood vessels constrict, and platelets are activated to form a blood clot, which prevents further bleeding and initiates leukocyte recruitment. This is followed by the inflammatory phase, where neutrophils and macrophages infiltrate to the wound and remove dead cells, bacteria, and debris, while fibroblasts begin to appear and contribute to the healing process. Fibroblasts proliferate and synthesize extracellular matrix components, while angiogenesis occurs to form new blood vessels and support the granulation tissue formation in proliferation phase. Finally, in the remodelling phase, the epidermis regenerates, the scab detaches, and the extracellular matrix is reorganized. Excess capillaries and fibroblasts disappear, leading mature scar tissue formation and restoration of skin structure and function [22].

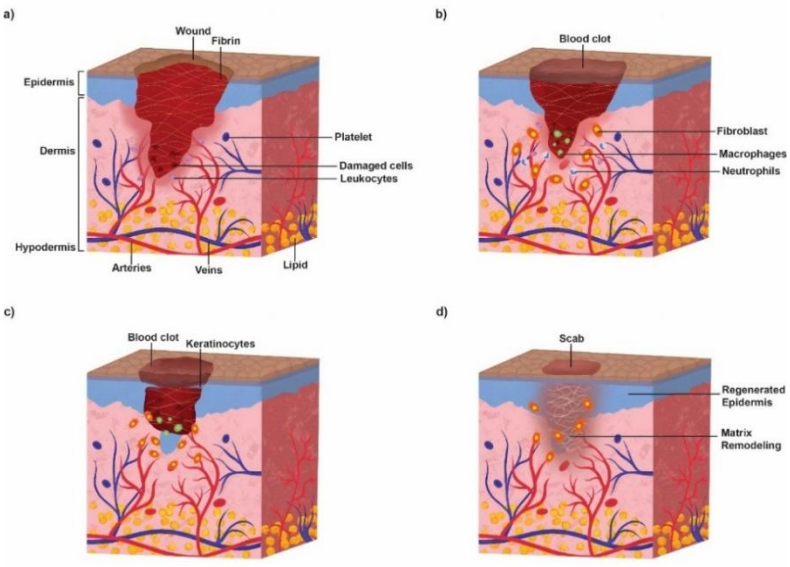

Figure 2 A schematic representation of wound healing, illustrating its 4 continuous phases. The 4 sequential and intersecting phases of wound healing: (a) hemostasis, (b) inflammation, (c) proliferation, and (d) remodeling. This figure is modified from Tavakoli and Klar [22].

Chitosan

Basic structure

Derived from chitin, chitosan is a linear polysaccharide composed of β-(1→4)-linked D-glucosamine and N-acetyl-D-glucosamine units (**Figure 3**). The key difference between chitosan and chitin is their degree of acetylation. Chitin has a high degree of acetylation, making it insoluble in aqueous acidic media due to its rigid polymer structure. In contrast, chitosan, with a higher degree of deacetylation, becomes soluble in acidic solutions as its primary amine groups (-NH_2) undergo protonation [24,25]. Chitosan contains 3 reactive functional groups: primary amino groups (-NH_2), primary hydroxyl groups (-OH) at the C6 position, and secondary hydroxyl groups (-OH) at the C3 position [23,26].

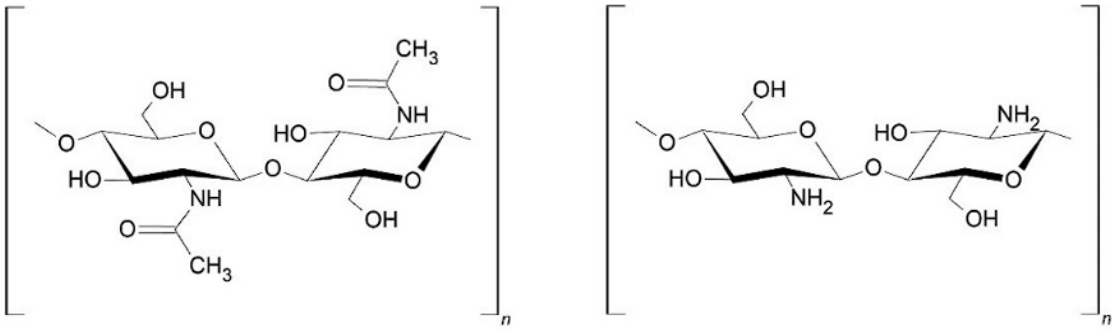

Figure 3 Chemical structure of chitin and chitosan [23].

Degree of deacetylation

Chitosan's degree of deacetylation (DD) significantly influences its solubility, charge density, and bioactivity. The combination of DD and molecular weight plays a crucial role in determining the polymer's solubility [24,26]. A greater DD promotes antibacterial efficacy and biocompatibility, boosts positive charge density for better interaction with negatively charged molecules, and increases solubility in acidic conditions because of the availability of free amino groups [27,28]. The following formula is employed to characterize the degree of chitosan deacetylation:

$$\frac{\mathrm{n(GlnN)}}{\mathrm{n(GlnN) + n(GlnNsc)}} \times 100\%$$

where n(GlcN) is the average number of N-glucosamine units, n(GlcNAc) is the average number of N-acetylglucosamine units, and DD is the degree of deacetylation [17]. Depending on certain parameters and needs, like the type of cells utilized, the desired mechanical qualities of the scaffold, and the intended use, the ideal DD for articular cartilage tissue engineering. Nonetheless, it is generally accepted that a DD range of 50% to 95% is appropriate for articular cartilage tissue engineering [23].

Molecular weight variations

The molecular weight (MW) of chitosan plays a critical role in determining its mechanical strength and biological performance, significantly impacting its overall properties and applications. Based on its spectrum of molecular weights, chitosan is classified as low (50 - 90 kDa), medium (190 - 310 kDa), or high (310 - 375 kDa) [23]. Chitosan, with a low molecular weight has less mechanical strength and viscosity, but it may be more soluble and bioactive, which makes it appropriate for uses like medication distribution. Chitosan exhibits antimicrobial activity against both bacteria and fungi, with its efficacy influenced by molecular weight and degree of deacetylation [29,30]. High molecular weight chitosan, on the other hand, has superior mechanical integrity and viscosity, which makes it more suitable for structural uses such scaffolds and wound dressings [31,32]. Additionally, molecular weight changes as a result of the deacetylation process, which further impact chitosan's solubility and mechanical properties [31,33]. The chemical modification to enhance its mechanical, solubility, and bioactive properties such as wound healing and antibacterial capacity which further be discussed in this review [16,34-39].

Biocompatibility and biodegradability of chitosan

Cytotoxicity studies on human skin cells

For a biomaterial to be approved, it must be biocompatible, adhere effectively, resemble natural tissue, be bioinert, bacteriostatic, bactericidal, and most importantly, non-toxic to the surrounding cells [15,40]. Chitosan, a biodegradable and biocompatible biopolymer with inherent bactericidal and bacteriostatic properties, has been widely applied in tissue engineering to partially or fully replace tissues by regulating cell growth and releasing bioactive compounds [15,41].

The cytotoxicity of chitosan has been extensively studied in various human cell types, including gingival fibroblasts and human pulp cells, to evaluate its safety and effectiveness in biomedical applications [15,42]. According to the literature, chitosan exhibits dose-dependent cytotoxic effects, with concentrations above 0.19% significantly reducing cell viability [15]. Higher concentrations notably decrease the viability of human gingival fibroblasts, whereas lower amounts maintain cell health and exhibit anti-inflammatory properties.

This dual action suggests that chitosan may help regulate inflammation while promoting cell survival, which is crucial for skin health and wound healing. Therefore, careful concentration control is essential. Additionally, chitosan has shown promise in enhancing the biocompatibility and performance of scaffolds used in tissue engineering when combined with other biomaterials, further expanding its potential in regenerative medicine [15].

Biodegradation rate in physiological conditions

The mechanical properties and degradation rates of chitosan are carefully engineered to align with the intended duration of bone repair, reducing the risk of implant failure and enhancing the programmed release of growth factors and drugs [23,43]. Despite being insoluble in water and organic acids, chitosan readily dissolves in diluted acids with a pH below 6 [43]. Its degradation behavior is influenced by molecular structure while higher molecular weight also corresponds to reduced degradation rates [23,24]. Chitosan breaks down through multiple pathways, including enzymatic digestion by specific and non-specific enzymes, oxidative and reductive depolymerization, nitrous acid depolymerization, acid hydrolysis, and ultrasonic reaction [24,44]. Enzymatic reaction primarily occurs through lysozyme and chitinase, with lysozyme playing a crucial role in breaking down acetylated chitosan in human saliva, serum, and tears [45,46]. Chitosan is primarily broken down by lysozyme, acidic conditions, digestive enzymes, and the microbial flora of the colon in the human body [43,47]. In addition to enzymatic degradation, chitosan is highly susceptible to thermal cleavage. It cannot withstand temperatures above 200 - 220 °C. Thermal analysis reveals that chitosan degradation follows 2 distinct stages: An initial weight loss beginning at 220 °C, continuing until 320 °C, where 50% of its mass is lost, as measured by derivative thermal analysis equipment [48-50].

Antimicrobial and hemostatic properties

Antibacterial mechanism

Chitosan demonstrates significant potential in antibacterial applications due to its intrinsic antimicrobial properties, acting through multiple mechanisms [51,52]. These include the formation of thick coatings on microbial surfaces, disruption of the cell wall or membrane, interaction with microbial DNA, and nutrient chelation, all of which contribute to its bacteriostatic and bactericidal effects [51,52].

Chitosan's amine groups are protonated in acidic conditions, leading to positively charged ammonium groups that engage in electrostatic interactions with the negatively charged surfaces of microbes. This interaction alters cell permeability, leading to membrane lysis and eventual cell death [52]. The dominant theory suggests that the positively charged amine groups (NH^{3+}) in glucosamine engage with negatively charged bacterial surfaces, triggering the release of intracellular components and disrupting microbial integrity [53]. A similar process occurs in fungal and viral envelopes, as they also possess negatively charged components that interact with chitosan.

Chitosan's chelating ability further amplifies its antibacterial properties by sequestering essential metal ions and nutrients necessary for bacterial survival. Due to its strong metal-binding capabilities, the amino groups of chitosan interact with divalent metal ions such as Ca^{2+} and Mg^{2+}, inhibiting toxin synthesis and bacterial proliferation [51,53]. Additionally, chitosan adheres to microbial surfaces and forms a protective film, effectively preventing bacterial growth and

colonization. This multi-faceted antimicrobial mechanism highlights chitosan's versatility and effectiveness in controlling microbial infections [52,54].

Spectrum of antimicrobial activity

Chitosan demonstrates varied inhibitory efficacy against a wide range of bacteria and fungi. Chitosan's antimicrobial mechanism is categorized as intracellular or extracellular, depending on where it acts within the cell of the bacteria. The intracellular effect arises when chitosan penetrates bacterial cells and binds to their DNA, altering transcription, translation, and mitochondrial activity. The extracellular effect is defined by chitosan's capacity to adhere to the bacterial cell wall's outer surface, resulting in a polymer membrane that blocks nutrient transport into the bacteria. This binding causes the leaking of intracellular contents, which eventually kills the bacteria [55].

The mechanism of antibacterial properties is an intricate process that varies between Gram-positive and Gram-negative bacteria due to variations in cell structure [52,53]. Gram-positive bacteria have thicker peptidoglycan layers than gram-negative bacteria, which have greater amounts of lipopolysaccharide (LPS) [56,57]. The cell wall of Gram-positive (G^+) bacteria contains teichoic acids, which contribute to the overall negative surface charge. In contrast, Gram-negative (G^-) bacteria exhibit a negatively charged surface primarily due to the presence of lipopolysaccharides in their outer membrane [51]. Teichoic acids in Gram-positive bacteria carry a negative charge, primarily due to the phosphate groups within their structure [53]. Interestingly, the inhibition of teichoic acid biosynthesis in *Staphylococcus aureus* has been shown to increase resistance to chitosan. This finding implies that chitosan's antibacterial mechanism involves more complex interactions beyond simple electrostatic attraction [52].

Furthermore, chitosan demonstrates strong antifungal properties, effective against a wide spectrum of fungal infections in both plants and humans. This action is mostly due to its ability to interact with and damage the integrity of the fungal cell wall and membrane [52,58-61]. However, the minimum inhibitory concentrations (MICs) of chitosan against fungal pathogens differ widely, depending on several key factors such as its degree of deacetylation (DD), molecular weight (MW), the particular fungal species being targeted the pH of the solvent used [61-63]. Beyond its antifungal action on the extracellular, low-MW chitosan capable of infiltrating the fungal cell wall and outer membrane, disrupting essential cellular processes. This interference leads to the impeding of DNA replication, DNA transcription, and translation, ultimately suppressing fungal growth and viability [53,57,64,65].

Electrostatic interaction takes place between the positively charged chitosan and the negatively charged surfaces of microorganisms. Since negative charges primarily found in lipopolysaccharides (LPS) in Gram-negative bacteria, while in Gram-positive bacteria, they are mainly associated with teichoic acids, also the phosphorylated mannosyl in fungi, chitosan effectively kills all of them. Additionally, the nutrients and ambient ions needed for bacterial viability are chelated by chitosan. When low-molecular-weight (MW) chitosan and oligo-chitosan enter the cytoplasm through the cell wall and membrane, they may have an impact on the production of proteins or DNA/RNA. Moreover, oligo-chitosan and low-MW chitosan suppress ATP synthesis and mitochondrial activity in fungi (**Figure 4**).

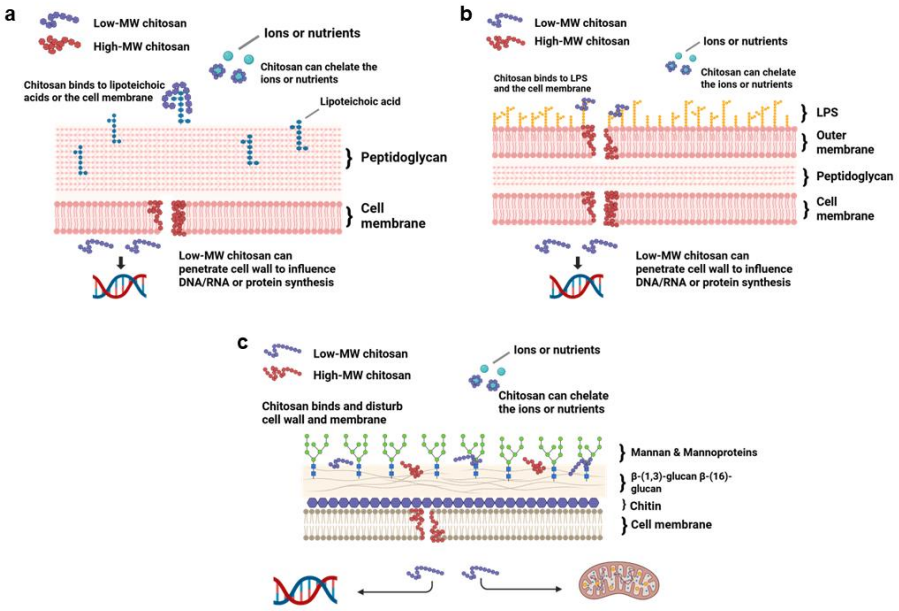

Figure 4 Antibacterial mechanisms of chitosan against (a) Gram-positive bacteria, (b) Gram-negative bacteria, and (c) fungi. The figure is modified from Ke *et al.* [52] using BioRender.

Hemostatic mechanism

The hemostatic process of chitosan is driven by its ability to interact with red blood cells and platelets, leading to cross-linking that forms a mucoadhesive physical barrier at the bleeding site [66,67]. This hemostatic effect is achieved through multiple mechanisms working in conjunction [67-69]. One key mechanism involves electrostatic interactions between erythrocytes and chitosan, where the positively charged glucosamine groups of chitosan bind to the negatively charged surface of red blood cells. This interaction induces agglutination, thereby facilitating coagulation [67,70,71].

The binding between chitosan and red blood cells is further influenced by chitosan's molecular weight and degree of entanglement, which is attributed to specific intermolecular hydrogen bonding and electrostatic repulsion among polyelectrolyte chains [70,72]. Another significant mechanism involves the formation of a blood protein-membrane barrier at the place of hemorrhage. This barrier is established through covalent and hydrogen bonding, as well as chitosan and plasma proteins display reversible hydrophobic interactions, a process known as interpolymer complexation [73,74]. Unlike standard blood coagulation, this mechanism leads to initiation of independent coagulation, making it particularly suitable for individuals with coagulopathies [75,76].

Comparison with traditional wound-dressing

Conventional wound covers, such as gauzes and cotton-based composites, are mainly applied in the initial phase of wound care to facilitate hemostasis and to cleanse and dry wounds. While bandages and gauze are extremely absorbing and helpful in controlling dry to mildly exudating wounds, they must be changed frequently, which results in pain and discomfort once replaced. Additionally, they offer poor adhesion and inadequate wound drainage, limiting their overall effectiveness [77,78].

Although these dressings are cost-effective, they have several drawbacks, including low gas permeability and potential damage to newly formed epithelial tissue. Their fibers are able to adhere to granulation tissue, leading to pain during dressing changes, disruption of new tissue formation, and delayed healing [8,79].

Due to these limitations, traditional dressings have increasingly been replaced by advanced alternatives that provide better wound drainage, enhanced permeability, and superior healing support. Modern dressings promote the development of granulation tissue and aid the migration of epithelial cells from the wound's periphery to the center, accelerating the healing process [78,80].

Among modern wound dressings, hydrogels are particularly effective due to their ability to copy the tissue's native ECM. Owing to their soft texture and high-water content, they may preserve moist wounds, which is essential for the best potential healing [81]. Hydrogels offer excellent biocompatibility and anti-inflammatory properties while enhancing immune responses with minimal toxicity or allergenicity. Their ability to adhere to tissue creates a stable protective barrier while facilitating interactions with wound exudates and surrounding tissues [82-84]. Furthermore, hydrogels support essential healing mechanisms, including migration, adhesion, and proliferation of fibroblasts. They accelerate the healing process by promoting autolysis, which helps clear necrotic and granulation tissue, while also facilitating the transport of oxygen and nutrients necessary for tissue regeneration [82,84-86]. However, their high-water content creates an environment conducive to microbial colonization, posing a risk of infection.

The materials exhibiting ideal characteristics for hydrogel wound dressing are natural polymers, including dextran, agarose, hyaluronic acid, collagen, alginate, gelatin, cellulose, starch, chitosan, and xanthan [86]. Specific polymer combinations, such as chitosan with gelatin, have been shown to enhance hydrogel stability and resistance to enzymatic degradation, making them particularly advantageous for wound healing applications [82,85]. These innovative materials continue to advance wound care by providing a more effective alternative to traditional dressings, offering both antimicrobial and healing-promoting properties.

Chitosan-based hydrogels

Water absorption and swelling behaviour

Hydrogels consist of 3-dimensional polymeric networks formed by cross-linked polymers, either uniform or varied in composition, that are highly capable of absorbing and retaining substantial amounts of water [87,88]. The hydrogel's characteristic to absorb water is largely due to the presence of hydrophilic functional groups, such as hydroxyl (-OH), amine (-NH_2), amide (-CONH-, -$CONH_2$), and sulfate (-SO_3H), which promote water absorption and contribute to forming a 3-dimensional network structure [47,88]. The degree of swelling of hydrogels is determined by the polymer's hydrophilic density and influenced by surrounding factors, including temperature, pH, chemicals, light, pressure, and electric charge [89,90].

Chitosan's cationic nature, attributed to its amine groups, allows it to engage with mucosal glycoproteins that are negatively charged, making it an effective bioadhesive material [47]. As a bioadhesive polymer, chitosan enhances the residence time of drug-loaded systems, facilitating localized drug delivery. Additionally, it mediates paracellular drug transport, significantly impacting drug delivery efficiency [91-93]. With its biocompatibility, biodegradability, and structural versatility, chitosan is a promising drug carrier for various routes of administration. The following sections explore key chitosan-based drug delivery strategies [43,47].

Oxygen permeability

In wound healing stages like skin cell division, granulation, re-epithelialization, angiogenesis, and tissue regeneration, oxygen plays a crucial role [94,95]. Oxygenation techniques that successfully raise oxygen levels at the wound site are advantageous for improving wound healing. Numerous cellular functions, such as the synthesis of collagen, skin tissue regeneration, stimulation and migration of immune cells, and the formation of new blood vessels, are supported and maintained by an adequate oxygen supply. These processes are all fundamental for wound healing [95,96].

When the level of oxygen in tissues falls to 1% - 2%, it is recognized as hypoxia, or oxygen deficiency, and it triggers cellular damage and impaired function. Due to disturbed blood circulation and elevated oxygen need from inflammatory cells related in the healing process, the wound site is hypoxic during the early phases of wound healing [95,97]. These cells are found in low-oxygen environments and serve as vital factors for the granulation and proliferation of skin cells during tissue regeneration [94].

Synthesis and crosslinking strategies

Chitosan hydrogel may be synthesized through a physical or chemical crosslinking method [98]. Physical methods consist of electrostatic interaction, metal ion coordination, and hydrophobic interaction. The reaction in this method is reversible compared to the physical method. The chemical method consists of the

application of a crosslinking agent, resulting in permanent interaction by the covalent bond [99].

Chitosan-based hydrogels exhibited excellent properties to promote wound healing. Chitosan has excellent biocompatibility, biodegradability, and antimicrobial activity. Furthermore, it has been demonstrated that chitosan promotes homeostatic activity [100]. Chitosan also stimulates fibroblast proliferation and collagen synthesis, resulting in accelerated wound healing. As a hydrogel, chitosan has the capacity to absorb exudate from wounds and retain a moist environment to accommodate tissue regeneration [101]. Moreover, hydrogels have good flexibility, thus conforming to the wound shape and producing a protective barrier [102].

Electrostatic interaction

Electrostatic crosslinking occurs when anionic and cationic polyelectrolytes interact. Chitosan's amine group act as a cationic molecule, bonding with an anionic molecule shown in **Figure 5** [103]. As such, there are many possible anionic molecules to be interrogated into chitosan hydrogels.

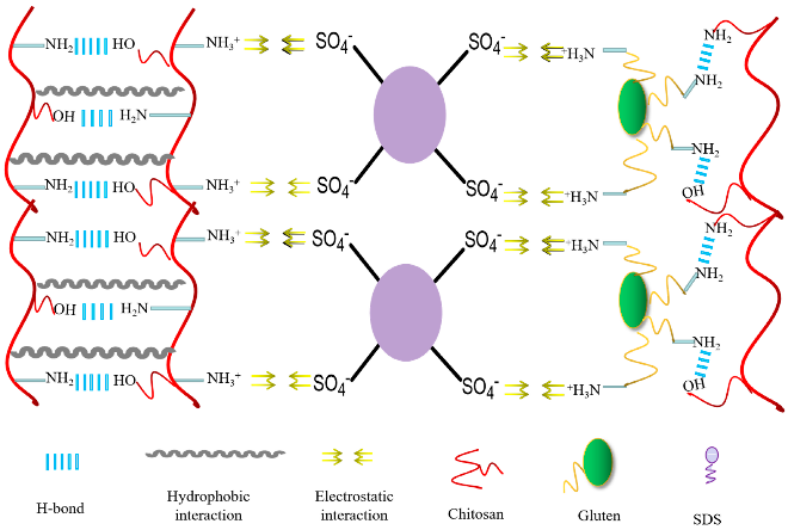

Figure 5 Hydrophobic interaction and electrostatic interaction of chitosan [103].

A hydrogel consisting of gluten/ sodium lauryl sulfate (SDS)/chitosan (**Figure 5**) showed a change in zeta potential, the absolute potential difference value increase in pH 3.0 - 9.0 indicating an electrostatic repulsion and increase in pH 9.0 - 12.0 indicating electrostatic attraction between chitosan and SDS [103]. Within the synthesis, no harsh chemicals or extreme temperatures are required, making them ideal for sensitive biomolecules such as proteins, DNA, or cells [104,105]. Additionally, the protonation of chitosan also gives pH-responsive behavior such as swelling at a certain change of pH. Therefore, the hydrogel is applied to deliver the targeted drug in pH-sensitive environment like acidic pH of the stomach or basic pH of the intestines [106]. The positive charged chitosan also could interact with negatively charged molecules, such as cell membranes, tissues, negatively charged drugs, or biomolecules such as DNA and protein [104,105,107].

Hydrophobic interaction

Hydrophobic interactions occur as attractive forces between non-polar groups, causing these groups to aggregate into larger molecules within a solution and form a crosslinking network. To leverage this method, chitosan must be integrated with hydrophobic groups like alkyl chains or aromatic rings, or with hydrophobic polymers. The hydrophobic groups will cluster together to evade water, while the hydrophilic groups will remain on the surface, interacting with the water [99,108,109]. Hydrophobic interactions represent non-covalent bonds that may be reversed, enabling the development of stimuli-responsive behaviors in hydrogels. Additionally, hydrophobic groups have been shown to enhance the elasticity of the hydrogel to promote cell adhesion and growth. Importantly, this interaction

avoids cytotoxicity, making it suitable for biomedical applications [110].

Metal ion coordination

Metal ion coordination utilizes chitosan as a ligand to bind various metal ions (e.g., Zn^{2+}, Fe^{2+} and Cu^{+}). Chitosan's amino and hydroxyl groups are able to bind covalently with metal ions through the lone electron pairs on nitrogen and oxygen [111,112]. This coordination enhances mechanical properties and introduces new functional characteristics. Previous study has developed carboxymethyl chitosan (CMCS) and glycyrrhizic acid (GA) hydrogels (GA-CMCS-based hydrogel) that exhibit antibacterial activity by using Fe^{2+} and excellent quality of self-healing, swelling, and water retention [113]. While commonly used metals like Zn^{2+} are biocompatible (**Figure 6**), other metals like Cu^{2+} and Ag^{+} become toxic in high concentrations [114,115].

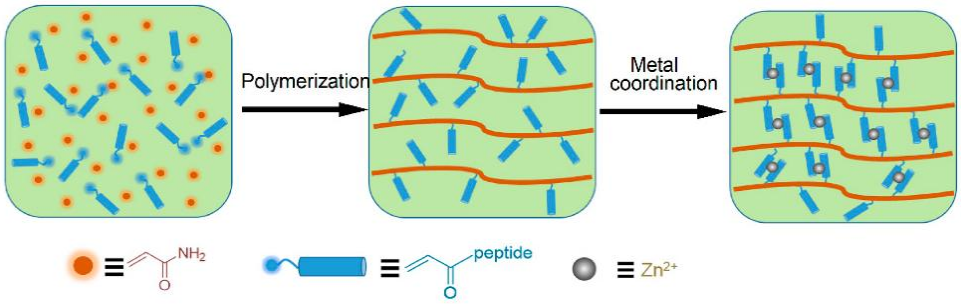

Figure 6 Polymerization and metal coordination between chitosan and Zn^{2+} [111].

Crosslinking agent

This method utilizes chemicals to interact with the amino and hydroxyl groups in chitosan and form a hydrogel with a gel structure that is more rigid and stable [116]. The most common crosslinker agents are glutaraldehyde, genipin, tripolyphosphate (TPP), dialdehydes (e.g., glyoxal), and derivatives of polyethylene glycol (PEG) [116-120]. Emani *et al.* Chitosan hydrogel synthesis using 1,3,5-benzene tricarboxylic acid (BTC) as a crosslinkera enhances the rheological properties of chitosan using [121]. The resulting hydrogel has good stability, exhibited by high storage modulus (G' values) that imply a strong cross-linking, also confirming the elastic behavior. Tannic acid has been previously investigated by complexing with the hydrogen bond between the oxygen on the hydroxyl group of chitosan with tannic acid. Moreover, glyoxal added to form covalent bond between chitosan molecules in chitosan hydrogel **(Figure 7**) [119].

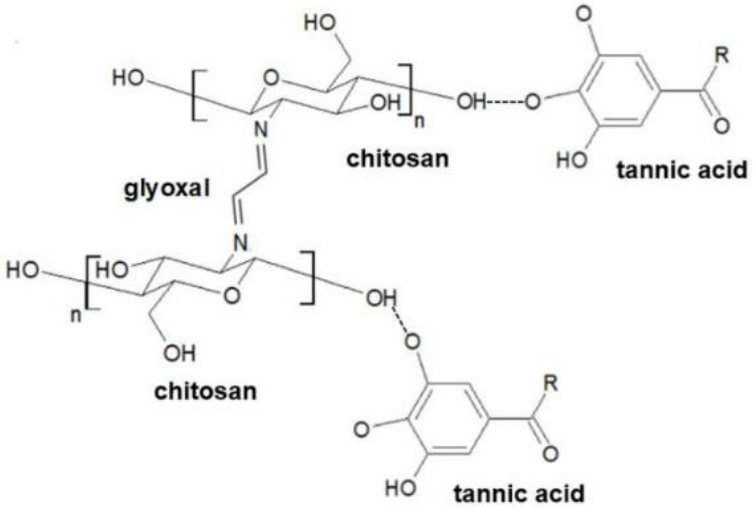

Figure 7 The cross-linking mechanism between chitosan and glyoxal [119].

Radiation

The radiation method employs free radicals to create a crosslinking network. Previous study applied this method to engineer carboxymethyl chitosan (CMCS) hydrogels through recombination of CMCS's side methyl groups [101]. However, this results in a partial crosslinking network. In order to strengthen the hydrogel with controlled swelling, poly(ethylene glycol) diacrylate (PEGDA) is added. The double bond of PEGDA reacts with CMC macroradicals, creating an intramolecular bond. Another benefit of the radiation method is sterilisation of the materials.

Fabrication methods of chitosan-based films and membranes

Solvent casting

Solvent casting methods create a film by dissolving the chitosan into a solution, then pouring it into a mold. Afterwards, the solution needs to be dried to remove the solvent. As the solvent dries up, the chitosan network film is formed and can be peeled off (Figure 8). This method ensures that the chitosan biocompatibility is still intact and avoids thermal degradation. Moreover, the method doesn't require harsh chemicals and is simple in its equipment as the mold commercially available in the market [31,122].

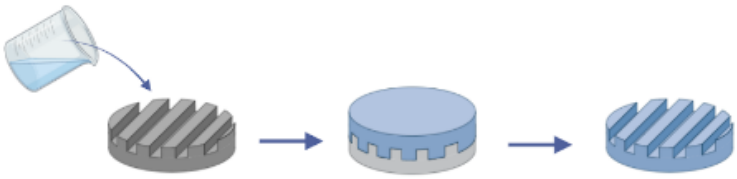

Figure 8 Schematic illustration of solvent casting method. This figure was adapted from Masi *et al.* [122] using BioRender.

Electrospinning

Electrospinning is a method to create fibrous material using a high electrostatic voltage field from a charged polymer solution. Initially, an electric force is created between the needle of the Taylor's cone and the collecting plate. Then, the solution is put into the syringe then spun to create Taylor's cone at the needle part (**Figure 9**). A charged droplet from Taylor's cone is ejected from the tip and put in the collecting plate [40]

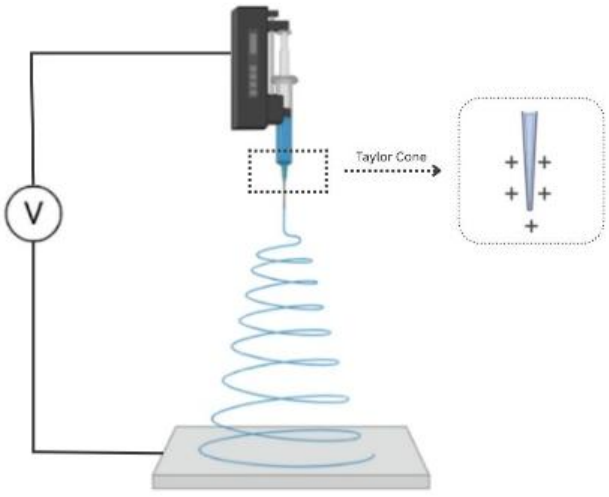

Figure 9 Schematic illustration of electro-spinning method. This figure was adapted from Chen *et al.* [40] using BioRender.

Freeze-drying

The freeze-drying method involves preparation steps, freezing, and drying. The process of freeze-drying consists of 3 steps, which are freezing, primary drying, and secondary drying [123]. The freezing step causes the solution to cool until there is a nucleation of ice and forming of ice crystals. The frozen water is then removed in the primary drying step using optimal temperature and pressure. Another drying step, or the secondary drying step, is added to remove residual water in products with amorphous characteristics. This process is applicable to porous hydrogel fabrication, illustrated by **Figure 10**

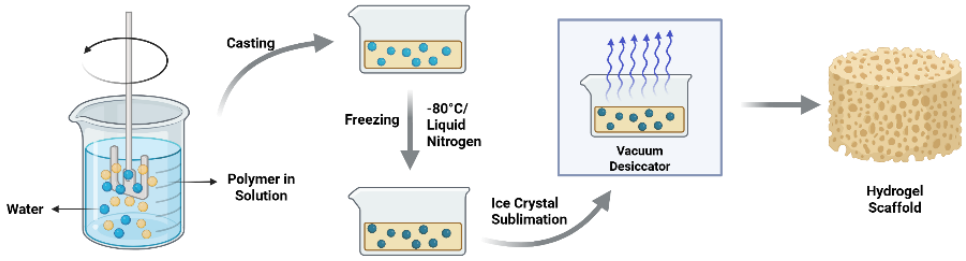

Figure 10 Schematic illustration of hydrogel fabrication using lyophilization process. This figure was created using BioRender.

This method is applied to synthesize porous chitosan hydrogel beads [124]. The chitosan hydrogel bead was frozen. In frozen temperatures below the point of water crystallization, chitosan molecules fill in the space of the ice crystal. Afterward, the chitosan hydrogel bead dried up and caused sublimation of the ice crystal. This created a sponge-like structure with large pores such as utilize the same concept of freeze-drying to create a 3D porous film of chitosan/pectin/ZnO nanoparticles (NPs) [18].

Chitosan functionalization for enhanced wound healing

Chemical modification for improved bioactivity

Chitosan biofilm retains the characteristics of chitosan, such as biocompatible, biodegradability, supporting cell regeneration, and antimicrobial activity [40]. Chitosan biofilm has enhanced mechanical strength, such as good tensile strength and elongation at break [18]. However, it has limited moisture retention. Therefore, chitosan biofilm is more suitable for dry or minimally exuding wounds [125].

Chitosan has been utilized extensively in biomedical research. The chemical modification of chitosan is possible due to the presence of hydroxyl and amino groups within its structure, which exhibit chemical activity (**Figure 11**). This property allows for the enhancement of the physical and chemical properties of chitosan through chemical modification. The chemical reaction has been shown to enhance the properties of chitosan while maintaining its sustainability.

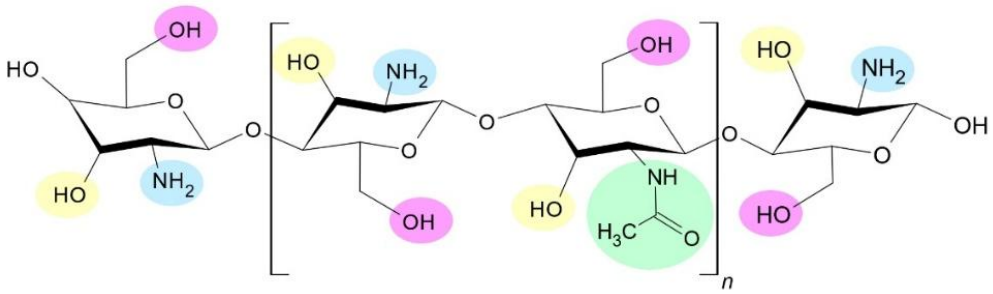

Figure 11 Molecular structure of chitosan and its functional groups; Pink: Primary hydroxyl group; Yellow: Secondary hydroxyl group; Blue: Primary amine group; Green: acetamide group. This figure was adapted from Ojeda-Hernández *et al.* [126].

The chemical modifications of chitosan may be implemented in the functional groups of chitosan, such as the primary hydroxyl group (**Figure 11**), which is usually conjugated with melatonin [127] and TiO_2 [128] The secondary hydroxyl group is usually conjugated with Ag^+ [129], Zn^{2+} [130], and TiO_2 [128]. In addition, the amino group of chitosan (**Figure 11**) is typically conjugated with various substances, including curcumin [131], gentamicin [132], simvastatin [133], Ag^+ [129], Zn^{2+} [130], TiO_2 [128], and has also been observed with growth factors [134].

Incorporation of bioactive agents

Growth factors

Growth factors were secreted by fibroblasts, inflammatory cells, endothelial cells, epithelial cells, and platelets, which are synthesized peptides. Growth factors that are frequently used for wound healing are EGF, FGF, TGF-β, PDGF, and VEGF. Growth factors were divided into several families based on their characteristics [135]. Hydrogels are frequently incorporated with growth factors or cytokines to enhance wound healing effectiveness [136]. The growth factors or cytokines that were added to the hydrogels include:

Epidermal Growth Factor (EGF)

EGF is a protein that may stimulate the synthesis of extracellular matrix (ECM), granulation tissue, and the growth and motility of fibroblasts and keratinocytes. However, EGF's loading capacity in a hydrogel is extremely limited [136]. It has been developed that incorporating EGF accelerates fibroblast migration, which in turn encourages the proliferation phase. In the initial phases of wound healing, EGF expression increases [137]. According to preliminary investigations, encapsulated epidermal growth factor (EGF) in nanocomposites could boost angiogenesis, which might render wound healing more efficient.

Additionally, the *in vitro* study proved that incorporating EGF into hydrogels promoted the best possible proliferation of L929 fibroblast cells. In particular, hydrogels containing EGF were compared to samples that did not contain it to treat skin wounds. These compositions were found to be promising substitutes for the development of medicine and the study of wound healing [138].

Fibroblast Growth Factor (FGF)

It has been demonstrated that fibroblast growth factors (FGFs) enhance the proliferation and migration of most cell types implicated in the process of wound healing. These cell types encompass such entities as capillary, vascular, and fibroblast cells, as well as keratinocytes and epithelial cells. This stimulation occurs within experimental settings *in vitro*, in addition to *in vivo* settings. In experimental models, FGF-2, known as FGF, has been revealed to induce a series of biological responses, including epithelialization, neovascularization, collagen synthesis, and granulation tissue formation [139]. Also, FGF stimulates vascularization through the activation of capillary endothelial cells and fibroblasts.

Incorporating FGF into chitosan-based hydrogels was a critical step in ensuring the controlled release of FGF at the wound site and maintaining its stability there.

A series of studies have indicated that the incorporation of fibroblast growth factors (FGFs) and basic fibroblast growth factors (bFGFs) into chitosan-based hydrogels effectively enhances the healing of chronic wounds [135].

Vascular Endothelial Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is secreted by many different kinds of cells, including platelets, macrophages, fibroblasts, and keratinocytes, during the wound-healing process. The process of wound angiogenesis, or the development of new blood vessels in the wound area, depends heavily on this growth hormone. It has been established that VEGF acts on endothelial cells, promoting vascular growth and aiding in the healing process.

Vascular permeability, basement membrane degradation, endothelial migration, and vascular cell proliferation within the wound bed have all been demonstrated to be accelerated by VEGF. It has been shown that providing exogenous growth factors accelerates up the healing process of wounds. However, these aspects need to be used locally consistently for this intervention to be successful. The short half-life of growth factors, their quick dilution in the body, and the occurrence of unfavorable effects at high systemic levels are the reasons for this requirement [139].

Antibacterial agents

Bacterial inhibitors are mostly used in hydrogels to promote their antimicrobial capability, while in the hydrogel materials presence chitosan which inherently possess bacteriostatic properties [136]. One of the most frequently used in hydrogels is Ag^+. Ag^+ is a potent antimicrobial agent and has been used on various antimicroorganisms, including bacteria, viruses, and fungi. Ag^+ has bacteriostatic activity [136]. Besides that, Ag^+ has been demonstrated to possess bactericidal properties. However, its effectiveness is subject to bacterial type, with Gram-negative bacteria displaying a greater response to Ag^+ at equivalent doses when compared with Gram-positive bacteria. Ag^+ has a multi-targeted mechanism of action within the bacterial cell, which is typically divided into 2 stages, one occurring externally and the other occurring internally. The initial phase entails the immobilization of Ag^+ on the bacterial membrane's surface, is to facilitate binding to the membranes of bacterial proteins, thereby inducing damage to the bacterial cell wall. The result of this interaction facilitates the leakage of potassium ions from the bacterial cell, leading to its demise.

Another antibacterial agent is gentamicin sulphate, a broad-spectrum aminoglycoside antibiotic which works by inhibiting bacterial protein synthesis through electrostatic binding [85]. Specifically, gentamicin has been observed to promote healing in purulent wounds infected with *Escherichia coli* and *Staphylococcus aureus*. There is also a synergy exhibited between gentamicin sulphate and chitosan that is crosslinked by quaternary ammonium salt. This effect is demonstrated by the increase of antibacterial activities in the cross-linked chitosan quaternary ammonium salt (CTMCSG) hydrogel films, which suggests that these films have potential use as antibacterial wound dressing. Another study by Yan and colleagues [140] has shown a good response for repairing skin scald using chitosan-gentamicin hydrogels. The hydrogels have an improved healing rate compared to wet burn ointment. It also has higher collagen fiber content than the blank group. These studies indicated a high degree of promise for chitosan-gentamicin combination as a research material in the medical field.

Hormones

Other antioxidant molecules such as endogenous hormone melatonin (MLT; 5-methoxy-N-acetyl-tryptamine) which is a powerful antioxidant used for treating skin burns and improving the proliferation and differentiation of skin cells [141]. MLT also increases antioxidative enzyme activity. This enhances the first line of defense against oxidative damage to cells by increasing the endogenous antioxidant defense capacity and inducing upregulation of gene expression. Because of these properties, it has been proposed as a potential treatment for wound healing, being more effective than vitamins C and E. Aside from the anti-inflammatory effect, it also has some benefits over other antioxidants, such as being endogenous and being able to enter cells etc.

Drug molecules

Drug-loaded hydrogels hold considerable promise in bioengineering research with tunability of the chemical structure of hydrogels allows a wide variety of

encapsulated pharmaceutical products, including antibiotics, anticancer drugs, and macromolecular drugs with adjustable release properties [142]. Here is a drug that is loaded in hydrogels such as:

Simvastatin

Simvastatin is a pharmaceutical agent employed in the treatment of dyslipidemia. It functions by acting as a competitive inhibitor of HMG-CoA reductase, the enzyme responsible for the conversion of HMG-CoA to mevalonate. Reports indicate that simvastatin enhanced VEGF production, thereby stimulating angiogenesis. Simvastatin has also been reported to stimulate microvascular function, impede oxidative stress, and enhance endothelial function, thus enhancing wound healing effectiveness. The substance has been demonstrated to manifest anti-inflammatory properties, manifesting in 2 distinct forms of wounds, acute and chronic. This phenomenon is achieved through the upregulation of pro-inflammatory cytokines such as IL-1, IL-6, and IL-8, as well as TNFα. The substance has also been shown to inhibit the migration of transendothelial cells, including leukocytes. This effect occurs by reducing the expression of adhesion molecules, such as intercellular adhesion molecule ICAM-1, thereby leading to a reduction in inflammation [143].

Curcumin

Curcumin has several pharmaceutical activities, including anti-inflammatory, antibacterial, antifungal, antiviral, antioxidant, and anticancer [144-148]. Curcumin's antioxidant, anti-infectious, antibacterial, anti-inflammatory, and analgesic activities have been attributed to its contribution in wound healing. It is effective in modulating the inflammation and proliferation phases in the healing process, while also inducing and promoting angiogenesis [147]. Curcumin has also been shown to significantly protect keratinocytes and dermal fibroblasts of humans from hydrogen peroxide damage [149-151].

Another reported mechanism of curcumin's activity is by decreasing the level of lipid peroxides (LPs) and increasing the activity of catalase and glutathione peroxidase (GPx), which are responsible in accelerating the rate of wound healing. Its mechanism behind the antioxidant activity is electron transfer or hydrogen atom donation from 2 methoxyphenol groups. The various functional groups of curcumin, such as diketone, are also responsible for its antioxidant activity by an electron transfer mechanism. The diketone serves as a functional group that makes curcumin bind to metals. The phenolic hydroxyl groups contained in curcumin are the responsible functional group behind curcumin's ROS-scavenging ability [150,151].

Stimuli-responsive chitosan material

pH responsive

These hydrogels, which exhibit sensitivity to pH, are frequently employed in targeted and controlled drug delivery across a range of pH values, considering the varying pH levels present within the human body's internal bodily fluids and organs [152]. pH-responsive polymers are polyelectrolytes that have weak acidic or basic groups in their structure that accept or release protons in response to a change in the environmental pH. pH-responsive polymers, polymers that have acid or basic groups like carboxyl, pyridine, sulfonic, phosphate, and tertiary amines, which undergo ionization of the groups with pH change, resulting in a change in the structure (**Table 1**) [153].

Typically, normal skin has a pH below 5, however, when the skin surface is damaged, the underlying tissue becomes exposed, which has a pH of 7.4. Chitosan is one of the pH-responsive natural polymers that are suitable for chemical modifications and provide better materials for drug delivery systems. The strategy for pH-responsive polymers is grafting onto the polysaccharide backbone. Chitosan, which has a pH of approximately 6.5, exhibits responsiveness to these changes in environmental pH. When the chitosan hydrogel is applied to the wound, in the initial phases of wound healing, chitosan hydrogels induce expansion of the hydrogels within the acidic environment of the wound, thereby promoting cellular infiltration, proliferation, and oxygen permeation. This hydrogel demonstrates promise in facilitating the release of anti-inflammatory agents during the primary phase of wound healing (**Tabel 1**) [153,154].

In addition, to influence the solubility of chitosan, pH also affects the electrical charges of the chitosan molecules [155,156]. This property allows the chitosan molecules to bind via electrostatic interactions. pH-responsive hydrogels are divided into:

Anionic hydrogels

Anionic hydrogels contain carboxylic acid or sulfonic acid chains with negative electric charges, and cationic hydrogels contain amine chains. When the surrounding medium is higher than its pKa, the ionized structure increases the electrostatic repulsion between the chains and the hydrophilicity of the hydrogel's network (a network of water-attracting parts of the gel). Under these conditions, the hydrogel will be able to absorb a large amount of water and will form a very loose structure.

Cationic hydrogels

In cationic hydrogels, the amino group changes from NH_2 to NH_3^+ because the electrostatic repulsion is enhanced when the pH is lower than pKb, thus increasing the hydrophilicity and swelling rate. Chitosan exhibits low solubility in organic solvents and water at its physiological pH, thereby limiting its applications. However, due to its high abundance, biocompatibility, and antibacterial activity, the use of chitosan to make chitosan in biomedical applications is particularly advantageous. The presence of a wide range of functional groups on the backbone of the chitosan renders it suitable for modification to suit a variety of needs and applications. The protonation of the high-density amino groups ($-NH_3^+$) of the chitosan molecules results in the conversion of the quaternary form, thereby imparting a positive charge to the polymer. This, in turn, increases the intermolecular electric repulsion, resulting in a polycationic macromolecule. The protonation of the high-density amino groups ($-NH_3^+$) of the chitosan molecules results in the conversion of the quaternary form, thereby imparting a positive charge to the polymer. This, in turn, increases the intermolecular electric repulsion, resulting in a polycationic macromolecule. Chitosan displays polycationic characteristics under acidic conditions, with an optimal pH range below 6. These characteristics facilitate solubility in water. Conversely, as the pH declines, there is a concurrent enhancement in the degree of adsorption of chitosan on bacterial surfaces. This is an essential requirement for interacting with negatively charged substances, including proteins, fatty acids, and phospholipids, that are some components of the bacterial cell [157,158].

Temperature responsive

Hydrogels exhibit a temperature-dependent phase transition, known as the sol-gel transition, at physiological temperatures [156,158]. This phenomenon is characterized by the presence of a UCST (upper critical solution temperature) and LCST (lower critical solution temperature) that offers significant advantages, such as enhancing the efficiency of drug mixtures and facilitating the targeted delivery. In the present study, stem cells, growth factors, pharmaceuticals, and peptides are to be mixed homogeneously with the polymer solution in a liquid phase. In the following step, the mixture is administered to the wound via injection or spray. The blend undergoes a phase transition to a gelatinous state in response to fluctuations in temperature, thereby facilitating a regulated release and the process of wound repair. Chitosan is converted into a temperature-sensitive material using thermo-reversible chemical agents, such as N-isopropyl acrylamide (NIPAAm), via a grafting method. NIPAAm is hydrophilic below its LCST of 32 °C and hydrophobic above it. This is due to hydrophobic interactions and collapsing hydrogen bonds between NIPAAm functional groups and water molecules (**Table 1**).

Thermosensitive hydrogels have been demonstrated to change in size in response to alterations in temperature. While research on the critical temperature has demonstrated that hydrogels show transition between phases or volumes when the temperature changes, the design of hydrogels is predominantly informed by the lower critical solution temperature (LCST). It has been established that when the ambient temperature exceeds the LCST, the strength of the hydrophobic interaction between the non-polar groups in the hydrogel polymer chain increases, which leads to a reduction in the size of the polymer chain and increase in the density of the polymer matrix. Nevertheless, in circumstances where the ambient temperature is lower than the LCST, the strength of a hydrophobic interaction between the hydrogel polymer chains is observed. This interaction leads to a weakening of the interaction. The result of this weakening is an expansion in network structure, which creates a more open structure [145]. As mentioned above, NIPAAm as a thermo-reversible chemical agent, undergo chemical modification to modify its LCST. Chitosan is able to

shrink and release the bioactive substance into the surrounding environment at the hydrophobic state [158].

It is suggested that the thermo-sensitive hydrogels have the potential to be utilized as an alternative to conventional wound dressings. Thermosensitive chitosan-based hydrogels show potential as therapeutic drug delivery systems to promote skin tissue repair and regeneration of the skin, and engineered as micro nanoparticles, self-assembled micelles, and in situ hydrogels as a platform. *In vivo* delivery of drug-structures is facilitated through these systems as a platform. This method ensures the controlled and sustained release of loaded drugs. Additionally, their injectability affords superior adaptability to a range of irregular skin wounds, thereby enhancing their efficacy in addressing diverse clinical presentations (**Table 1**) [154,158].

Enzyme responsive

Enzyme reaction systems have been shown to possess excellent sensitivity and distinct selectivity in tissues or cells *in vivo* when compared with other stimulation systems. In optimum conditions, the enzyme system may be more effective than other conventional stimuli (e.g., pH, temperature, and light) [155].

According to Chandrawati [159], to successfully obtain enzyme-responsive materials, 3 fundamental principles are required, there are: (1) The materials must have been recognized by the enzymes. This is achieved as long as the materials contain recognized elements or are able to mimic the original substrate. (2) Substrates anchored in material must be accessible to enzymes. This factor significantly influences the rate of enzyme-catalyzed reactions. (3) Enzyme-substrate reactions must be converted to alterations in material properties. This intends to compromise the internal factors of material, such as the degradation or transformation in morphology properties of biomaterials. (4) The benefit of enzyme-responsive stimuli is the excellent sensitivity and distinct selectivity in tissue or cells with overexpressed enzyme. The enzyme has the capacity to transform the synthesized amphiphilic biocarriers into non-synthetic structures, allowing for the subsequent disassembly of these structures, and then the drug is released.

For example, enzyme-sensitive chitosan removes the time-consuming requirement in wound infection tests, because it is due to its ability to detect the presence of enzymes in wound infections in real time. Therefore, releasing the drug immediately upon the onset of bacterial infection. Besides that, enzymatic hydrogels are primarily used in drug delivery to the colon. The major advantage of using enzyme-reactive hydrogels is their ability to reduce proteolytic activity in the colon compared to the small intestine. The development of enzyme-responsive hydrogel nanoparticles, magnetic hydrogels, drug-responsive hydrogels, and hydrogels for dual protein delivery is among the smart hydrogel delivery systems. So, it was important to further develop the enzyme-responsive chitosan hydrogels as a potential mechanism in smart wound dressing (**Table 1**) [155,158].

Light responsive

Light-responsive hydrogels were once a tempting option for drug delivery because of their abilities to provide a non-invasive, controlled, remote, and instant delivery method [160]. Light-responsive hydrogels exhibit structural and conformational changes in response to light irradiation [161]. According to Tian and Liu [155], the development of light-responsive hydrogels involves the utilization of photonic waves that have an appropriate wavelength and an adequate intensity. Some examples of typical light sources employed in such applications are near-infrared (NIR), visible light (Vis), and ultraviolet light (UV) (**Table 1**).

Photo-responsive hydrogels are a class of materials that demonstrate the capacity to undergo changes in their properties when exposed to light. To modify the hydrogel into photo-responsive is achieved through 3 mechanisms. In the initial stage, the hydrogel was modified with photosensitive functional [155]. The photosensitive functional groups, known as photosensitizers, are applied for developing stimuli-responsive systems in hydrogels. These photosensitizers link their functional groups to chitosan physically or chemically. But the photosensitizers have a toxic characteristic, so the photosensitizers have been limited to application in clinical stages [158]. After the photosensitive functional groups are incorporated into chitosan hydrogel, the hydrogel absorbs selective energy photons then the response is induced by the phase transition. The most common response is light responsive (**Table 1**) [155].

The second mechanism, hydrogel catalyzed by the photoactive molecules to produce ions, which subsequently interface within the hydrogel structure, affecting a modification in osmotic pressure, and leading to hydrogel expansion. After this second mechanism, the photoactive compound contained within the hydrogel exhibited the capacity to modify the hydrogel's characteristics, employing photon energy absorption in response to alterations in the environment. The hydrogel structure is characterized by the presence of macromolecular pores, which facilitate high permeability for nutrients, oxygen, and cellular [155]. The light-responsive has some potential advantages, most notably including smart drug-delivery systems, simultaneous cancer imaging, and efficient tumor growth inhibition [162].

Hydrogel physical properties are modified through various methods, altering the soft tissue's mechanical properties in the process. The application of photo-stimuli is possible via direct delivery, then the delivery of light stimuli is metered with a high degree of precision. This suggests that photo-responsive hydrogels might offer significant therapeutic benefits over the available products [155]. Nanocarriers for the light-responsive materials have been applied for drug release include photo-induced-thermal effect, photo-isomerization, or photo-cleavage of chemical bonds in the nanocarriers matrix [162]. One example, the capacity of sol-gel stimuli to be delivered in real-time, emphasizes the significance of photo responsive hydrogels in various applications, like ophthalmic drug (**Table 1**) [155,162].

There are some light sources that are commonly used for light-responsive hydrogels are:

(1) Ultraviolet (UV): The utilization of UV as a light source is frequently observed due to the abundance of UV-responsive photosensitizers, but usually used for *in vitro* experiments, because it causes cytotoxic, and due to its low tissue penetration. Under UV radiation, hydrogels expand discontinuously and shrink after the removal of UV light [155,161].

(2) Near-infrared (NIR): NIR has been demonstrated to be a more viable option for *in vivo* studies, with the capacity to induce drug release within deep tissues. Material science has recently witnessed a proliferation of novel photosensitizers, which demonstrate responsiveness to near-infrared (NIR) light [155,161].

(3) Visible (Vis): Visible light is a readily attainable form of illumination that is characterized by its safety, cleanliness, affordability, and ease of operation. Although the effect of light stimulation is immediate, the response of hydrogels to this effect is still slow. In most cases, the conversion of light into heat must occur before the reorganization of the polymer chains due to the temperature change [155,161].

Table 1 Stimuli-responsive chitosan-based hydrogels.

Stimulus	Mechanism	Applications	Limitations
pH-responsive	▪ Consist of weak acidic/basic groups (e.g., -COOH, -NH_2) that ionize with pH change [153]. ▪ Chitosan becomes protonated in acidic environments, leading to elevate swelling and positive charge [155,156]. ▪ Electrostatic repulsion leads to expansion and improved water absorption [155,156]. ▪ Classified into anionic (acid groups) and cationic (amine groups) hydrogels [155,156].	▪ Targeted drug delivery [152]. ▪ Wound healing: Swelling in acidic pH promotes cellular permeation and drug release [155,156].	▪ Limited solubility at physiological pH [152]. ▪ Swelling behavior mainly dependent on narrow pH range [155,156]. ▪ Electrostatic interactions may show unstable in mixed biological fluids [157,158].
Temperature-responsive	▪ Chitosan grafted with thermo-reversible agents (e.g., NIPAAm) [156,158]. ▪ Exhibits sol-gel transition around LCST (~32 °C) [156,158].	▪ Injectable wound dressing [156,158]. ▪ Skin regeneration and tissue engineering [154,158].	▪ Mechanical strength is frequently low [144]. ▪ May require chemical modification to optimize LCST [158].

Stimulus	Mechanism	Applications	Limitations
	▪ Hydrophobic and shrinks above LCST, releasing encapsulated drugs [158]. ▪ Gel state forms at body temperature, allowing localized, sustained delivery [154,158].	▪ Prolonged drug delivery systems [154,158].	▪ Deep tissues present a slower thermoresponse [154,158].
Enzyme-responsive	▪ Recognition of enzyme substrates integrated in the hydrogel [155]. ▪ Enzyme-catalyzed cleavage modify hydrogel morphology (e.g., degradation or disassembly) [159]. ▪ Triggered release happens where enzymes are overexpressed (e.g., infection or colon tissues) [155,158].	▪ Site-specific drug delivery (e.g., colon) [155,158]. ▪ Responsive wound dressings (infection real-time) [155,158]. ▪ Dual protein delivery systems [155,158].	▪ Requires presence of specific enzymes [155]. ▪ Response rate relies on enzyme activity and substrate accessibility [159]. ▪ Enzyme levels vary between individuals [155,158].
Light-responsive	▪ Modified with photosensitive groups (e.g., UV/NIR/Vis-activated moieties) [155,161]. ▪ Light exposure induces phase transitions or bond cleavage [162]. ▪ Produce ions to differ in osmotic pressure or trigger thermal effect for drug release [155]. ▪ Responsive to UV (rapid, *in vitro*), Vis (safe), NIR (deep tissue) [155,161].	▪ Remote, precise drug delivery [160]. ▪ Imaging and cancer treatment [162]. ▪ Ophthalmic medications [155,162].	▪ UV light is cytotoxic and has low penetration (mainly *in vitro*) [155,161]. ▪ NIR systems require advanced sensitizers [155,161]. ▪ Photosensitizers are potentially toxic [158]. ▪ Response delayed due to heat conversion requirement [155,161].

***In vitro* and *in vivo* evaluation**

Cell compatibility and proliferation assays

A chitosan hydrogel appears to be great for cutaneous wound healing because it looks like tissue structures and controls the release of loaded substances [72,163]. However, some studies have shown that modified hydrogels induce self-healing of wounds without adding any other substances [72,74]. When evaluated *in vitro*, chitosan-based hydrogels exhibit great hemocompatibility, biocompatibility, also exhibit immunomodulatory properties via inducing immunological and epithelial cell activation. Proliferative cellular signals, including growth factors and interleukins, and the activation of matching cells, are examples of how wound healing is stimulated [72,163].

In addition to maintaining its biocompatibility, the chitosan derivative, in addition to maintaining its biocompatibility, the chitosan derivative developed enhanced anticoagulant activity and the capacity to bind TGF-β and IL-6. To promote inflammation, IL-6 has been shown to induce megakaryopoiesis and aid in regulating the human myeloid cell line, whereas TGF-ß carries out crucial mitotic and chemotactic actions that are necessary for granulation tissue throughout the process of wound healing [164]. Hydrogels based on chitosan have been shown to possess antibacterial and anti-inflammatory properties. TNF and IL-1 family cytokines are among the many cytokines and substances that contribute to the inflammatory response [164,165]. Hydrogels composed of chitosan possess the capacity for self-healing, which means that they regain their original shape following mechanical manipulation. The suppression of pro-inflammatory cytokines expression, including TNF-α, IL-6, and IL-8, self-healing hydrogels help patients experience less severe inflammation [165,166].

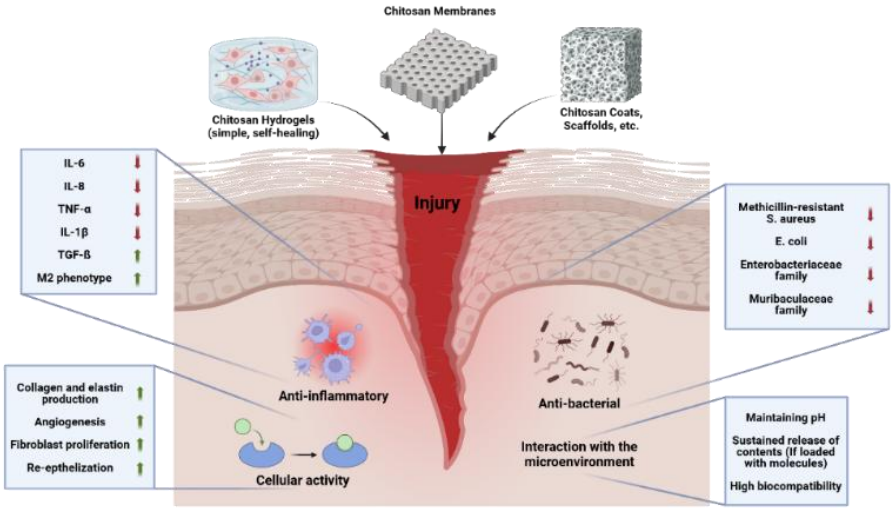

Figure 12 Chitosan biomaterial activities on skin injury. This figure was adapted from Kim *et al.* [164] using BioRender.

Chitosan has been demonstrated to play a significant role in the process of skin wound healing through various biological mechanisms. Chitosan is applied in various forms including cell cultures, porous scaffolds, and sponges depending on the wound site and healing needs. After application, chitosan provides anti-inflammatory effects by decreasing pro-inflammatory cytokines such as IL-6, IL-8, TNF-α, and IL-1β, and increasing the expression of TGF-β and the M2 phenotype of macrophages. These phenomena contribute to tissue healing. In addition, chitosan supports cellular activities that are important in tissue regeneration, including increased collagen and elastin production, stimulation of angiogenesis (formation of new blood vessels), fibroblast proliferation, and the re-epithelialization process, which is the re-formation of damaged skin layers. Chitosan also has antibacterial properties that are effective against various pathogens such as methicillin-resistant Staphylococcus aureus (MRSA), E. coli, and several bacteria from the Enterobacteriaceae and Muribaculaceae families, thereby preventing wound infection. In addition, chitosan interacts well with the wound microenvironment, helps maintain a stable pH, allows gradual release of active substances when combined with drug molecules, and has high biocompatibility so it is safe to use in wound therapy. With these properties, chitosan is a very potential biomaterial to accelerate the repair process of wounds while ensuring safety and efficacy (**Figure 12**).

Cell adhesion and proliferation

Cells' passive attachment to static substrates, include culture flasks and Petri dishes, has been demonstrated to be caused by spesific membrane integrin-binding interactions that form a mechanical link between intracellular actin and the extracellular matrix [167,168]. Recent research has investigated the role of charge-dependent interactions in mediating the interaction of chitosan with cell membranes, that regulates cell adhesion behavior. The adhesion of hyaluronic acid-chitosan films was improved using PC-3 prostate cancer cell line, within the pH range of 3.0 to 5.0 [41,167].

Bovine chondrocytes exhibited better attachment to sodium hydroxide-neutralized chitosan films, which affected cell proliferation and adhesion on scaffolds. The extent of acetylation in chitosan has been demonstrated to modulate a significant influence on its effects on cell adhesion and proliferation. This phenomenon is attributed to alterations in both hydrophobicity and its protonated groups availability for cell adhesion [167,169]. The increased levels of acetylation of the chitosan utilized in the films resulted in increased hydrophobicity and reduced surface charge, which decreased the adhesion of fibroblasts and chondrocytic cells [41].

***In vivo* biocompatibility**

Ideal hydrogels must be biocompatible in order to exhibit the function properly, preventing cellular damage and immune responses [7,38,170]. After days of

in vivo injection, the subcutaneous tissue shows no signs of irritation, indicating that the newly developed hydrogel is suitable as an innovative injectable platform for drug administration and tissue engineering [38,171]. A study in rats employing an infected full-thickness skin wound model showed that the versatile hydrogels were remarkably successful in healing infected wounds [38,172,173]. The *in vivo* animal tests demonstrated that the hydrogel dressing facilitated angiogenesis and collagen matrix deposition, resulting in enhanced wound healing and regeneration [38,174].

Nonetheless, the newly formed tissue is not invariably like healthy cartilage, potentially due to cell modification during the in vivo culturing phase and requires an extended recovery period for complete tissue formation [23]. *In vitro* and *in vivo* studies further confirmed that the hydrogel possesses enhanced antibacterial properties and minimal cytotoxicity, making it a viable choice for obstetric wound care [16,34].

Challenges and future directions

Chitosan-based wound dressings have been extensively evaluated for their effectiveness in managing chronic refractory wounds. Chitosan-based gauze demonstrated significant advantages, including enhanced wound healing, reduced pain and itching, decreased wound size, and lower frequency and overall cost of dressing changes clinically [175-178]. Another study has identified over 100 clinical studies assessing the safety and efficacy of chitosan-based products and nanoparticles, with approximately 95% being interventional, while the remaining 5% are observational [179]. Regulatory approval for Chitosan-based products varies globally. While the FDA has authorized chitosan for specific medical device applications, its pharmaceutical approval remains limited due to concerns regarding supply consistency, purification methods, and potential immunological responses [179].

Chitosan-based hydrogels have been developed to have excellent and tunable properties for accelerating and improving wound healing. Chitosan hydrogel is designed to respond to internal and external stimuli as a biocompatible, biodegradable, and sustainable polymer. Smart hydrogels are designed to enhance wound care quality and have a significant therapeutic effect. Responsive stimulus chitosan-based hydrogels also face significant challenges for translational research from experimental to clinical applications. Since the pH of chronic wounds is slightly higher than that of normal skin, pH-stimulated response chitosan hydrogels are a feasible strategy to deliver drugs to the wound site [155].

Recently, soft robotic chitosan-based hydrogel for wound dressings has arisen as a smart strategy for specific medical cases for precise and convenient applications. For instance, a magnetic chitosan hydrogel robot has controlled precise medication by magnetic-driving deformation [180]. Moreover, chitosan/PVA hydrogel provides a smart wearable application at joints and breathing [180]. Bio-inspired soft robotic hydrogel offers smart design with intelligent behaviors, including perception, motion, and response to specific environmental changes [107]. The future direction of hydrogel for excellent wound dressing must exhibit flexibility, convenient application, and antibacterial activity with adaptive characteristics in the wound environment.

To be applied for clinical purposes, hydrogels have to be biocompatible, have sufficient mechanical properties, and be biodegradable without activating unwanted immune responses. Most of the clinically approved injectable hydrogels are applied for joint or skin regeneration, as well as drug delivery. However, hydrogels for cell therapy have not yet been approved by the United States Food and Drug Administration (FDA), except cell-laden applications for wound healing [181]. Apligraf® is a bovine collagen-I hydrogel scaffold filled with neonatal fibroblasts and topped with a layer of neonatal keratinocytes. It is approved for the treatment of chronic wounds such as diabetic foot ulcers and venous leg ulcers and is applied to wound sites for several weeks before being reapplied as needed. Despite studies showing minimal cell DNA detection after 4 weeks and no engraftment, Apligraf® remains effective, most likely due to the secretion of pro-healing cytokines [181-186].

Conclusions

A promising emerging field in advanced wound care is chitosan-based hydrogels, which combine biocompatibility, biodegradability, and adjustable physicochemical characteristics. Their potential as next-generation wound dressings is highlighted by their

capacity to support tissue regeneration, preserve a moist wound environment, and share therapeutic agents in a controlled manner. With continuous advancements like pH-responsive systems and soft robotic applications improving their therapeutic accuracy and flexibility, clinical investigations have increasingly confirmed their effectiveness in treating chronic and refractory wounds. The incorporation of smart characteristics, like robotic actuation and stimuli-responsiveness, places chitosan-based hydrogels at the forefront of biomedical materials, overcoming regulatory and translational obstacles. To fully achieve their clinical promise, future research should concentrate on resolving regulatory obstacles, guaranteeing reproducible purity, and overcoming scale-up restrictions. Chitosan-based hydrogels have the potential to revolutionize wound healing techniques, moving toward more individualized, effective, and efficient care through sustained interdisciplinary efforts.

Tailored chitosan hydrogels are designed to be ideal wound dressings for better wound healing activity. Chitosan is a highly versatile biomaterial platform for wound healing applications due to its tunable structure, multifunctional properties, and capacity for bioactive molecule delivery. As illustrated, tailored chitosan hydrogels are engineered through various structure modifications such as carboxylation, crosslinking, and graft copolymerization, which enhance their mechanical strength, mimic the extracellular matrix (ECM), and improve biocompatibility and antimicrobial activity. These modifications allow chitosan hydrogels to be bioactive, soluble, and effective in supporting tissue regeneration (**Figure 13**).

Furthermore, chitosan hydrogels are capable of being conjugated with a wide range of bioactive molecules, including growth factors (EGF, FGF and VEGF), antibacterial agents, hormones, and drug molecules, enabling targeted and sustained therapeutic delivery at the wound site. The incorporation of smart hydrogel design features, such as responsiveness to light, temperature, pH, and enzymes, further enhances their adaptability to dynamic wound environments and enables controlled release of therapeutic agents. These combined characteristics facilitate the support of all key phases of the wound healing process, such as hemostasis, inflammation, proliferation, and remodeling. Thus, tailored chitosan hydrogels offer significant promises for advanced wound care, providing a multifunctional platform that addresses the complex requirements of tissue repair and regeneration (**Figure 13**).

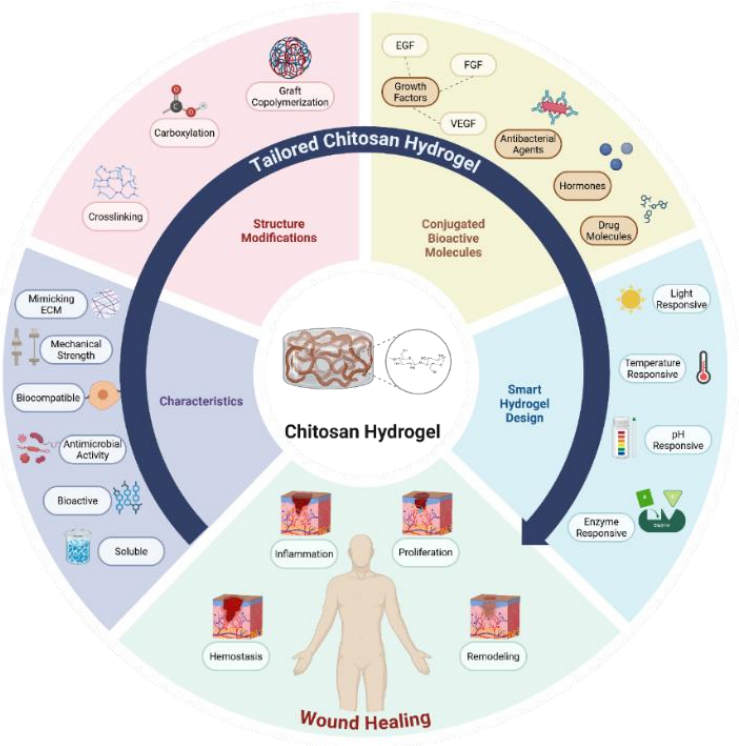

Figure 13 Schematic overview of tailored chitosan hydrogel design, characteristics, and bioactive modifications for enhanced wound healing applications. This figure was drawn with BioRender.

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