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Review article

How the combination of alginate and chitosan can fabricate a hydrogel with favorable properties for wound healing

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ABSTRACT

Wound management has always been a significant concern, particularly for men, and the search for effective wound dressings has led to the emergence of hydrogels as a promising solution. In recent years, hydrogels, with their unique properties, have gained considerable importance in wound management. Among the various types of hydrogels, those incorporating chitosan and alginate, two distinct chemical materials, have shown potential in accelerating wound healing. This review aims to discuss the desirable characteristics of an effective wound dressing, explore the alginate/chitosan-based hydrogels developed by different researchers, and analyze their effects on wound healing through in vitro and in vivo assessments. In vitro tests encompass a wide range of evaluations, including swelling capacity, degradation rate, porosity, Fourier Transform Infrared Spectroscopy, X-ray diffraction analysis, moisture vapor transmission rate, release studies, mechanical properties, microscopic observation, antibacterial properties, compatibility assessment, cell adhesion investigation, blood clotting capability, cell migration analysis, water contact angle determination, and structural stability. Furthermore, in vivo assessments encompass the examination of wound closure rate, modulation of gene expression, as well as histopathological and immunohistochemical studies.

1. Introduction

Wound healing is a dynamic physiological process involving four molecular phases: hemostasis, inflammation, proliferation, and remodeling [1]. Hemostasis involves platelet aggregation and fibrin clot formation [2], with calcium ions (Ca^{2+}) playing a critical role in clot stabilization [3]. The inflammatory phase involves the influx of inflammatory cells and factors into the defective region [4]. At this stage, neutrophils debride the wound, while macrophages release cytokines at the wound site. In the proliferative phase, fibroblasts migrate into the wound, creating a new extracellular matrix to initiate re-epithelialization [2]. Remodeling is characterized by collagen deposition and wound contraction [5]. It can continue for up to 2 years after the wound appears [6]. Effective wound management presents ongoing challenges, necessitating careful evaluation of wound and dressing features, considering patient affordability [7].

During the wound healing process, infectious complications may arise. The management of chronic wound infections necessitates a

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multidisciplinary approach, with consideration given to underlying comorbidities and concomitant factors, along with appropriate antibiotics and antisepsis use [8,9]. Also, the prolonged duration of wound healing represents an additional challenge [10].

The search for effective wound dressings has a long history, with various materials used throughout human civilization [11].

Traditional wound dressings with structural and functional limitations turned out to be ineffective in wound healing [4]. These dressings, which include gauze, lint, and bandages, have limited capacity to handle wound drainage, and tend to adhere to the wound surface, causing trauma during removal [12,13]. Traditional wound dressings provide inadequate hemostatic effects and blood clotting time. In contrast, modern dressings feature a larger surface area, facilitating rapid aggregation of platelets on them. Furthermore, the interaction between the negative charge of the platelets and the positive charge of the applied material in modern dressings results in accelerated hemostasis [14]. Additionally, modern wound dressings are enhanced with materials such as silver to prevent bacterial infection, thereby reducing the duration of the inflammatory stage in the wound healing process [15]. Therefore, since the 1980s, significant advancements have been made in the composition of wound dressings [16]. The latest generation of wound dressings possesses unique features that help to create an optimal environment for wound healing. These dressings are composed of different polymers and can be categorized into six groups according to their physical formation, including film, foam, fiber, sponge, hydrocolloid, and hydrogel [3,17]. Among them, hydrogels have shown promise due to their biological, chemical, and physical characteristics.

Numerous combination hydrogels that have been recently formulated certify their inherent advantages. Through the incorporation of certain materials into hydrogels, they effectively fulfill distinct functions in the facilitation of wound healing [18].

Alginate exhibits a range of characteristics, including low immunogenicity, biodegradability, and an affinity for metal ions, leading to the formation of highly robust gels [18,19]. On the other hand, chitosan offers advantageous properties such as antibacterial and hemostatic effects [20], as well as biocompatibility, and non-toxicity [18]. Nevertheless, due to some drawbacks, chitosan is utilized in combination with other biomaterials [19].

Consequently, alginate and chitosan have been applied to promote hydrogels by addressing common challenges encountered in the wound healing process. The mentioned polymers possess highly advantageous properties, including antimicrobial activity, sufficient absorption capacity, biocompatibility, non-cytotoxicity, and affordability. Thus, alginate-chitosan hydrogels offer desirable water retention capabilities, efficient biodegradability, proper gaseous exchange at the wound site, and sustained drug release [17,21,22]. Additionally, chitosan serves as a polycationic skeleton that interacts with the polyanionic nature of alginate, leading to the stabilization of the forming polyelectrolyte complex [23]. This article focuses on the advantages of hydrogels and specifically analyzes experiments evaluating alginate-chitosan hydrogels as potential wound dressings.

2. Hydrogels

Hydrogels are versatile materials that can absorb and retain a significant amount of water within their three-dimensional network structure. They have found numerous biomedical applications, including wound repair, implantation, cartilage repair, and neurite growth [24]. Hydrogel dressings, in particular, create an ideal moist environment for wound healing due to their high water content. These dynamic dressings absorb wound secretions, protect against secondary infections, and exhibit favorable antioxidant properties [17].

Hydrogels can be classified based on their preparation method, especially the way they are crosslinked. The crosslinking process can be done physically or chemically. Among the physical methods of cross-linking, freeze-thawing and stereocomplex can be mentioned. Freeze-thawing is a method in which microcrystals are formed in the structure as a result of freezing and thawing. In stereocomplex formation however, the products are dissolved in water and the solution is mixed [25]. Physically cross-linked hydrogels can also be obtained by hydrogen bonds, ionic interactions, etc. [26]. On the other hand, chemical methods include chemical grafting, radical polymerization, high-energy radiation, and using enzymes. Chemical grafting involves the activation of macromolecular backbones by the reaction of a chemical reagent [25]. If using radiation polymerization, initiators, catalysts, or crosslinkers are not demanded anymore [27]. Other applied methods include photocrosslinking, polyelectrolyte complexation, electrodeposition, utilizing light, etc. [27,20].

Hydrogels can be classified into two broad categories based on their origin: natural hydrogels and synthetic hydrogels [28]. Natural hydrogels are derived from naturally occurring polymers, while synthetic hydrogels are made from artificial polymers [17]. Natural hydrogels are widely used due to their favorable properties. Alginate, chitosan, and collagen are examples of natural polymers. On the other hand, synthetic hydrogels are artificial polymers that are less effective than natural ones. Among them are polyacrylamide (PA) hydrogels and polyethylene glycol (PEG) hydrogels [28].

In terms of polymer composition, hydrogels can be divided into three categories:

homopolymeric, copolymeric, and multipolymer hydrogels. Homopolymeric hydrogels are derived from a single monomeric species (hydroxypropyl methylcellulose hydrogel by *Pan* et al. *2023*) [29]. Copolymeric hydrogels consist of two or more types of monomers with at least one hydrophobic component arranged in a random or block configuration along the chain of the polymer network (dialdehyde methylcellulose/chitosan oligomer hydrogel by *Ho Yeo and Ho Park, 2021*) [30]. Multipolymer hydrogels consist of two independent cross-linked synthetic or natural polymer segments that are connected to each other in a network form [31] (gelatin methacrylamide/alginate hydrogel modified with polydopamine on the surface by *Pacelli* et al. *2018*) [32,33].

2.1. Alginate

Alginate is a biocompatible marine-derived polysaccharide commonly obtained from brown algae. It possesses versatile properties

Table 1
Alginate/chitosan hydrogel in combination with other materials.

Year	The scaffold & composition	Advantages
Yang et al.(2023) [45]	fumaria officinalis extract-loaded chitosan nanoparticles incorporated into calcium alginate hydrogel	1) improving cell migration 2) higher wound closure and collagen deposition
Sanchez et al.(2023) [46]	lavender essential oil into alginate-chitosan membranes	1) biocompatibility
Rudyardjo and Wijayanto. (2017) [47]	chitosan-alginate hydrogel and lauric acid	1) higher thickness of the hydrogel 2) higher elongation of the hydrogel
Bagher et al.(2019) [48]	alginate/chitosan hydrogel loaded by hesperidin	1) favorable porosity 2) significant antibacterial property
Ebrahimi et al.(2020) [49]	alginate/chitosan hydrogel containing berberine and naringin nanoparticles	1) desirable swelling and weight loss value 2) improving nerve regeneration
Bayat et al.(2021) [50]	chitosan hydrogel loaded by sodium alginate-chitosan nanoparticles containing bromelain	1) decrease in necrotic tissue 2) stimulating re-epithelization
Zhang et al.(2021) [35]	alginate-chitosan oligosaccharide-zinc oxide composite hydrogel	1) sustained release of zn^{2+} 2) antibacterial property

and has the ability to form gels under mild conditions [34]. Due to its numerous physical and biological characteristics, alginate finds diverse applications in the food, biomedical, cosmetic, and health industries [2].

The chemical structure of alginate consists of an anionic polysaccharide called alginic acid, or alginate. It is composed of varying proportions of repeating blocks of [1,4]-linked β-D-mannuronic acid (M unit) and α-L-guluronic acid (G unit) [34,35].

Alginate is highly favored as a material for medical applications due to its various biomedical properties. Its sponge-forming structure, bioactivity, biocompatibility, and biodegradability make it ideal for wound dressings [4].

Alginate's stability and gelling ability under mild conditions allow for the creation of hydrogels, microspheres, fibers, and microcapsules, and therefore its use in wound healing, tissue engineering, and drug delivery systems [34]. While alginate beads and hydrogels exhibit swelling and disintegration properties in alkaline and normal saline solutions, they remain stable in acidic media [36]. Alginate mimics the characteristics of the native extracellular matrix (ECM), facilitating site-specific cellular behaviors [2].

In a study conducted by *Nazeri* et al. *(2015)*, honey-based alginate hydrogels accelerated the wound healing process [37]. Alginate can perform well in terms of drug delivery. According to *Huang* et al. *(2011),* insulin-loaded alginate microcapsules which have become magnetic, are promising for a magnetic-responsive drug delivery system [38].

While alginate possesses many desirable properties, its mechanical and antibacterial characteristics alone do not appear sufficient enough in alginate-based dressings [35,39]. Additionally, alginate does not exhibit sustained release behavior due to its short-term release in alkaline and neutral media [36]. Moreover, solid dressings made solely of alginate may not offer sufficient hemostatic properties [2]. To overcome these limitations, researchers and developers have explored the use of alginate in combination with other polymers as complementary materials.

2.2. Chitosan

Chitosan is a polycationic polysaccharide that is derived from the deacetylation of chitin. It is considered a quasi-natural polymer and exhibits unique properties, including the formation of polyelectrolye complexes. Amino polysaccharides with low molecular weight resulting from the deacetylation of chitin comprise the chemical structure of chitosan. It has a polymeric structure containing units of D-glucosamine and N-acetyl-D-glucosamine linked by β(1–4) glycosidic bonds [16,35,40]. Unlike chitin, chitosan is soluble in acidic aqueous solutions, which allows for the formation of hydrogels. In acidic conditions, chitosan displays a high affinity for various metal ions [16].

Chitosan is hemostatic and non-toxic, therefore widely regarded as an attractive substance [1,2]. It is utilized in tissue engineering as a drug delivery material [41], and the literature highlights its antibacterial properties [36]. This amino polysaccharide can create more active sites for red blood cells, thereby improving blood clotting and tissue repair. Additionally, chitosan could stimulate cell proliferation by promoting the synthesis of growth factors by fibroblasts. It can also regulate platelet adhesion and aggregation [1].

Chitosan's properties extend beyond the avoidance of wound dehydration and include hindering infection via inducing macrophages to release cytokines [4]. Chitosan's biodegradability and adhesion properties present it as a promising candidate for constructing a variety of wound dressings [1].

Chitosan powder has demonstrated antibacterial properties against various bacteria including Escherichia coli, Pseudomonas aeruginosa, Enterococcus faecalis,and Staphylococcus saprophyticus [42]. *Wiegand* et al. *(2010)* stated that applying chitosan would stimulate the secretion of interleukin 6 and interleukin 8 by human keratinocyte cell line HaCaT [43]. *Bhise* et al. *(2008),* manufactured chitosan-Naproxen sodium complexes and concluded that this scaffold is sufficient for controlled release of ionic drugs after performing experimental sections [44].

Table 1 illustrates a number of explorations of alginate and chitosan combined with other materials.

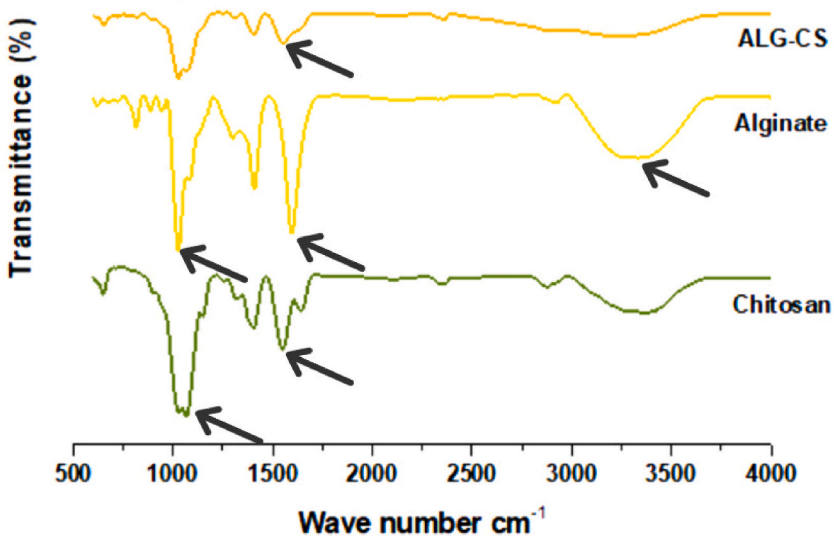


Fig. 1. Fourier Transform Infrared Spectroscopy (FTIR) for chitosan, alginate, and alginate-chitosan (ALG-CS): FTIR illustrates the spectral analysis of the sample, providing detailed information about the molecular composition and functional groups present in the material. This figure was adopted from the *Shyam Kaparekar* et al. *study (2021)* with some modifications [21]. Key absorption peaks are indicated by arrows.

3. An ideal wound dressing

The selection of a suitable wound dressing is crucial for optimal wound healing outcomes [16]. It must have an optimal moisture vapor transmission rate (MVTR) to prevent water loss through evaporation [35]. The dressing should also be capable of stimulating the migration and proliferation of various cells, like epithelial and endothelial ones [16]. Porous structure is another essential characteristic that allows gaseous exchanges crucial for wound healing [34]. Additionally, the swelling capacity of a dressing plays a fundamental role in its efficiency, as optimized fluid absorption leads to the removal of exudates [4]. The dressing must also provide a moist environment to allow for easy and harmless dressing changes [2]. Furthermore, the dressing must not exhibit cytotoxicity or antigenicity; the features that are essential for clinical usage [3,16]. Blood compatibility is also important due to the constant interaction between the dressing and blood [39]. An ideal dressing would possess antimicrobial activity [34] and perform as a suitable barrier against microorganisms [2]. It should represent intrinsic antimicrobial activity with improved sustained-release characteristics. The combination of bioactive materials in dressings can significantly affect the wound healing process and cause associated modifications in the wound area [36]. In addition, wound dressings should meet other criteria, including suitable mechanical properties, appropriate degradability, and effortless removal without trauma [2,35]. Considering the mentioned criteria, hydrogels have recently become desirable.

4. Assessments of hydrogels properties for wound dressings proposes

4.1. Swelling capacity

Hydrogel wound dressings are commonly evaluated using a range of criteria, including swelling capacity. The ability of a hydrogel to absorb water is determined by its swelling ratio, which is a critical factor influencing its therapeutic efficacy and the rate of uptake of wound exudates [35,39].

The pore size of hydrogels is directly associated with the entry of water molecules into the network of the hydrogel, thereby affecting the swelling rate. For example, N-carboxymethyl/sodium alginate gene activated matrix scaffolds with higher porosity have been found to exhibit the highest swelling rate [40]. In a recent study, sodium alginate/chitosan oligosaccharide hydrogels with or without zinc oxide nanoparticles demonstrated a swelling degree of approximately 150 % [35]. The higher porosity and hydrophilic nature of alginate significantly contribute to the high swelling rate of hydrogels, as observed in chitosan/alginate/hyaluronic acid sponges with less chitosan content, exhibiting a higher swelling percentage than others [4].

4.2. Biodegradability rate of Hydrogel

To ensure effective wound healing, the dressing's degradation rate should match the speed of recovery. If the dressing degrades more quickly than the healing process, healing can be hindered. Conversely, a slower degradation rate can impede tissue formation [51]. Alginate degrades more slowly than chitosan, as shown by *Vakilian* et al. *(2021)* [52]. Chitosan degradation is reduced with its reduction, according to *Meng* et al. *(2021)* [4].

4.3. Importance of porosity

Porosity plays a pivotal role in hydrogels, influencing their structural characteristics and functionality. The porosity of the dressing improves the migration of fibroblasts, micronutritional exchange, and absorption of excess exudates at the wound site [4]. This property could accelerate wound healing by facilitating oxygen exchange at the wound site [53].

High porosity is advantageous for preserving high water content and separating nutrients [39]. A prior investigation developed hydrogels made of oxidized alginate and carboxymethyl chitosan in different ratios. The results showed that with the increase in oxidized alginate content (with consistent carboxymethyl chitosan content), the rate of porosity increased as well [54].

4.4. Infrared spectroscopy analysis

The absorption peaks of chitosan, alginate, and alginate/chitosan hydrogel can be examined in the IR spectrum. At 3186 cm^{-1}, chitosan has an absorption peak attributed to OH group and N–H bonded stretching vibrations. In addition to that, it demonstrates another peak at 1633 cm^{-1} related to C═O stretching of amide I. C–O stretching frequency contributes to chitosan's transmittance peak at 1025 cm^{-1}. Sodium alginate demonstrates a noticeable peak at 1651 cm^{-1} associated with the vibration of the carboxylate C═O group. Also, the observed peaks at 3350 cm^{-1} and 1025 cm^{-1} were attributed to the O–H group and stretching of the C–O–C bond, respectively [21,55]. The chitosan/alginate spectrum showed an intensified amide II peak at 1562 cm^{-1}, with both amino group peaks of chitosan (1567 cm^{-1}) and carboxyl group peaks from alginate (1615 cm^{-1}) being absent, suggesting that the ionic interaction between carboxyl and amino groups is the reason for chitosan/alginate hydrogel formation (Fig. 1) [21,56].

4.5. Determination of the underlying crystal structure

X-ray diffraction (XRD) is a powerful analytical technique used to investigate the crystal structure and the degree of crystallinity in various materials, including hydrogels [57]. XRD is based on the principle of Bragg's law. It states that when X-ray photons reach the material, there are absorption and scattering effects. The X-ray photons scatter in multiple directions upon reaching all the atoms within the irradiated volume. However, the scattered radiation can undergo constructive or destructive interference. This occurrence results in distinct diffraction patterns, which can be analyzed to explore the crystal structure of materials [58]. Indeed, analyzing the diffraction patterns obtained from XRD analysis of hydrogel samples can provide valuable insights into their crystal structure. A research investigation performed by *Shyam Kaparekar* et al. *(2021)* examined the X-ray diffraction (XRD) analysis of hydrogels composed of alginate and chitosan. The findings of the study indicated that chitosan shows a crystal peak at 10° and 20.2°, while alginate depicts a crystal peak at 24.30°. Moreover, alginate-chitosan dressings demonstrated a weak, broad profile with no obvious peak in the XRD pattern. Based on further observations, the authors supposed that the incorporation of alginate into chitosan disrupted the crystal arrangement of chitosan and hindered the formation of hydrogen bonds between amine and hydroxyl groups, a reason why the structure of alginate-chitosan scaffolds became amorphous [21].

4.6. Moisture vapor transmission rate

Skin undergoes water loss via evaporation, and controlling this process is crucial for maintaining skin hydration. The water vapor transmission rate (WVTR) refers to the rate at which water vapor passes through a material, and it plays a crucial role in regulating water loss from the skin [35] and evaluating the effectiveness of wound dressings. Dressings with an optimal WVTR can maintain an appropriate moisture level, creating a favorable environment for the growth of epidermal cells and fibroblasts [39]. Insufficient WVTR, on the other hand, can lead to bacterial growth due to the accumulation of wound secretions. The WVTR of a hydrogel can be influenced by the composition of the applied material. *Zhang* et al. *(2021)* developed bilayer dressings containing chitosan nanoparticles loaded with Ag-Metal−organic frameworks and a mixture of polyvinyl alcohol, sodium alginate, and chitosan. The WVTR of these dressings was found to be directly associated with the chitosan content. Additionally, the degree of cross-linking in a hydrogel is believed to impact its WVTR. The aforementioned research showed that highly cross-linked hydrogels exhibited lower water vapor permeability, while less cross-linked hydrogels demonstrated higher permeability [1].

4.7. Release capacity of alginate/chitosan hydrogels

Investigating release rates is crucial in hydrogel research for evaluating drug delivery systems' safety and effectiveness. The release profile of a drug or bioactive compound from a hydrogel can provide valuable insights into its controlled release behavior [59]. *Ehterami* et al. *(2019)* fabricated a chitosan/alginate hydrogel containing vitamin E. The release profile of vitamin E demonstrated a 37.9 % release in the first 24 h. It was then sustained at a rate of 77.2 % over 14 days [60]. *Najafpour* et al. *(2022)* loaded solanum nigrum L. leaf extract into sodium alginate nanoparticles and incorporated them into chitosan hydrogel, reducing explosive release. In vivo analysis showed that the extract solution and chitosan hydrogel were more effective in reducing inflammation than the chitosan gel and positive control. The system trapped the extract due to electrostatic interactions between negatively charged extract and positively charged chitosan. The release of bioactive materials from hydrogels depends on factors like network structure, loading concentrations, and physicochemical properties [61].

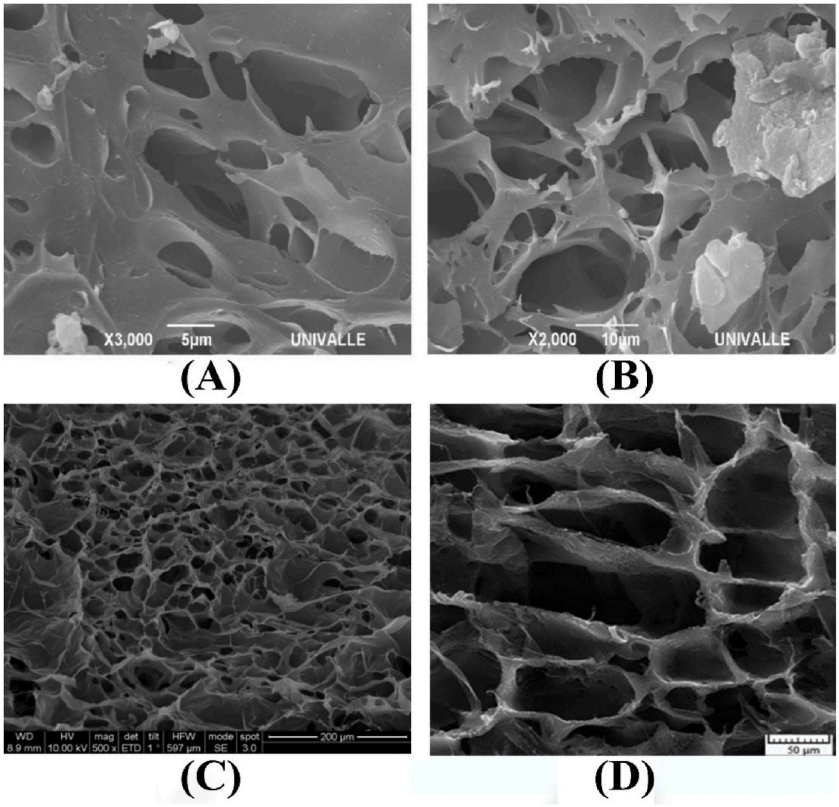


Fig. 2. SEM images of chitosan hydrogel cross-linked with dialdehydes: (A) glyoxal and (B) glutaric acid [65]. (C) SEM image of the cross-section of alginate hydrogel [66]. (D) SEM image of alginate/chitosan hydrogel cross-linked with N,N dicyclohexylcarbodiimide [67].

4.8. Mechanical properties

Evaluating the mechanical properties of materials, such as tensile strength, elongation at break, and elastic modulus, is crucial for understanding their structural integrity and performance [2,3].

According to *Meng* et al. *(2021)*, with more chitosan content in polyelectrolyte complexes, compression modulus and elongation at break increase, while elastic modulus decreases, resulting in the better mechanical strength of sponges based on chitosan under stressful conditions [4]. Additionally, chitosan nanoparticles boost tensile strength and lessen elongation at break [1,62].

Chemically cross-linked hydrogels exhibit stronger mechanical properties and a more stable structure when compared to physically cross-linked hydrogels [54,63]. The use of cross-linking agents such as calcium ions (Ca^{2+}) and zinc ions (Zn^{2+}) has been shown to significantly enhance tensile strength in chitosan/sodium alginate hydrogels [64].

4.9. Microscopic appearance

Scanning electron microscopy (SEM) is primarily used to examine the physical structure and surface properties of various materials, including dressings such as chitosan hydrogel (Fig. 2 (A and B)), alginate hydrogel (Fig. 2(C)), and alginate/chitosan hydrogel dressings. The technique allows researchers to observe the shape, size, and distribution of pores within the dressings (Fig. 2(D)) [16,35,41]. The polymer chains in a hydrogel are connected through both covalent and noncovalent bonds, leading to the formation of three-dimensional networks [65]. The internal structure of hydrogels and the degree of cross-linking are also investigated by SEM [66]. Alginate/chitosan hydrogel exhibits a highly porous structure and the pores are interconnected, with the pore size being within the interval of 45–141 µm [67], while chitosan hydrogels cross-linked with Sodium triphosphate pentabasic have a pore size in the range of 1–5 µm [33]. Also, alginate hydrogel contains pores with an average size of 56 ± 11 µm [68].

4.10. Antibacterial properties

The process of wound healing is intricate and multifaceted. The susceptibility to external microorganisms, for example, can lead to inflammation and chronic infections in the affected area [39]. These complications not only pose a health risk to patients but also lead to significant financial burdens. Biofilms, which are microbial populations formed by specific types of bacteria, can further complicate the healing process. Furthermore, the emergence of multidrug-resistant pathogens has heightened the risk of septicemia, particularly

in hospital settings where infections can be nosocomial, originating from hospital equipment or staff [17].

Hydrogels have shown promise as physical barriers against external organisms [69]. Antibiotic-free hydrogels can be classified into three categories based on their antibacterial mechanisms: intrinsic antibacterial hydrogels, hydrogels that act as reservoirs for antibacterial materials, and hydrogels that exhibit antimicrobial properties when exposed to extrinsic stimuli, such as phototherapies and nitric oxide-based antibacterial activity [70].

Recently, there has been a growing interest in enhancing the antimicrobial properties of hydrogels using natural polymers such as chitosan and alginate [71]. Chitosan, in particular, possesses inherent antibacterial properties due to the electrostatic interactions between its amino groups and bacterial cell walls, disrupting the permeability of bacterial walls to nutrients. Moreover, cationic polymers can penetrate the cell wall and bind to bacterial DNA, inhibiting transcription [54,70].

4.11. Biocompatibility

In order to ensure the safe and effective use of biomaterials for wound healing, it is necessary to evaluate their biocompatibility [1]. The CCK-8 method assay and MTT assay are valuable tools for assessing the biocompatibility of biomaterials used in wound healing [3,40]. The cytotoxic evaluation of drugs has traditionally relied on conventional colorimetric assays, such as CCK-8 and MTT assays, which utilize dye labels. These assays have gained popularity due to their exceptional sensitivity and convenience. Among these methods, the CCK-8 assay, which employs highly water-soluble tetrazolium salt, demonstrates superior detection sensitivity compared to MTT [72].

4.11.1. Cytotoxicity

Cytotoxicity tests can be conducted in 2D monolayer cell cultures or 3D cell cultures. In 2D monolayer cultures, traditional methods such as direct contact test or extract tests are commonly employed. The 3D in vitro model, however, contains four approaches: cell spheroids, cells on a chip, immobilized cells, and tissue cultures [73]. Chitosan and chitosan-containing hydrogels do not exhibit cytotoxicity [40,55]. In the case of alginate/chitosan hydrogels, they have been reported to promote proper cell proliferation and density while also demonstrating reduced cytotoxicity [35].

4.11.2. Hemocompatibility

It is essential to determine hemocompatibility when using materials that come into contact with blood. Studies have reported the hemocompatibility of alginate/chitosan hydrogels [51].

4.12. Cell adhesion investigation

The adhesion and clustering of platelets and red blood cells (RBCs) are crucial for blood clotting. Therefore, to evaluate the clotting potential of biomaterials, it is essential to carefully investigate the adhesion of platelets and erythrocytes to the surface of these materials. Chitosan has been observed to regulate platelet adhesion and aggregation, thus promoting blood coagulation [1]. Cell adhesion to chitosan depends on the interaction between its positively charged amino groups and negatively charged matrix molecules [74].

The morphology of the cells adhered to the surface of the dressing can be examined via scanning electron microscopy (SEM).

An internal three-dimensional structure is suitable for cell attachment, as evidenced by the strong adhesion and acceptable morphology of cells cultured on the dressing created by *Zhou* et al. *(2020)* [39].

Despite alginate, chitosan exhibits remarkable adhesive properties. Hydrogels composed of chitosan-alginate, which act as a coating for a drug core, as reported by *Patil* et al. *(2016)*, display superior mucoadhesive characteristics compared to dressings without chitosan [54,75]. However, pure chitosan is not appropriate for cell attachment studies due to its cationic nature. To overcome this limitation, *Baysal* et al. *(2013)* designed hydrogels using various ratios of chitosan and alginate. They observed that an increase in alginate content resulted in a more favorable environment for cell attachment. Therefore, creating cross-linked three-dimensional hydrogels that include chitosan and other polymers, particularly alginate, is a suitable strategy for generating scaffolds that promote cell attachment [76].

4.13. Blood clotting capability

The blood coagulation test is used to determine the duration of blood clotting, involving the intrinsic, extrinsic, and common pathways. The intrinsic pathway is the longest pathway of secondary homeostasis and consists of factors I, II, IX, X, XI, and XII, starting with the activation of factor XII. The extrinsic pathway consists of factors I, II, VII, and X, while the common pathway involves factors I, II, V, VIII, and X, beginning at factor X, which is activated to factor Xa [77].

In a study by *Meng* et al. *(2021)*, it was shown that a chitosan/alginate/hyaluronic acid sponge with a ratio of 2:3 chitosan/alginate (CAHS2) can activate both intrinsic and extrinsic pathways. The CAHS2 sponge can absorb liquid to accelerate coagulation factors and platelets, and the negative charge on its surface can activate coagulation factors [4]. Carboxymethyl chitosan/alginate (AC) and carboxymethyl Chitosan/Alginate/Kongfuxin (ACK) have demonstrated good and rapid coagulation effects. The hemoglobin concentration of the AC group was found to be lower than that in the alginate group, indicating better coagulation improvement [78]. Previous research has shown that carboxymethyl chitosan can activate platelets and clotting factors, and combining it with bioglass is effective in developing blood coagulation [79].

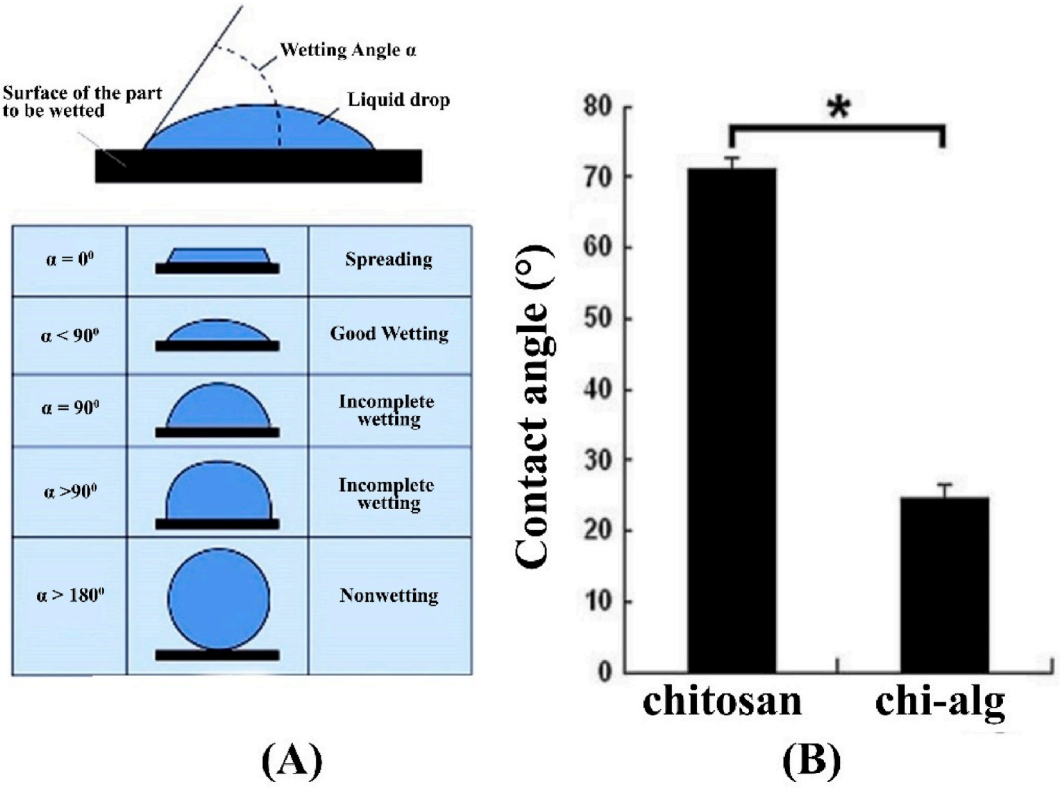


Fig. 3. (A) The angle formed between a liquid interface and the surface of a material is called the water contact angle. Water contact angles smaller than 90^{O} introduce a hydrophilic surface, whereas contact angles greater than 90^{O} show that the solid surface is hydrophobic [56,85]. (B) The contact angles of chitosan and chitosan-alginate hydrogels fabricated by *Wang* et al.*(2017):* Angles smaller than 90^{O} represent a hydrophilic material surface [56].

4.14. Cell migration analysis

The migration of fibroblasts is essential for effective wound closure [71]. The scratch assay is a commonly used method to assess the ability of dressings or biomaterials to stimulate fibroblast migration [69,80]. Prior investigations established that fibroblast and inflammatory cell migration is crucial for granulation tissue formation and extracellular matrix production, which in turn accelerates wound healing [71]. Hydrogels have been found to be effective in promoting cell migration owing to their porous structure [1,48], and chitosan activates cell migration by stimulating the release of interleukins and keratinocytes [81].

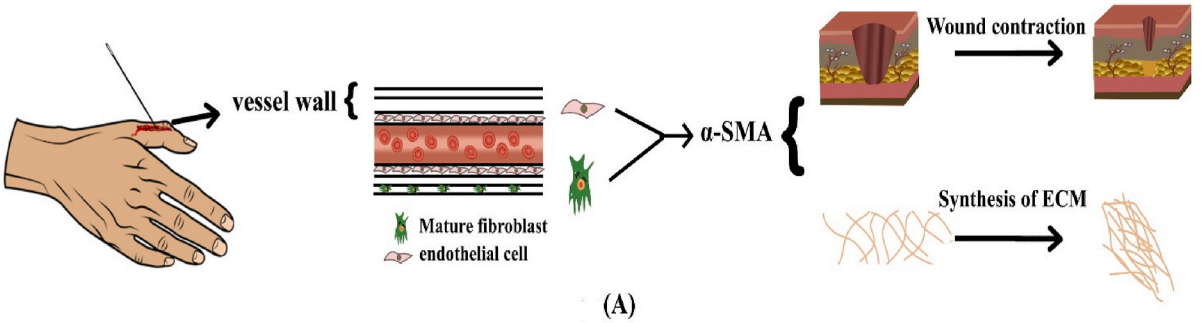
A study reveals that encapsulating polydeoxyribonucleotide (PDRN) in CaCO3 nanoparticles and subsequently incorporating them into an alginate/chitosan hydrogel resulted in the formation of Gel@PCNPs. Applying the medium containing Gel@PCNPs resulted in the acceleration of cell migration to the scratched region, leading to the complete disappearance of scratches within 48 h [69].

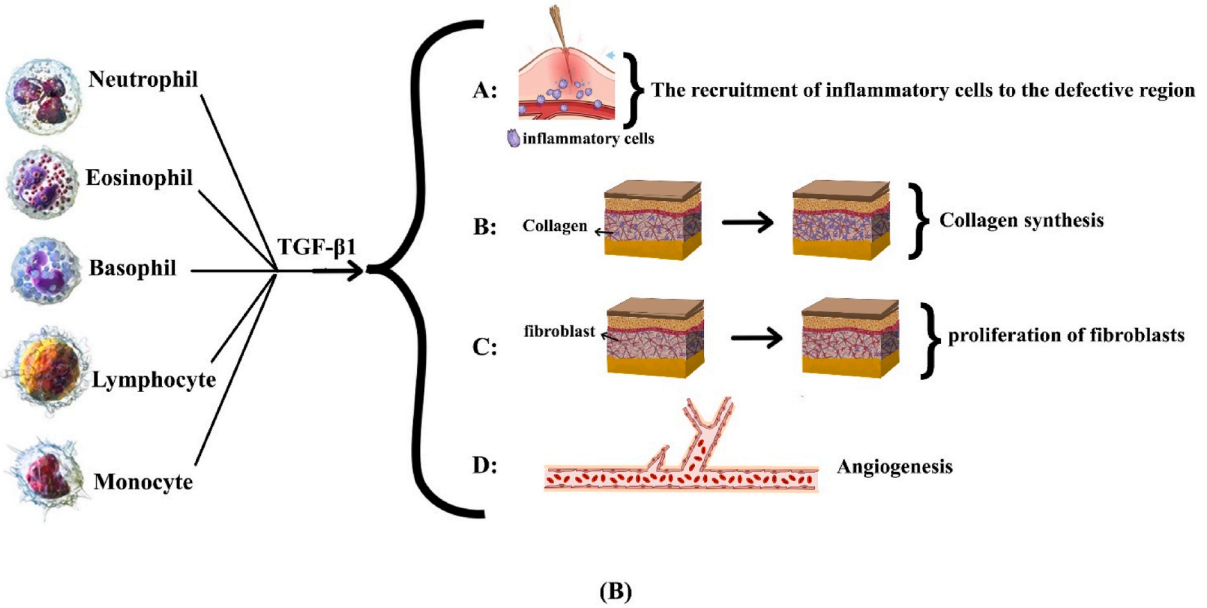
4.15. Wettability

Wettability is a critical characteristic for evaluating the hydrophilicity or hydrophobicity of hydrogels. The contact angle is defined as the angle formed between a liquid interface and the surface of a material (Fig. 3(A)) [56]. Alginate, chitosan, and their combination have different water contact angles and various water absorption rates (Fig. 3(B)) [56,82]. The contact angle decreases as solution absorption increases. Increasing carboxymethyl chitosan in hydrogels enhances hydrophilicity [83]. Moreover, alginate demonstrates a higher hydrophilicity than chitosan, therefore a smaller water contact angle [84]. Incorporating alginate into chitosan hydrogels significantly reduces the contact angle, making them more hydrophilic [56].

4.16. Structural stability

Assessing dressing stability is crucial to determining a hydrogel's ability to resist degradation in a wound bed environment. Coating alginate hydrogels with water-soluble chitosan hydrochloride (CH–Cl) increases their stability, as interactions are not affected by sodium exchange, resulting in delayed degradation [36]. Adding Aloe Vera and Dracaena Cinnabari to alginate/chitosan-based hydrogels also enhances stability [52], however, adding aloe Vera and honey decreases stability, but hydrogels are still suitable for use as wound dressings [86]. Bioglass added to sodium alginate and carboxymethyl chitosan hydrogels improves stability due to strong





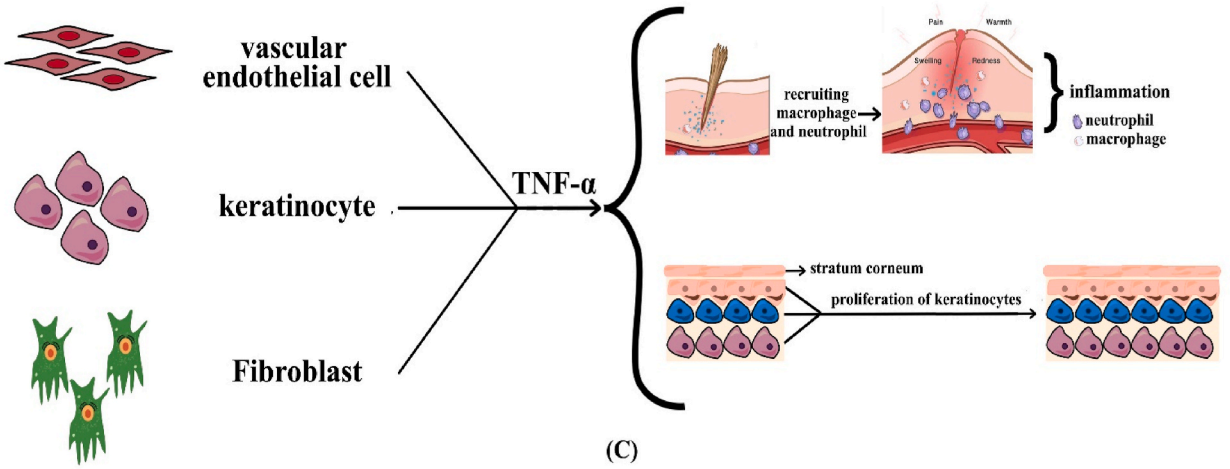


Fig. 4. (A) α-SMA causes the contraction of the wound and the synthesis of ECM after being expressed by fibroblasts and blood vessel endothelial cells. (B) How TGF-β1 expressed by leukocytes affects different parts of the wound healing. (C) TNF-α is released from vascular endothelial cells, keratinocytes, and fibroblasts, improving the proliferation of keratinocytes and extending inflammation in a higher content.

cross-linking between bioglass ions and sodium alginate particles. Some authors suggest that hydrogels containing sodium alginate (SA) and carboxymethyl chitosan (CMCS) exhibit low stability. To address this issue, *Wu and Li (2017)* added bioglass to the SA/CMCS hydrogel, resulting in improved stability due to strong cross-linking between the released ions from bioglass and sodium alginate

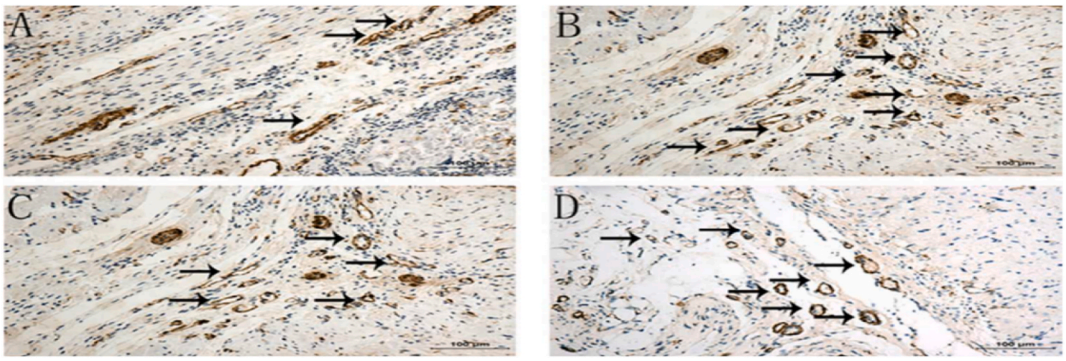


Fig. 5. The progression of angiogenesis within the damaged region via the presentation of the α-SMA immune-staining outcome after a 4-week treatment period. The small arrows indicate the marked blood vessels. Figures A–D correspond to the PBS-only, alginate-only, chitosan-only, and alginate-chitosan treatment groups, respectively [94].

particles [79].

5. *In vivo* studies

5.1. Rate of wound closure

The evaluation of wound healing entails in vivo studies to assess the efficacy of hydrogels as wound dressings [3]. Granulation tissue formation and collagen protein are two factors that can affect the wound area during the healing process. Granulation tissue aids in filling the wound and facilitates epithelialization, while collagen provides strength and elasticity to the wound healing process [40].

Alsharabasy et al. *(2015)* fabricated alginate/chitosan polyelectrolyte complexes and applied them to wounds. They observed a quicker reduction of wound size in the treatment group compared to the control group after a certain time. The materials of the dressings, such as chitosan, absorb protein molecules from the wound surface into their own structure. This process generates physical forces that contributes to a faster contraction of the wound area in wounds treated with alginate/chitosan hydrogel than in non-treated wounds [81].

5.2. The impact of alginate/chitosan hydrogels in gene expression modulation

Several studies have investigated the behavior of alginate/chitosan-based hydrogels containing growth factors, analyzing the release of these factors [87,88]. Also, some experiments have focused on the role of hydrogels in regulating the gene expression of different growth factors released by cells [71,74,89].

Alpha-smooth muscle actin (α-SMA) is expressed by mature fibroblasts and blood vessel endothelial cells. Mature fibroblasts differentiate into myofibroblasts, contributing to wound contraction and extracellular matrix (ECM) synthesis (Fig. 4(A)) [90,91].

TGF-β1 has a significant effect on the different phases of wound healing. This growth factor recruits inflammatory cells to the damaged area, promotes collagen synthesis, stimulates fibroblast proliferation, angiogenesis, and myofibroblast differentiation, and enhances re-epithelialization, thus facilitating the remodeling phase (Fig. 4(B)) [71,89,90].

During the healing process, cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-10 (IL-10) play a crucial role in regulating tissue regeneration. TNF-α affects keratinocytes by increasing their motility and proliferation, as well as promoting the expression of their intracellular adhesion molecules (Fig. 4(C)) [71,90]. On the other hand, IL-10 acts as an inhibitor with anti-inflammatory effects and influences the functioning of fibroblasts as well as endothelial progenitor cells [90].

In a different study, *Gossla* et al. *(2021)* fabricated a fiber-reinforced hydrogel by combining an alginate hydrogel with a chitosan flock scaffold, which caused overexpression of chondrogenic marker genes, including collagen II (COL II) and cartilage oligomeric matrix protein (COMP), compared to chondrocytes cultured in a pure chitosan flock scaffold [74].

Chitosan, when bound with nucleic acid in bacteria, can influence their DNA expression [92]. Meanwhile, the sustained release of growth factors is a crucial factor in managing cell growth and differentiation; therefore, the rate at which the growth factors are released is important. For instance, an initial burst release and the deactivation of the released growth factors are two major challenges [87].

If basic fibroblast growth factor (bFGF) is released from the hydrogel in a sustained manner, it can accelerate cell growth. *Ho* et al. *(2009)* created chitosan-alginate hydrogels that were functionalized with heparin and compared them to heparin-free hydrogels. The structures without heparin showed a faster release of bFGF, while those with more heparin content exhibited a slower release rate. The groups with certain levels of heparin led to the most efficient improvement in cell proliferation [93].

Matsusaki et al. *(2007)* developed alginate hydrogels incorporating vascular endothelial growth factor (VEGF), which were nano-coated with polyelectrolyte multilayer films consisting of chitosan and dextran sulfate. The non-coated VEGF-incorporated alginate hydrogel was compared to the coated hydrogel in terms of release behavior. The investigation revealed a sudden release of VEGF

within 6 h for the non-coated group, whereas the nano-coated hydrogel exhibited a continuous release of VEGF even after one month, without an initial burst. Furthermore, the controlled release percentage was dependent on the applied polyelectrolyte multilayer thickness. The growth factor remained undenatured, and the chitosan and dextran nano-coating on the alginate hydrogel prevented the sudden release of VEGF [87].

5.3. Histopathological and immunohistochemistry studies

Immunohistochemistry (IHC) staining is used to evaluate the molecular effects of the dressing on wound healing, including the staining of factors such as IL-6, EGF, FGF, VEGF, CD31, CD34, and TGF-β1 [16]. Research conducted by *Deng* et al. *(2019)* demonstrated that alginate-chitosan hydrogel enhances angiogenesis. They fabricated the hydrogel and measured blood vessel density in histological sections by immunostaining for α-SMA. The regions treated with alginate-chitosan hydrogel exhibited the highest degree of vascularization compared to the alginate-only and chitosan-only groups (Fig. 5(A-D)). Furthermore, the hydrogel prevented more inflammation than other groups, as evidenced by the lower number of $CD68^{+}$ macrophages using anti-CD68 staining. The alginate-chitosan hydrogel group demonstrated a much lower number of macrophages than the groups treated with only alginate or chitosan [94].

6. Limitations for using alginate/chitosan hydrogel as a wound dressing

Although alginate/chitosan hydrogels have emerged as promising materials for wound dressings, some studies implied limitations for their application as wound dressings such as:

Firstly, the mechanical properties of alginate/chitosan hydrogels can be a limitation. While these hydrogels are known for their excellent gel-forming capabilities and moisture retention [95], their mechanical strength may not be sufficient for applications on wounds under high tension or where significant structural support is [96]. This could potentially limit their use in areas subject to movement or shear forces.

Secondly, the rate of biodegradation poses another challenge. Ideally, a wound dressing should degrade at a rate commensurate with tissue regeneration [97]. However, the degradation rates of alginate/chitosan hydrogels can vary significantly based on factors such as crosslinking density and environmental conditions. If the degradation is too slow or too fast relative to tissue healing rates, it could either prolong inflammation or necessitate premature dressing changes.

Thirdly, while chitosan has inherent antimicrobial properties [98], alginate does not. Thus, alginate/chitosan hydrogel dressings might require additional modifications or incorporation of antimicrobial agents to prevent infection in wounds susceptible to bacterial colonization [99]. This adds complexity to dressing formulation and raises concerns about potential cytotoxicity from incorporated antimicrobial agents.

Lastly, the effectiveness evidence supporting alginate/chitosan-based dressings remains limited by small sample sizes and short follow-up periods observed across studies [97,100]. Most available studies provide very low-certainty evidence downgraded due mainly to risk of bias and imprecision, suggesting a need for large randomized controlled trials to establish robust conclusions regarding their clinical benefits.

7. Conclusion

Upon thorough analysis, it is evident that the physical and chemical characteristics of fabricated hydrogels vary depending on the materials incorporated. In the case of chitosan-alginate hydrogels, notable changes in the properties of the resulting materials have been observed. Various research studies have highlighted several beneficial features associated with chitosan-alginate hydrogels, including porosity, degradability, and adhesion. Further studies have demonstrated that achieving a precise proportion of chitosan and alginate in the hydrogel formulation can confer advantageous mechanical properties. Given the encouraging attributes of these hydrogels, there is considerable scope for further exploration and enhancement of their properties. This can be achieved by experimenting with various material types, including both natural and synthetic polymers. By conducting comprehensive evaluations and integrating diverse materials, it is plausible to engineer hydrogels with improved secretion rates, increased absorption capacity, and enhanced degradability. These advancements would contribute to the development of more effective and versatile hydrogel-based systems for wound healing and related applications.

Combining chitosan with alginate produces advanced hydrogel formulations exhibiting enhanced mechanical stability and bioactivity, making them highly suitable for wound dressing applications. Through innovative approaches, including incorporating additional functional components like MOFs nanoparticles or natural compounds such as curcumin, significant improvements in infection control, inflammation modulation, and tissue regeneration have been achieved, offering promising prospects for future clinical application. Nevertheless, continued research aimed at addressing current limitations and exploring novel combinations as well as functionalities remain crucial to fully realize the potential of these multifunctional materials. Looking forward, we anticipate that such progressions will be realized, serving to amplify the potential applications of hydrogels across multiple domains.

8. Search strategy

A systematic search was conducted in databases including PubMed and Web of Science using the terms: (((((((Alginate[Title/Abstract]) OR (Alginic Acid[Title/Abstract])) OR (Acid Alginic[Title/Abstract])) OR (Sodium Alginate[Title/Abstract])) OR (chitosan

Table 2
Search strategy and article screening.

Step	Description	Number/Detail
Database Selection		PubMed, Web of Science, Scopus
Key Concepts Identified		- Alginate, Sodium Alginate, Alginic Acid - Chitosan - Hydrogel - Wound healing - Wound Dressing, Wound coverage, Bandage
Search Query	(((((((Alginate[Title/Abstract]) OR (Alginic Acid[Title/Abstract])) OR (Acid Alginic[Title/Abstract])) OR (Sodium Alginate[Title/Abstract])) OR (chitosan[Title/Abstract])) AND ("wound healing"[Title/Abstract])) AND (((wound dressing[Title/Abstract]) OR (wound coverage[Title/Abstract])) OR (bandage[Title/Abstract]))) AND (hydrogel[Title/Abstract]) Filters: in the last 20 years	
Filters Applied		- Last 20 years (2004–2024) - English language
Initial Articles Retrieved		341
Title/Abstract Screening		81 excluded
Full-texts Excluded		191 with reasons
Total Articles in Review		260

[Title/Abstract])) AND ("wound healing"[Title/Abstract])) AND (((wound dressing[Title/Abstract]) OR (wound coverage[Title/Abstract])) OR (bandage[Title/Abstract]))) AND (hydrogel[Title/Abstract]); limited to the last 20 years. Articles were further screened based on title, abstract, and full-text relevance. Additional articles were sourced from reference lists of key studies (Table 2).

Ethics approval and consent to participate

The research was conducted following the highest ethical standards. The data presented in this manuscript are accurate and authentic to the best of our knowledge.

Availability of data and materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

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Consent for publication

Not applicable.

CRediT authorship contribution statement

Mostafa Saberian: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Raha Safari Roudsari:** Writing – original draft, Software, Resources, Investigation, Data curation. **Neda Haghshenas:** Writing – original draft, Resources, Investigation, Data curation. **Ali Rousta:** Writing – original draft, Visualization, Software. **Shaban Alizadeh:** Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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