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Review Article

Recent Advances in Chitosan-Based Metal Nanocomposites for Wound Healing Applications

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Chitosan (CS) has been extensively studied as a natural polymer, in the field of wound repair, due to its useful properties, which include a lack of toxicity and stimulation, excellent biological affinity, degradability, and promotion of collagen deposition. However, inferior mechanical strength and moderate antibacterial properties are the drawbacks restricting its further clinical application. Many researchers have adopted the use of nanotechnology, in particular metallic nanoparticles (MNPs), in order to improve the mechanical strength and specific antibacterial properties of chitosan composites, with promising results. Furthermore, chitosan naturally functions as a reducing agent for MNPs, which can also reduce cytotoxicity. Thus, CS, in combination with MNPs, exhibits antibacterial activity, excellent mechanical strength, and anti-inflammatory properties, and it has great potential to accelerate the process of wound healing. This review discusses the current use of CS and MNPs in wound healing and emphasises the synergy and the advantages for various applications in wound healing.

1. Introduction

As the largest external organ of the human body, skin plays an essential role in protecting the body from mechanical damage and microbial invasion. The skin is composed of the epidermis, dermis, and subcutaneous tissue; damage to the integrity of skin results in the formation of a wound. Depending on the depth of the injury, a skin wound can be classified into the categories of a superficial defect, partial injury, or full-thickness injury [1].

Wound healing consists of the following four stages: (1) haemostatic stage, (2) inflammatory response stage, (3) cell proliferation stage, and (4) collagen fibre remodelling period [2] (Figure 1). During the haemostatic phase, platelets and fibrin clot successively to form thrombi, which cause the skin wound to contract and prevent further blood loss [3]. At the same time, platelets can induce chemotaxis in inflammatory cells, such as polymorphonuclear leucocytes (PMN) and endothelial cells by releasing growth factors [4]. The inflammatory phase is marked by the infiltration of immune cells, mainly the early neutrophils and late macrophages, to resist foreign substances and provide favourable conditions for the subsequent proliferation of fibroblasts [5, 6]. Thereafter, the cell proliferation phase occurs, where fibroblasts rapidly divide in response to various growth factors and secrete collagen fibres [7]. As the collagen fibres grow and mature, the remodelling phase, which is the final phase, comes to an end [8]. Although the epidermal tissue is naturally self-healing, the repair process may fail due to excessive depth of the wound. If the skin wound is infected by external pathogens, at any of the stages outlined above, then a high level of clinical care and treatment is required, which will undoubtedly cost considerable time, money, and resources. Therefore, it is particularly essential to prepare wound dressings that can promote rapid wound healing [9, 10].

The ideal wound dressing should possess the following characteristics: (1) nontoxic and nonirritating, (2) biodegradable in vivo, (3) excellent antibacterial properties to prevent wound infection, (4) good moisture and air permeability to avoid repeated changes, and (5) sufficient mechanical strength to prevent wrinkling [11, 12]. Natural

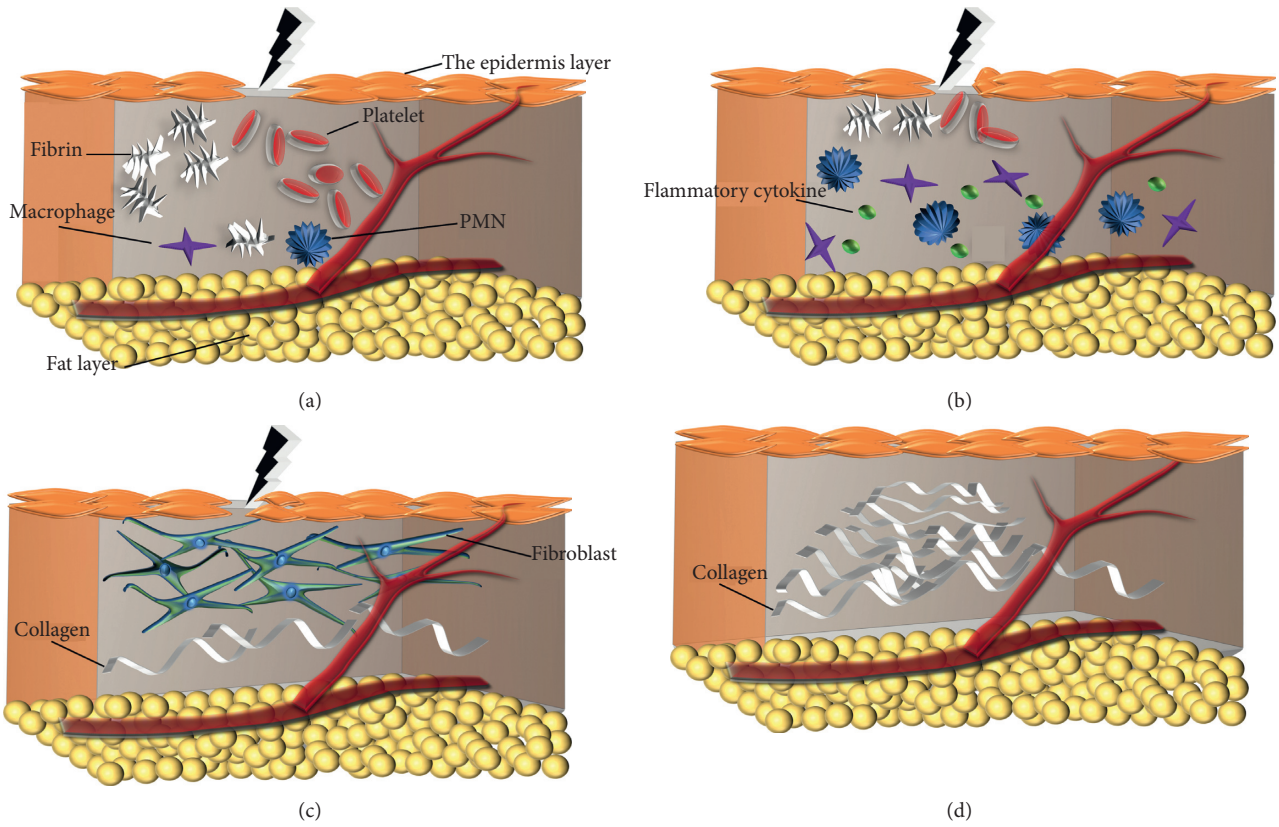


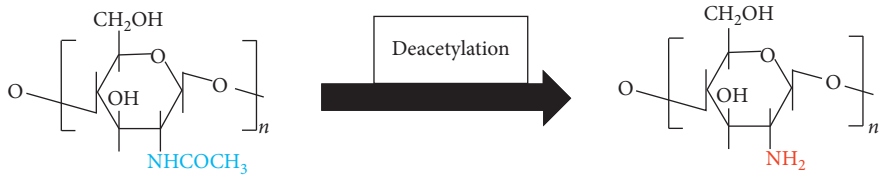
Figure 1: The process of wound healing: (a) haemostatic stage; (b) inflammatory response stage; (c) cell proliferation stage; and (d) collagen fibre remodelling period.

polymers, including natural polysaccharide polymers (such as alginate, hyaluronic acid, starch, chitosan, and cellulose) and natural proteins (e.g., silk fibroin, keratin, gelatine, and collagen), have been widely developed as wound healing materials due to their excellent biological properties [13]. This review focuses on chitosan (CS), the second most abundant polysaccharide in nature. Previous studies have shown that CS dressings can accelerate collagen deposition and angiogenesis and also inhibit inflammatory responses [14].

CS is a straight-chain copolymer of d-glucosamine and N-acetyl-β-D-glucosamine produced by the deacetylation of chitin (Figure 2). Deacetylation results in the formation of cationic amine groups, which is a prerequisite for the modest antibacterial performance of CS [15]. Unfortunately, several factors limit the performance [16], and as such, many studies have focused on optimising the antimicrobial activity of CS wound dressings. Zhou et al. constructed a gentamicin coating of CS as a nanovalve for controlled release [17], whereas other studies have proposed adding metallic nanoparticles (MNPs) to CS. Guo et al. combined CS with different concentrations of Ag, and all tested Ag coatings exhibited effective and long-lasting antibacterial properties [18]. Furthermore, there have been reports of the addition of bacteriostatic natural compounds and extracts to CS [19]. Interestingly, Sauperl et al. developed a CS nanofibre membrane with honey for wound healing [20]. The antibacterial tests proved that the resulting film dressing possessed improved antibacterial properties relative to those without added honey.

MNPs and engineered nanoparticles are widely used in wound dressings due to their unique physicochemical properties and specific repair effects [21, 22]. By incorporating metal nanomaterials within the natural polymers, ideal nanocomposite systems with enhanced repair performance can be designed. This model has received considerable interest in the field of wound healing for two main reasons: (1) the presence of MNPs significantly enhances the physical properties and antibacterial activities of natural polymer materials, and (2) the cytotoxicity of metal nanomaterials is significantly reduced when they are wrapped in natural polymers [21, 23, 24]. Currently, Ag, Au, Cu, and ZnO have demonstrated potential applicability to the field of wound repair [22, 25].

Numerous papers on wound healing have been published describing the use of CS-metal nanocomposites [26, 27]. Nonetheless, there are relatively few reviews on the subject. In this review, we will focus on systems combining CS and MNPs, which are beneficial for infection control and the healing process. First, we will critically evaluate the advantages and disadvantages of CS and MNPs in wound healing, which is a prerequisite for constructing the systems. Next, we will review the known examples of CS nanocomposites containing MNPs. Finally, we will discuss the challenges that hinder the application of this category of nanocomposite and explore perspectives on its promising future.



Chitin Chitosan

Figure 2: Structural representation of the deacetylation reaction producing chitosan.

2. Application of CS in Wound Healing

CS fulfils the requirements of an ideal wound-healing material and has good prospects for clinical application at this time [28]. CS is a linear polysaccharide polymer derived from chitin, a bone extract from crustaceans, through deacetylation [29]. In addition to its inherent biocompatibility and nontoxicity, it also possesses effective antibacterial, healing, haemostatic, and analgesic effects associated with the cationic amine group [30]. Khanna et al. studied the effect of deacetylation on wound healing [31], and the results of in vivo experiments showed that the higher the degree of deacetylation, the faster the rate of wound surface healing. CS can also be used to prepare different types of dressings, including hydrogels, fibres, films, membranes, scaffolds, and sponges, for various types of wounds; this is convenient for clinical applications [32–34]. Different types of wound dressings are listed in Table 1.

2.1. Antibacterial Performance. Wounds are prone to infection during the process of healing, and the action of microorganisms can lead to delayed healing of wounds, resulting in a vicious cycle. Therefore, the antibacterial property of the wound dressing is particularly important [48]. The exact mechanism regarding the antimicrobial properties of CS is not fully understood. Various studies have proposed several hypotheses, among which a number are generally accepted [49–52] (Figure 3). The most convincing mechanism involves the combination of cationic amine groups in CS and anionic phospholipids on the surface of the bacteria, which promote cell disintegration [49, 53]. Some authors propose that CS, in particular, low molecular weight derivatives, invade bacteria, thereby inhibiting transcription and translation by binding to DNA or RNA [50]. Another possibility is that CS, utilising its exceptional metal-binding ability, interacts with divalent ions in the bacterial cell wall to prevent the cells from performing their normal functions [51]. The final hypothesis is that CS acts as a blocking agent, forming a membrane on the surface of bacteria that prevents nutrients and oxygen from entering the cell [52]. Although CS has been proven to possess favourable antibacterial properties, it also has many limitations; these include low pH, a high degree of deacetylation, and low molecular weight [54–56]. Many studies have focused on the possibility of optimising the antibacterial properties of CS through various modifications that have been proposed [57, 58]. For example, Liu et al. postulated that the surface grafting of gentamicin molecules improved the antibacterial properties of CS [59]. Dananjaya et al. developed AgNP- (Ag nanoparticle-) embedded chitosan films with more optimal antimicrobial properties [60].

2.2. Healing Potential. As mentioned above, wound healing is a complicated and lengthy process during which the wound gradually shrinks, cells continuously multiply, and collagen fibres gradually deposit. CS exhibits strong tissue adhesion, which provides a reasonable three-dimensional structure for cell proliferation [61, 62]. In addition, following degradation, CS may release a substance that initiates the proliferation of fibroblasts and the synthesis of collagen [63]. CS can be clinically prepared in a variety of forms to meet the needs of different individuals and achieve better healing effects [64].

2.3. Haemostasis. It is essential to achieve haemostasis at the earliest opportunity after the integrity of the skin has been compromised. Thus far, numerous studies validate that CS exhibits excellent haemostatic function, both in vitro and in vivo. On the one hand, Zielinska et al. prepared two forms of haemostatic agents—CS/alginate lyophilised foam and CS/alginate impregnated gauze—and verified their feasibility in vitro [65]. On the other hand, Hemcon and Celox synthesised CS-derived haemostatic agents that have been approved by the FDA for external applications [66]. The haemostatic properties of CS are presumably dependent on its positive charge, which can rapidly support cross-linking with the negatively charged membrane of erythrocyte, resulting in a tight binding to the wound surface that is independent of the classical coagulation cascade [67]. Furthermore, reports show that CS can also be attracted to platelets, leading to platelet activation and thrombosis [68]. Although there have been experimental and clinical confirmations concerning the haemostatic efficacy of CS [69, 70], the influence of molecular weight, concentration, and other factors on the activity remains unclear, thus contributing to challenges in the optimisation process [71].

2.4. Analgesic. When Ohshima applied CS as a skin dressing in a clinic (1987), the material unexpectedly revealed excellent pain-relieving effects [72]. Consequently, many studies have investigated the potential mechanism for this effect [73]. In previous studies, the absorption of inflammatory agents, such as bradykinin, into CS has been associated with pain relief. Bradykinin is a mediator substance related to pain. Huang et al. modelled a second-degree scald

Table 1: The application of chitosan-based nanocomposites in wound healing in recent years.

MNPs	Matrix	Delivery strategies	Results	Reference
Ag	Gelatine/CS	Scaffolds	(1) Good mechanical properties, water absorption, and moisture retention (2) High antimicrobial activity against *S. aureus* and *E. coli* (3) No cytotoxicity on fibroblast cells (L929)	[35]
Cu	Sodium alginate/ cellulose/CS	Films	(1) High antibacterial properties against both MRSA and *E. coli* (2) No cytotoxicity on fibroblast cells (L929) and human dermal fibroblasts	[36]
Ag	Collagen/CS	Scaffolds	Accelerated the process of wound healing, the proliferation of fibroblasts, neovascularization, and collagen deposition in mice	[37]
Ag	Moxifloxacin- (Mox-) loaded CS	Films	(1) Presented higher swelling ratio and lower tensile strength (TS) and better elongation at break (EB) (2) Good antibacterial properties against *S. aureus*, *P. aeruginosa*, and MRSA	[38]
Ag	Sanghuang polysaccharides/CS	Sponges	(1) Excellent swelling and water retention properties (2) No cytotoxicity on L929 cells (3) Promoted wound contraction and internal tissue growth in mice	[39]
Ag	CS	Films	(1) Showed significant antibacterial activity against Gram-negative *E. coli*, *P. aeruginosa*, and Gram-positive *S. aureus* (2) Human fibroblasts (HF) displayed cell viability higher than 90%	[40]
Ag	PVA/CS	Hydrogels	(1) Higher antioxidant activity (2) Low cytotoxicity effect against Chinese hamster ovary (CHO-K1) cells (3) Significantly promoted wound healing in mice	[41]
Ag	PVA/graphene/CS	Hydrogels	Showed a significant decrease in inflammatory cells on histological examination	[42]
Ag/ Cu	CS/PVA/hydroxyapatite	Nanofibres	Rapid haemostasis in mice	[43]
Cu	CS	Films	Released nitric oxide and promoted wound healing	[44]
ZnO	Collagen/CS	Nanofibres	Accelerated the healing of burn wounds in rats	[45]
ZnO	Keratin/CS	Hydrogel	Assisted rapid skin cell construction along with collagen development	[46]
ZnO	CS/PVA	Nanofibres	Effectively healed diabetic wounds faster	[47]

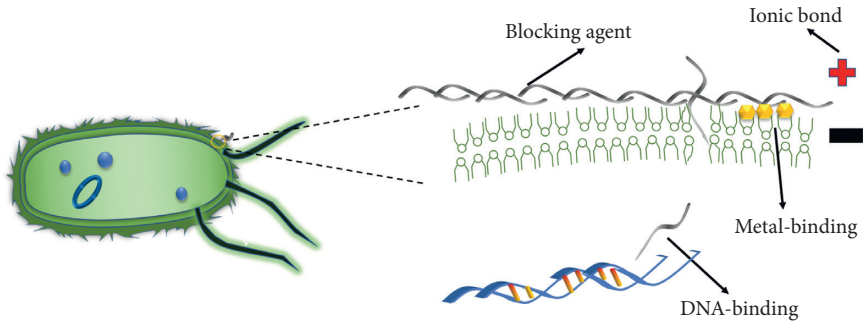


Figure 3: Hypothesis of bacteriostatic mechanism of chitosan.

in rats, which was subsequently treated with CS [74]. The concentrations of bradykinin and the 5-hydroxytryptophan in CS were significantly lower than that of the control. However, in recent years it has been suggested that the analgesic effect is due to intraperitoneal administration of acetic acid [75]. CS can absorb protons from acetic acid, which is presumably associated with pain. Also, the addition of CS potentially causes the pH to decrease due to the acidity of CS [76]. However, the specific mechanism still requires further exploration.

Despite the previously mentioned advantages, drawbacks and limitations for CS include inferior mechanical strength and moderate antibacterial properties [77]. In attempts to enhance mechanical strength, CS has been chemically modified in many previous studies to increase intermolecular forces and enhance chemical bonding. Iacob et al. postulated that arginine-modified CS possesses better mechanical strength due to the presence of peptide bonds [78]. In other studies, CS has been combined with inorganic nanoparticles to improve mechanical strength. Wen et al. improved the mechanical strength of CS hydrogels with silver nanoparticles [79]. Moreover, in order to prepare materials with better antibacterial properties, CS has been grafted to cationic molecules in numerous studies to enhance the effect of the positive charge [80]. CS has also been utilised in cooperation with other antimicrobial agents, such

as antibiotics, metallic nanomaterials, and some natural antibiotics [81, 82]. Therefore, the addition of MNPs can mitigate the disadvantages of CS. The strategies above have provided new ideas and improved treatment approaches for constructing ideal materials for wound healing.

3. CS-Metal Nanocomposites

Currently, the rapid development of nanotechnology provides a more facile synthesis of nanocomposites for use in wound healing [83]. Additionally, MNPs, such as silver (Ag), copper (Cu), and zinc oxide (ZnO), have exhibited good prospects for wound healing [84]. CS matrices, incorporated with metal nanomaterials, have been well studied in the literature. The mechanical properties and bacteriostatic properties of the CS matrix were enhanced by MNPs, while bioactive CS reduced the cytotoxicity of MNPs through encapsulation [22]. MNPs and CS may be combined as needed in a variety of forms, including hydrogels, fibre membranes, films, scaffolds, and sponges. In general, this multifunctional nanocomposite has superior physical properties and biological activities when compared to the base counterparts, as depicted in Table 1.

3.1. CS/Ag. Silver has been used directly in the form of silver ions to treat skin defects topically for centuries [85]. Silver sulfadiazine is notably the gold standard for the treatment of local burns [86]. With the development of nanotechnology, many studies have shown that silver nanoparticles exhibit prolonged and controlled release of silver ions; this can improve biological safety and reduce the frequency of clinical dressing replacements required with the use of silver salts [87]. Silver nanoparticles have strong bactericidal properties that have been observed in both in vivo and in vitro experiments [88–91]. More importantly, silver is a broad-spectrum antibiotic that is active against fungi and bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE). Peng et al. demonstrated the inhibitory effect of silver nanoparticles on MRSA through bacteriostatic annulus experiments and conducted relevant in vivo experiments that exhibited results consistent with those from in vitro experiments [92]. There are different opinions regarding the mechanism of the antibacterial activity of silver nanoparticles. The inhibition of the respiratory chain and the generation of reactive oxygen species lead to cell death, which causes the stable binding of silver ions to the sulphur group, the main component of the respiratory chain [93]. Other studies have also suggested that silver ions are highly active in damaged skin and bind easily to negative potential proteins such as DNA, thereby inhibiting transcription and translation [94]. In recent years, studies have suggested that silver ions can accumulate in bacteria, eventually reaching lethal concentrations and achieving the goal of sterilisation [95]. Microbial resistance to silver alone has rarely been reported, but it is known in clinical practice. Resistance is not triggered by high concentrations of silver but occurs at minimum inhibitory concentrations (MIC) (2–4 mg/L Ag^+) and below [96]. Thus, it is evident that uncontrolled use of silver at low concentrations may result in the emergence of resistant strains.

The addition of silver nanoparticles can also significantly improve the mechanical strength of the CS system. In the experiments of Xie et al., reduced silver nanoparticles were added to a CS-based hydrogel and resulted in the optimisation of the overall mechanical strength [97]. The compressive strength was more than 100 times higher than that of the CS control sample, reaching 15.95 ± 1.95 MPa.

In addition to its antibacterial properties and its effect on mechanical strength, silver has exhibits other wound healing properties. Sandri et al. reported that AgNP-loaded CS/glycosaminoglycan scaffolds were characterised by a strong tendency to promote the proliferation of fibroblasts [98]. Excess levels of matrix metalloproteinases (MMPs) degrade fibre junctions and peptide growth factors, delaying wound repair. Silver-based materials can promote wound repair by specifically downregulating the production of MMPs [99]. Immunohistochemical evidence obtained by Ibrahim et al. on essential metal-binding proteins suggests that silver can induce high expression of these proteins, thereby increasing local concentrations of zinc and copper [100]. Both metals are essential for epithelial cell proliferation. Li et al. hypothesised that AgNPs-loaded nanofibre promotes wound healing by activating the TGF beta 1/Smad signalling pathway [101]; quantitative real-time polymerase chain reactions and Western blot analyses were used to verify the aforementioned. Additionally, the addition of AgNPs can effectively and conclusively inhibit an inflammatory response by regulating inflammatory mediators. Oryan et al. demonstrated that Cs/Ag had an inflammatory inhibitory effect stronger than that observed in either monomer by significantly reducing interleukin-1 levels [102].

Admittedly, silver is dose-dependently toxic to both cuticle cells and fibroblasts, and the accumulation of silver in the liver, kidneys, and other major metabolic organs can lead to organ damage and failure [103]. To date, however, there has been limited agreement on acceptable dose levels. Surveys such as that conducted by Hernández-Rangel et al. have shown that primary human fibroblasts (HF) cultured on films with AgNP concentrations <0.036 wt% displayed cell viability rates greater than 90% [40]. Nguyen et al. prepared CS-based hydrogels containing different concentrations of AgNPs, and data from in vivo results have shown that the hydrogel with 30 ppm AgNP shows the highest healing rate [104]. Shah et al. prepared and evaluated CS-sericin-silver nanocomposite films with AgNP concentrations of 0.0085 mg/mL [105]. After applying the membrane to the burnt skin of rats for seven days, no silver ions were detected in the animals' blood. Fortunately, the application of a CS coating can diminish the toxicity of AgNPs. Peng et al. applied different AgNPs to mice with MRSA-infected wounds [92]. After 14 days of continuous application, mice treated with CS-AgNPs had lower levels of alanine aminotransferase and aspartate aminotransferase, indicating a significant reduction in liver dysfunction when compared with subjects receiving uncoated AgNPs.

3.2. CS/nCu. Copper is an essential element in enzymes and transporters associated with wound healing, but systemic copper poisoning can occur if large doses of copper come in direct contact with the epidermis and easily diffuse into the blood vessels. Additionally, the production of oxyradicals can also lead to the oxidative stress reaction and death of fibroblasts. Ren et al. showed that copper nanoparticles coated on CS release copper ions slowly and persistently, which is the key to avoiding systemic toxic reactions [106]. The Cu/CS framework released 33% of the total copper content within 1 h and 54% within 9 h. In addition, Ren et al. used in vitro cell survival experiments to prove that copper-embedded CS had obvious cytotoxic and apoptosis-inducing effects on human breast cancer (MCF-7) cells, in contrast with the lower cytotoxicity on the noncancerous HEK-293T cells.

As a typical inorganic metal nanoparticle, copper also has excellent antibacterial properties. Basumallick et al. treated a CS fibre membrane with copper sulphate in order to enhance its antibacterial potential towards common skin microbes [107]. The results showed that the addition of copper nanoparticles significantly improved the bacteria-inhibiting performance, which was the strongest when the concentration was 0.3 mg/mL. In addition to bacteriostasis, the combination of both can even achieve a sterilisation effect. It was reported that when the copper concentration was 100 μg/mL (1.57 mM), the copper-CS composite material had a lethal effect on both Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*). At the same concentration, pure CS and pure copper showed no antibacterial activity [107]. The antibacterial mechanism for copper nanoparticles is generally believed to hydrolyse the peptidoglycan layer and thus destroy the cellular structure of bacteria [108, 109]. It has also been suggested that this is due to the thiophilic behaviour of copper, which is sufficiently competitive to destroy cytoplasmic iron-sulphur enzymes [110]. Exploration of the underlying mechanism would lead to better optimisation of the bacteriostatic performance.

The addition of copper nanoparticles can also improve the physical and chemical properties of CS. Mishra et al. prepared CS porous scaffolds doped with copper nanoparticles by using a freeze-drying method [111]. With the addition of copper nanoparticles, the porosity increased to a level more suitable for skin tissue engineering. Kumari et al. evaluated the mechanical strength of CS-polyethylene glycol hydrogels with varying Cu concentrations [112]. The results showed that the mechanical stability of the hydrogel increases with increasing Cu concentration without affecting the absorbability of wound exudate.

Copper can directly or indirectly stimulate cytokines or growth factors, which can reduce inflammation, accelerate fibroblast proliferation, angiogenesis, and collagen deposition and thus accelerate wound healing. An inflammatory reaction is a result of antagonism between proinflammatory (e.g., IL-1, TNF-α, and IL-6) and anti-inflammatory (e.g., IL-4, IL-10, and IL-13) agents. Despite the excellent biocompatibility of CS, previous studies have also found a significantly high expression of TNF-α in CS-treated rats at an early healing stage [113]. Declining TNF-α levels were found in rats treated with CS/CuNPs, suggesting that copper reduced the inflammatory response caused by CS. In the meantime, the increase of anti-inflammatory mediators such as IL-10 further supplemented the anti-inflammatory capacity [114]. In other studies, VEGF and TGF-β1 were related to angiogenesis and collagen deposition, respectively, and showed high expression under the influence of CS/CuNPs [115, 116]. Figure 4 shows the effects of copper on various cytokines associated with wound healing [113]. The accelerated wound healing benefits from CS/CuNPs for the regulation of cytokines and growth factors.

It is worth noting that, given the preordained role of copper in vascularisation and its possible association with cancer induction, it is strongly suggested that copper causes malignant lesions of wounds. Lee et al. reported that patients undergoing long-term copper chelation therapy were prone to colon malignancy [117]. Further research is needed to understand the underlying mechanistic relationship between copper and angiogenesis and to address similar issues pertaining to in vivo metabolic processes.

3.3. CS/ZnO. Zinc oxide (ZnO) is a promising metal oxide for NPs with extensive applicability in various fields due to its unique optical and semiconducting properties. Similar to the above MNPs (Ag and Cu), ZnO NPs can also release Zn ions to achieve antibacterial action. In addition, the photocatalytic generation of hydrogen peroxide is another primary antibacterial mechanism available to ZnO NPs. As shown in various studies, an electron-hole pair is formed in ZnO NP semiconductors upon irradiation by light with wavelengths corresponding to an energy >3.2 eV [118]. Positively charged holes and negatively charged electrons are surrounded by water and air on the surface and may produce reactive oxygen species such as the superoxide radical anion ($O_2\bullet-$), hydroxyl radical ($\bullet OH$), and hydrogen peroxide (H_2O_2), which cause lipid peroxidation, protein oxidation, and nucleic acid damage in bacteria. The formation of electron-hole pairs and the production of reactive oxygen species are illustrated in Figure 5. Several studies have evaluated the improvement of antimicrobial activity of CS upon the addition of ZnO. Visnuvinayagam et al. characterised the ZnO-incorporated CS (ZnO-CS) and its antimicrobial activity [119]. In scanning electron microscopy images, ZnO-NPs were observed with nanosheet shapes. In addition, ZnO-treated MRSA and *Pseudomonas aeruginosa* demonstrated cell membrane rupture and cell shrinkage, respectively. CS combined with ZnO has an inhibition zone that is 5–15 mm more than that of CS alone. Yuvaraja et al. used polyvinyl alcohol (PVA) to prepare CS/PVA/ZnO beads as a novel antibacterial agent for wound healing [120]. The hexagonal crystal structure of ZnO was observed by transmission electron microscopy. The antibacterial activity and healing effect of CS/PVA/ZnO in mice skin wounds were significantly better than were those of CS and PVA. The results strongly support the feasibility of the nanocomposite system for applications involving wound healing. However, the effect of particle size on the antibacterial properties of ZnO has been the focus of debate in recent years. da Silva

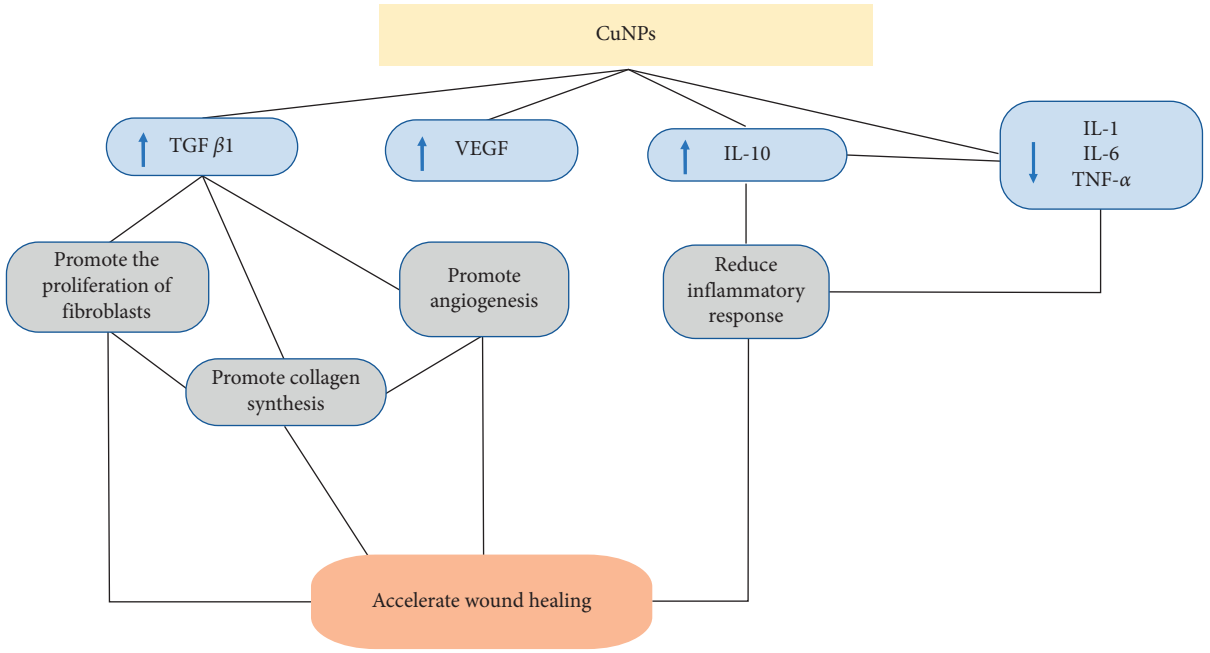


Figure 4: Effects of copper on different cytokines associated with wound healing.

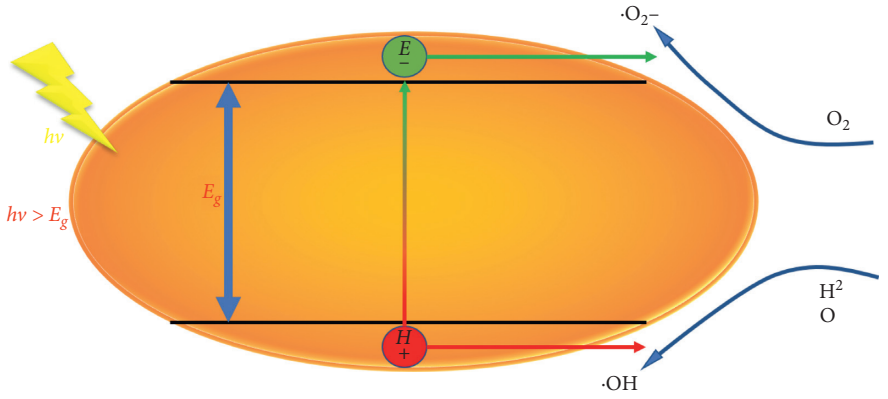


Figure 5: Schematic representation of the energetics of ROS formation.

et al. believe that the bacteriostatic performance is largely affected by the particle size, with smaller particle sizes corresponding to stronger bacteriostatic performance [121]. Stankovic et al., however, prepared ZnO of various sizes and with different surface stabilisers, and the results showed that the bacteriostatic properties were not affected by particle size [122].

In probing the effect of ZnO on the physicochemical properties of CS, Li et al. verified that the addition of 0.1 wt% ZnO significantly enhanced the tensile strength of the CS/hydroxyapatite (HAP) scaffold [123]. Khorasani et al. introduced hydrogels of CS/PVA/ZnO as a wound dressing, which showed improvements in properties such as swelling ratio, water vapour transmission rate, pore size, and mechanical and thermal properties, as compared to hydrogel wound dressings without ZnO [124]. Moreover and compared with other MNPs, ZnO is biocompatible and biodegradable. Kumar et al. developed a microporous CS/ZnO composite bandage via the incorporation of ZnO into a CS hydrogel [125]. Cell viability studies showed that neither the positive control (0.001–0.01% ZnO) nor the CS control group showed cytotoxicity after contact with normal human dermal fibroblast cells (HDF) for 24, 48, or 72 h. Subsequently, in vivo experiments showed that the nanocomposite bandages facilitate epithelialisation and collagen deposition. In order to explore the effect of a higher concentration of ZnO on cytotoxicity, Kumar et al. studied the cell viability of HDF cells [126]. After twenty-four hours of incubation, the results proved that bandages with ZnO concentrations of 0.025 and 0.01% showed 90% viability, whereas bandages with higher concentrations of ZnO (0.05 and 0.1%) showed 50–60% cell viability. Zinc is an essential element for fibroblast proliferation and collagen deposition, and, in several in vivo experiments with CS/ZnO nanocomposites, histopathological features were significantly improved by the presence of ZnO [127–129].

Due to the unique optical and semiconductor properties of ZnO, ultraviolet light (UV) can produce photocatalytic reactions to enhance its bacteriostatic properties. However, clinical studies examining the effects of UV on wound healing are limited. Liu et al. demonstrated that epithelial formation was significantly inhibited under UV irradiation, leading to delayed wound healing [130]. Therefore, the effective use of Cs/ZnO nanocomposites and UV irradiation for wound healing may be a subject of future research.

4. Conclusions

In this review, the addition of various MNPs to CS was discussed as a novel and promising method for developing wound healing applications. It has been reported in many articles that the combination of CS and MNPs performs better than the individual components in wound healing. First, in terms of preparation, CS stabilised the MNPs to a certain extent without adding other cytotoxic reductants (such as sodium citrate; sodium borohydride). In addition, the uncontrolled release of metal ions by MNPs and their potential cytotoxicity can be mitigated by CS encapsulation. At the same time, the modest antibacterial properties of CS and the inferior mechanical strength can be enhanced by the addition of MNPs. Furthermore, in many studies, the acceleration of wound healing is attributed to the promotion of collagen deposition, angiogenesis, and the anti-inflammatory effects of both. In general, the two materials can work together to yield a nanocomposite with biocompatibility, excellent antibacterial activity, and appropriate physical properties to accelerate wound healing.

Although numerous experiments in vitro and in vivo have demonstrated the feasibility of the nanocomposite in promoting wound healing, the implementation of further preclinical and clinical trials remains. It is worth noting that the mechanism of action of MNPs is dose-dependent, and excessive concentration can produce significant cytotoxicity. The load efficiencies and release rates of MNPs vary according to the fabrication process. Therefore, further exploration of dosage and the rational design of preparation methods are essential preconditions for clinical application. Moreover, despite the chemical interactions between CS and MNPs, they are of two functional types; one provides chelation, and the other provides electrostatic interactions. Unfortunately, limited studies have explored these interactions at the molecular level. In order to understand the possible interactions, overexpressed genes or proteins must be thoroughly evaluated. Overall, nanocomposites have a promising future in the field of wound healing and, with ongoing research and optimisation, should ultimately fulfil clinicians' expectations and meet the needs of patients.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Kai Wang wrote the manuscript and prepared the original draft; Chuan Fu curated the data; Su Pan reviewed and edited the manuscript; Xiaoyu Yang supervised the process.

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