

Potential Antimicrobial Applications of Chitosan Nanoparticles (ChNP)

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Polymeric nanoparticles are widely used for drug delivery due to their biodegradability property. Among the wide array of polymers, chitosan has received growing interest among researchers. It was widely used as a vehicle in polymeric nanoparticles for drug targeting. This review explored the current research on the antimicrobial activity of chitosan nanoparticles (ChNP) and the impact on the clinical applications. The antimicrobial activities of ChNP were widely reported against bacteria, fungi, yeasts and algae, in both in vivo and in vitro studies. For pharmaceutical applications, ChNP were used as antimicrobial coating for promoting wound healing, preventing infections and combating the rise of infectious disease. Besides, ChNP also exhibited significant inhibitory activities on foodborne microorganisms, particularly on fruits and vegetables. It is noteworthy that ChNP can be also applied to deliver antimicrobial drugs, which further enhance the efficiency and stability of the antimicrobial agent. The present review addresses the potential antimicrobial applications of ChNP from these few aspects.

Keywords: Chitosan, nanoparticle drug delivery, chitosan nanoparticle, microbial infection

Introduction

Chitosan, a derivative of chitin is a cationic polysaccharide composed of N-acetyl glucosamine and D-glucosamine [1]. Interestingly, chitosan consists of several functional groups which can be modified for numerous applications. Chitosan has been recognized as a versatile biopolymer because of its non-toxicity, biocompatibility, low allergenicity and biodegradability properties [2]. Chitosan is also classified as safe to be used by the US Food and Drug Administration (FDA) for wound dressing applications [3]. However, chitosan is a weak base and it has low solubility in neutral and alkaline media which limits its applications. Nevertheless, this limitation can be overcome by improving the formulation [4]. The most significant characteristic of this biopolymer which makes it able to adhere to the scaffold is the high porosity characteristic [5]. In addition, chitosan is the only biodegradable polymer that exhibits cationic character because of its primary

amino groups [6]. Furthermore, chitosan and chitin have been employed as a base material in nanotechnology application. Besides, chitosan shows significant antimicrobial properties because it binds the negatively charged residues of the bacterial cell wall. Electrostatic force between positively charged chitosan promotes a closer interaction with negatively charged bacteria cell wall, that leads to the penetration of drug through the bacteria cell wall. This is because bacterial cell wall is made up with a layer of peptidoglycan which is rich in negatively charged carboxyl and amino groups [7]. So, the potential of chitosan to gather at the site of infection will increase. Chitosan is also able to alter the electron transport chain of bacterial membranes [8]. This system is responsible to generate electron carriers and builds a proton gradient in the inner membrane of mitochondria, which is important for ATP production [9]. The antimicrobial activity of chitosan depends on the type of chitosan, degree of deacetylation and most importantly, the molecular weight [10]. Other

than that, lower molecular weight chitosan can enter the cell, binds to DNA and inhibits RNA and protein synthesis [9]. Since chitosan could adsorb the electronegative substances in the cell and flocculate them, it disturbs the physiological activities of the microorganism which eventually leads to death of the cells [11].

Drug Delivery

For decades, polymers such as chitosan have been utilized as carriers to provide a safe passage for delivery of bioactive compounds. Due to their good biodegradability and versatility, they can breakdown into non-toxic metabolites that can be eliminated easily [12]. Due to its high porosity, chitosan is also widely used as wound dressing and tissue scaffolding material. Chitosan also exhibits muco-adhesive, permeation-enhancing and efflux pump inhibitory properties [13]. Nanoparticles offer a novel delivery system in order to improve the distribution of drugs in different target routes and overcome the cellular barriers [14]. Chitosan improved the delivery of drugs in therapeutic studies. There are several possible drug release mechanisms of chitosan nanoparticles including diffusion of adsorbed drug, swelling of polymer matrix and erosion of chitosan matrix [11]. Chitosan nanoparticle size and surface profiles can be easily modified either as passive or active drugs targeting the different target routes [9]. In case of drug release, nanoparticles are established to exhibit sustained drug release, and also to reduce toxicity and side effects of drugs on the therapeutics system [12]. The role of chitosan nanoparticles in antimicrobial drug delivery is summarized in Table 1.

There are several common techniques to prepare chitosan-based nanoparticles such as ionic gelation, covalent cross linking, precipitation, polymerization, self-assembly, chitosan-drug complexes and spray-drying [15]. Furthermore, several types of chitosan drug delivery systems are available for different application sites, for example; oral, ocular, nasal, vaginal, buccal, vaccine, parental and intra-vesical drug delivery [16]. Other than that, doxorubicin chitosan-based nanoparticle displayed higher encapsulation efficiency with up to 70% doxorubicin encapsulated [17]. Similarly, another study also obtained a higher encapsulation efficiency of Protein/siRNA in chitosan, compared to other biopolymers [18]. In contrast, an encapsulation efficiency of less than 50% was reported on 5-fluorouracil-loaded chitosan nanoparticles reported

by Sun and colleagues [19]. Nevertheless, the nanoparticles exhibit a sustained-release pattern compared to 5-fluorouracil solution, which can significantly improve the resident time of drug in the human body. Banik *et al.* [20] prepared nanoparticles of chitosan-montmorillonite loaded with isoniazid drug to study the effect of particle size on the release profile. Their results showed that with the decreasing of nanoparticle sizes, the cumulative percentage of drug release was increased but the cytotoxicity also increased. However, the cytotoxicity can be decreased with incorporation of clay into the system. Furthermore, chitosan was utilized for nano-encapsulation of rosemary essential oil by Hussein *et al.* [21]. They reported a significant improvement in thermal stability of rosemary essential oil after the nano-encapsulation process. Chitosan has a suitable matrix for the encapsulation due its cationic characteristic.

Antifungal Activity

Generally, chitosan nanoparticles are effective in inhibiting spore germination and radial mycelial growth [22]. Numerous studies have been conducted on yeast and molds associated with food and plant contamination. Using ionic gelation method, Saharan *et al.* [23] prepared chitosan nanoparticles loaded with copper (Cu) ions to test antifungal efficacy against pathogenic fungi on tomato. The nanoparticles exhibited positive charge (+22.6 mV) zeta potential thus improving stability of particles and enhancing more electrostatic interaction with cell membrane pathogenic fungi. Cota-Arriola *et al.* [24] suggested the efficacy of ChNP antifungal activity on *Aspergillus parasiticus* was associated with particle size and matrix between chitosan and sodium tripolyphosphate. Pilon *et al.* [25] examined the use of chNP as a coating material to control microbial growth on fresh-cut apples. Their results showed that nanoparticles with a diameter of 110 nm were most effective in inhibiting the growth of mesophilic and psychrotrophic microorganisms. Recently, a comparative study was performed by Kheiri *et al.* [26] to evaluate the antifungal activity of chitosan and chNP against *Fusarium graminearum*. In this study, chitosan showed a weaker antifungal activity compared to chNP, which was evidenced by the higher minimal inhibitory concentration 50% (MIC₅₀) value. Due to its small size, chitosan nanoparticles are easily diffused into the cell membrane of microorganisms. Antifungal activities of chitosan with different molecular weights were compared by Hernandez-Lauzardo *et al.* [27]

Table 1. The role of ChNP in antimicrobial drug delivery.

Materials	Preparations	Particle size	Applications/results	References
ChNP loaded with 5-fluorouracil	Ionic gelation method	283.9 ± 5.25 nm	5-fluorouracil-loaded ChNP sustained longer drug release time compared to non-encapsulated drug in vitro and in vivo.	[19]
Chitosan-montmorillonite nanoparticles loaded with isoniazid	Ionic gelation method	282.2 and 451.8 nm	The release of isoniazid increased as the pH of medium decreased. The nanoparticles are suitable as drug carriers in gastric pH.	[20]
ChNP loaded with rosemary essential oil	Homogenization technique	10 – 20 nm	The nano-capsulation process increased the thermal stability of rosemary essential oil with antioxidant potential.	[21]
ChNP	Nanoprecipitation and nanoencapsulation methods	406.6 ± 25.5 nm	ChNP inhibited spore germination and radial mycelial growth on <i>Colletotrichum gloeosporioides</i> and <i>Alternaria</i> species.	[22]
ChNP loaded with copper (Cu)	Ionic gelation method	150.0 nm	In vitro antifungal efficacy of Cu–ChNP showed significant inhibition against pathogenic fungi on tomato.	[23]
ChNP	Iontropic gelation method	80.0 nm to 20.0 µm (depends on concentrations)	ChNP inhibited <i>Aspergillus parasiticus</i> in terms of radial growth, spore germination, and morphological changes.	[24]
ChNP	Polymerizing citric acid in chitosan solution	110.0 nm	ChNP reduced microbial growth on fresh-cut apples, while maintaining the food quality compared to conventional gel coating.	[25]
ChNP	Ionic gelation method	80.9 ± 35.5 nm	ChNP inhibited the growth of <i>Fusarium graminearum</i> by 77.5%.	[26]
ChNP	Ionic gelation method	240 nm	The antibacterial efficiency was affected by molecular weight of chitosan, sonication condition and type of cross linker.	[28]
ChNP loaded with ferrous (Fe ²⁺ , Fe ³⁺)	Ionic gelation method	206.4 and 195.2 nm	ChNP loaded with Fe ²⁺ , Fe ³⁺ showed significantly better antimicrobial activity than metal ions.	[29]
ChNP loaded with gold nanoparticles (CS-Au@MMT/gelatin)	Chitosan as a reducing and stabilizing agent in the synthesis of nanoparticles	8.32 ± 1.97 nm	CS-Au@MMT/gelatin dressing showed significant antibacterial activity against methicillin-resistant <i>Staphylococcus aureus</i> -associated wound infection.	[30]
ChNP	Samples were dissolved in 0.1 M sodium acetate pH 4.0 and irradiated in an ice bath with an ultrasonic probe	Low molecular weight: 425 nm Medium molecular weight: 403 nm	ChNP inhibited the growth of <i>E. coli</i> with same MIC and MBC values.	[31]
ChNP loaded with hybrid copper	Copper nanoparticles were synthesized into a chitosan, starch and ascorbic acid bio-friendly system	131 ± 36 nm	ChNP loaded with hybrid copper exhibited significant antibacterial activity against cariogenic <i>Streptococcus mutans</i> that cause tooth decay.	[32]
ChNP loaded with nisin	Ionic gelation method	86 ± 0.23 nm	Microscopic observation showed that the cell membranes of <i>S. aureus</i> and <i>E. coli</i> were destroyed when treated with ChNP loaded with nisin.	[33]

against *Rhizopus stolonifer* (Ehrenb.:Fr.) Vuill. The results revealed that chitosan with low molecular weight exhibited the most significant inhibition of mycelial growth while

chitosan with high molecular weight disrupted spore shape, sporulation and germination.

Antibacterial Activity

The antibacterial activity of ChNP was documented against foodborne *Escherichia coli* O157:H7. The antibacterial efficiency was affected by the molecular weight of chitosan, sonication condition and type of cross linker [28]. Other than that, chitosan loaded with ferrous and ferric sulphate exhibited better antimicrobial activity against *E. coli* and *Staphylococcus aureus* compared to ChNP [29]. Moreover, in vivo rabbit wound healing model was used to study the efficiency of chitosan-gold nanoparticles blended with gelatin (CS-Au@MMT/gelatin) dressing [30]. They discovered the CS-Au@MMT/gelatin exhibited a significant growth reduction against methicillin-resistant *S. aureus* associated wound infection. In addition, ultrasound irradiation was used to study the antibacterial activity of chitosan nanoparticles on *E. coli* [31]. They discovered that the antibacterial efficiency of ChNP depends on the molecular weight of chitosan and pH of medium at 5.0. The antibacterial mechanisms of ChNPs involve cell wall interfering and penetration into the nuclei of the microorganisms. In the same year, hybrid copper-chitosan nanoparticles prepared by Covarrubias *et al.* [32] were reported to exhibit a significant antibacterial activity against cariogenic *Streptococcus mutans* that cause tooth decay. Microscopic observation showed that the cell membranes of *S. aureus* and *E. coli* were destroyed when treated with chitosan nanoparticles loaded with nisin [33].

In conclusion, ChNPs are one of the most versatile polymers used for the development of antimicrobial chemotherapies in therapeutics study. Based on the review, ChNP exhibited significant antimicrobial activity on a wide spectrum of microorganisms. Recently, in order to achieve the synergistic antimicrobial effect, ChNP was used to deliver various antimicrobial agents. As a sufficiently wide application of ChNP is foreseen, fundamental studies should be carried out to investigate the antimicrobial mode of action as well as the synergism mechanisms of ChNP.

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Conflict of Interest

The authors have no financial conflicts of interest to declare.

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