Antibacterial Activity of Chitosan-Based Systems

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Abstract

Chitosan and its derivatives can be called environmental purification functional materials as they can effectively control the growth and reproduction of hazardous bacteria and also control toxic pollutants. From the basic science to the latest developments and innovations, starting with the history of the material, this chapter presents a facile way to understand the antibacterial activity of the chitosan, together with other materials, to the reader. This chapter also summarizes the general developments in the study of antimicrobial applications. In the light of the current situation of the research and the progress in the related fields, this chapter discusses the differences among influencing factors in detail and compares the antimicrobial activity between different physical states of chitosan. Also, this chaper discusses the more recent processes and applications.

Keywords

Chitosan · Antibacterial materials · Antibacterial mechanism · Affecting factors

15.1 Introduction

Antibacterial and antimicrobial agents and its disinfected systems are becoming important day by day. They have been studied for possible use in a variety of healthcare environments, industries, laboratories, and even houses (Ali et al. 2015) (Hosseinnejad and Jafari 2016). Most importantly, the use of these materials is to sterilize medical environments and equipments in order to prevent thousands of deaths due to hospital-acquired infections, such as linens and clothing where bacteria

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could grow and infect the human body. Therefore, a suitable environment should be provided in order not to let infectious diseases spread from fungi or viruses (Yılmaz Atay and Çelik 2017).

According to the Centers for Disease Control and Prevention (CDC), each year in the United States, 48 million people get sick, 128,000 are hospitalized, and 3000 die due to foodborne diseases. Therefore, ensuring microbiological safety of the products, while maintaining their nutritional and organoleptic properties, is still a priority nowadays (Severino et al. 2014). Another option is the use of antimicrobial packaging to provide an increased margin of safety and quality. This packaging may prevent the growth of microorganisms on the product's surface and, hence, lead to an extension of its shelf life (Cha and Chinnan 2004). Therefore, it can be said that antibacterial and antimicrobial systems are highly important not only in the hospital and healthcare environments but also for laboratory, home, marine and some industrial applications (Hosseinnejad and Jafari 2016; Lin et al. 2015).

15.2 Antibacterial Materials

Antibacterial materials are being developed to prevent harmful bacteria and viruses from spreading (Chang et al. 2015). These materials can effectively control the growth and reproduction of hazardous bacteria and also control toxic pollutants (Claesson and Ninham 1992). They contain an antimicrobial agent that inhibits the ability of microorganisms to grow in the material. Such materials are becoming more widely investigated for possible use in various settings including clinics, industry and even the home. The most common and important use of antimicrobial coatings has been in the healthcare setting for sterilization of medical devices to prevent hospital-associated infections, which have accounted for almost 100,000 deaths in the United States. In addition to medical devices, linens and clothing can provide a suitable environment for many bacteria, fungi and viruses to grow when in contact with the human body which allows for the transmission of infectious disease (Chapter 26: Microbial Growth Control 2018).

Antibacterial materials could be classified into two groups: inorganic and organic materials. Inorganics are metals, metal oxides and metal phosphates (Tuncer 2007). Among the inorganic materials, metal oxides such as TiO2, ZnO, MgO and CaO are of particular interest as they are not only stable under harsh process conditions but also generally regarded as safe materials to human beings and animals (Teli and Kale 2011). Organics are phenols, halogenated compounds and quaternary ammonium salts; in recent years, studies about antibacterial materials have been focused on natural materials, such as chitosan (CTS) and chitin (Hosseinnejad and Jafari 2016).

Ren et al. (Chen and Chou 2005) characterized and investigated copper oxide (CuO) nanoparticles with respect to potential antimicrobial applications. They generated nanoscaled CuO by thermal plasma technology. CuO nanoparticles were effective in killing a range of bacterial pathogens involved in hospital-acquired infections. In addition, Cioffi et al. (Chien et al. 2015) manufactured polymer composites including copper nanoparticles with antifungal and bacteriostatic

properties. Polymer/metal nanocomposites are a viable choice, as a spinnable coating capable of releasing metal species to a broth of living organisms in a controlled manner is an extremely interesting material for a number of biotechnological applications. At their study, a polymer-based nanocomposite loading stabilized copper nanoparticles is proposed as a biostatic coating, and systematic correlations between material properties and biological effects are established. Researchers in Japan (Chung et al. 2004) have discovered a nickel-alloy coating with antibacterial properties, which is believed to be effective at reducing the SARS coronavirus. SARS is believed to be spread through close contact, such as coughing, sneezing or contact with faeces of patients. It can also be transmitted when people touch surfaces contaminated with the virus. The use of this antibacterial material has the potential to radically alter the quality and cleanliness of laboratory and pharmaceutical workplaces. In laboratory tests, the nickel-alloy coating developed by Kobe Steel reduced the growth of mouse hepatitis virus (MHV, or mouse coronavirus), which is a close relative of the SARS (severe acute respiratory syndrome) coronavirus (CoV). Both MHV and SARS CoV are in the same group of coronaviruses (Chung et al. 2004; Yilmaz Atay 2013).

In our previous study, silver-supported materials and titanium dioxide photocatalyst materials were investigated as inorganic environmental purification functional materials by applying an in vitro test. The bactericidal activity for these bacterial cells was estimated by zone of inhibition on the nutrient agar plates. Zone of inhibition is produced by the silver-loaded polymer coating against the bacteria representing its antibacterial effect. All Ag nanoparticle-reinforced polymer composites showed a good inhibition zone for *S. aureus* and *E. coli*. This antibacterial activity of silver is thought that it results from the interaction of silver and thiol groups in bacteria proteins (Yılmaz Atay et al. 2015).

Another material we work on this area is an active biomolecule: chitosan [poly-(b-1/4)-2-amino-2-deoxy-D-glucopyranose]. Recently, the research on this material has been increased dramatically because of its great potential for a wide range of applications. Due to its biodegradability, biocompatibility, antimicrobial activity, non-toxicity and versatile chemical and physical properties, chitosan has a significant role in food application area in view of recent outbreaks of contaminations associated with food products as well as growing concerns regarding the negative environmental impact of packaging materials currently in use. Chitosan-based polymeric materials can be formed into fibres, films, gels, sponges, beads or even nanoparticles (Chung et al. 2003; Yilmaz Atay 2013). In the following sections, the antibacterial properties and applications of chitosan will be analysed thoroughly.

15.2.1 Antibacterial Activity of Chitosan-Based System

One of the most investigated properties of chitosan is its antimicrobial effect embracing from biomedical to cosmetic and from food to agriculture applications. To make use of the antimicrobial activity of chitosan together with its peculiar features in order to produce self-preserving materials, many studies have been

conducted up until now. This has led to the design of a large range of products containing chitosan as beads, films, fibres, membranes and hydrogels that are intended for various uses (Perinelli et al. 2018).

15.2.2 History

Chitosan has been investigated as an antimicrobial material against a wide range of target organisms like algae, bacteria, yeasts and fungi in experiments involving in vivo and in vitro interactions with chitosan in different forms (solutions, films and composites) (Kong et al. 2010). Ever since the broad-spectrum antibacterial activity of this material was first proposed by Allan and Hardwiger (Allan and Hardwiger 1979), along with great commercial potential, the antimicrobial property of chitosan and its derivatives have been attracting great attention from researchers. Investigation of the antimicrobial properties of chitosan has been a long journey of scientific exploration and technological development. The journey began two decades ago, with studies on the biological phenomena arising from foodborne and soilborne pathogenic fungi in the food and agriculture industries (Rabea et al. 2003). In light of their intimate relationship with human activities, bacteria rightly began to receive more attention in the search for efficacious antimicrobials. The studies at that time were typically carried out via chemical, biochemical, microbiological and medical assays of chitosan and its derivatives. In some cases, but rarely so, molecular and cell approaches were utilized. The outcomes obtained through this period suggested that antimicrobial activities of chitosan and its derivatives relied on numerous intrinsic and extrinsic factors, such as pH and molecular weight (Mw). Some basic hypotheses about underlying antimicrobial mechanisms were also proposed (Zivanovic et al. 2004). Based on the outcomes, various antimicrobial agents based on chitosan or its derivatives emerged. At the same time, since biocide-resistant bacteria and fungi, growing public health awareness of pathogenic microorganism raised demands for safe and efficacious agents that were less prone to stimulating development of resistance. In addition to tremendous advancements in molecular biological, pharmaceutical, cell biological technologies and detecting methods, nanotechnology emerged and began playing an extraordinary role, carrying the potential to extend antimicrobial treatment to the atomic level. The many approaches that have been used in studying antimicrobial activities of chitosan and its derivatives have given rise to various physical forms of chitosan in differing methods, from the original solution applied in agriculture to film structure in food sector and to ubiquitous pharmaceutical nanostructure materials (Kong et al. 2010).

15.2.3 Sources of Chitosan

Chitosan is a natural antimicrobial agent found in the shells of crustaceans, such as crab, shrimp, squid pen and crawfish (No et al. 2002). Recently, some studies have

pointed to the possibility of chitosan production from fungi. In one study, chitosan was extracted from cell wall of filamentous fungus, *R. oryzae*, by Jeihanipour et al. (Jeihanipour et al. 2007), and its antimicrobial properties were studied against *E. coli*, *K. pneumoniae* and *S. aureus* (Hosseinnejad and Jafari 2016).

15.2.4 Water Soluble

Although chitin and chitosan have been confirmed as attractive biomacromolecules with relevant antimicrobial properties, applications are somewhat limited due to both being water insoluble. Water-soluble chitosan derivatives can be obtained by the introduction of permanent positive charges in the polymer chains, resulting in a cationic polyelectrolyte characteristic independently of the pH of the aqueous medium. This can be accomplished, for instance, by the quaternization of the nitrogen atoms of the amino groups (Goy et al. 2009).

15.2.5 Derivatives of Chitosan

Due to its unique polycationic nature, chitosan and its derivatives have been recommended for applications in agriculture, food, biomedical, biotechnology and pharmaceutical fields. However, the antibacterial functions of chitosan are limited because amino groups on chitosan backbone can only function as relatively weak positive charge centres. To improve the antimicrobial activity of chitosan, it is reasonable to enhance the strength of positive charges on the chitosan molecules by endowing it with some more positively charged groups (Xiao et al. 2011). Therefore, in the past two decades, extensive investigations have been carried out to increase solubility of chitosan in water and broaden its applications by preparing functional derivatives of chitosan such as carboxymethyl chitosan and quaternized carboxymethyl chitosan, chitosan-N-arginine by reacting amino groups of chitosan with arginine, N-alkylated disaccharide chitosan, water-soluble maltose chitosan derivative and water-soluble quaternary. Chitosan derivatives are obtained by N-acylation with betaine and water-soluble oligochitosans (Hosseinnejad and Jafari 2016).

15.2.6 Degree of Deacetylation

Chitosan is produced commercially by deacetylation of chitin. In the process of deacetylation, acetyl groups from the molecular chain of chitin are removed to form amino groups. The degree of +, Mg-2 + 2 deacetylation, which determines the content of free amino groups in polysaccharides, can be employed to differentiate between chitin and chitosan. It is very well known that the degree of deacetylation is one of the most important chemical characteristics, which could influence the performance of chitosan in many applications (Hosseinnejad and Jafari 2016).

15.3 Mechanism of Antibacterial Activity

The exact mechanism of antibacterial activity is yet to be fully understood. It is known that chitosan's antimicrobial activity is influenced by a number of factors that act in an orderly and independent fashion. The most prevalent proposed antibacterial activity of chitosan is by binding to the negatively charged bacterial cell wall causing disruption of the cell, thus altering the membrane permeability, followed by attachment to DNA causing inhibition of DNA replication and subsequently cell death (Nagy et al. 2011). Another possible mechanism is that chitosan acts as a chelating agent that electively binds to trace metal elements causing toxin production and inhibiting microbial growth (Divya et al. 2017).

The polycationic structure of chitosan is a prerequisite for antibacterial activity. As environmental pH is below the pKa of chitosan and its derivatives, electrostatic interaction between the polycationic structure and the predominantly anionic components of the microorganisms' surface (such as Gram-negative lipopolysaccharide and cell surface proteins) plays a primary role in antibacterial activity (Kong et al. 2010).

The polycationic structure forms unnecessarily in acidic conditions, because the grafted groups of specific derivatives may change the pKa of chitosan and cause protonation at higher pH value (Yang et al. 2005). When the positive charge density of chitosan strengthens, the antibacterial property will increase consequently, as is the case with quaternized chitosan and chitosan metal complex (Xie et al. 2007). On the contrary, if the polycationic property of chitosan is deprived or reversed, the corresponding antibacterial capacity will be weakened or lost. Besides protonation, the number of amino groups linking to C-2 on chitosan backbones is important in electrostatic interaction. Large amount of amino groups are able to enhance the antibacterial activity. Accordingly, native chitosan with higher DD shows a stronger inhibitory effect than a molecule with a lower DD. Moreover, it has been reported that asparagine N-conjugated chitosan oligosaccharide that possesses two positively charged sites provides strong interaction with carboxyl-negative charges on the bacterial cell wall (Jeon et al. 2001). Another attempt to increase the amount of amino groups via substituting amino by formamidine obtained a guanidinylated chitosan, which showed better antibacterial activity than chitosan (Hu et al. 2007a, b).

HMw water-soluble chitosan and solid chitosan including larger size nanoparticles interact with cell surface instead and alter cell permeability resultingly (Leuba and Stossel 1985) or form an impermeable layer around the cell, thus blocking the transport of essential solutes into the cell. Experiments conducted with *E. coli* treated with CM and oleoyl-chitosan nanoparticles (OCNP) have revealed that the same microbial species can display significant differences in mode of action depending on the two different dimensions of chitosan particles (Xing et al. 2009).

As shown in Fig. 15.1, the cells located on the surface of chitosan microsphere showed various states: some were intact, some were leaking intracellular substances, and some had already ruptured leaving only the membrane. These results are consistent with the idea that CMs kill bacteria through an interfacial contacting

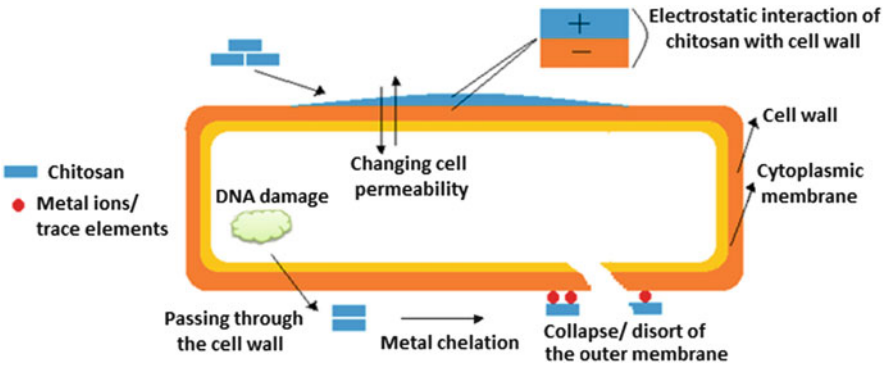


Fig. 15.1 Schematic representation of antimicrobial mechanisms of chitosan and its derivatives (Hosseinnejad and Jafari 2016)

inhibitory effect that occurs on the surface of the microspheres (Kong et al. 2008). Chitosan undergoes a surface-to-surface and local reaction mode, rather than a thorough contacting mode that occurs in liquid state (Hosseinnejad and Jafari 2016).

Similar to bacteria, the chitosan activity against fungus is assumed to be fungistatic rather than fungicidal with a potential to communicate regulatory changes in both the host and fungus. Generally chitosan has been reported as being very effective in inhibiting spore germination, germ tube elongation and radial growth. Most of the studies have been done on yeasts and moulds associated with food and plant spoilage. For these, in the presence of chitosan, several biological processes are activated in plant tissue, where chitinases are induced with action on biotrophic and necrotrophic mycoparasites, entomopathogenic fungi and vesicular arbuscular mycorrhizal fungi (Ghaouth et al. 1992; Goy et al. 2009).

15.4 Factors Affecting Antibacterial Property

Variations in chitosan's bactericidal efficacy arise from various factors. Several properties are reviewed in Fig. 15.2. They are explained in detail as follows:

15.4.1 Concentration of Chitosan

At lower concentrations, chitosan binds to the negatively charged cell surface, disturbs the cell membrane and causes death of the cell by inducing leakage of intracellular components, whereas, at higher concentrations, the protonated chitosan may coat the cell surface and prevent the leakage of intracellular components. In addition, the positively charged bacterial cells repel each other and prevent agglutination (Lim and Hudson 2004). An antimicrobial packaging material was prepared

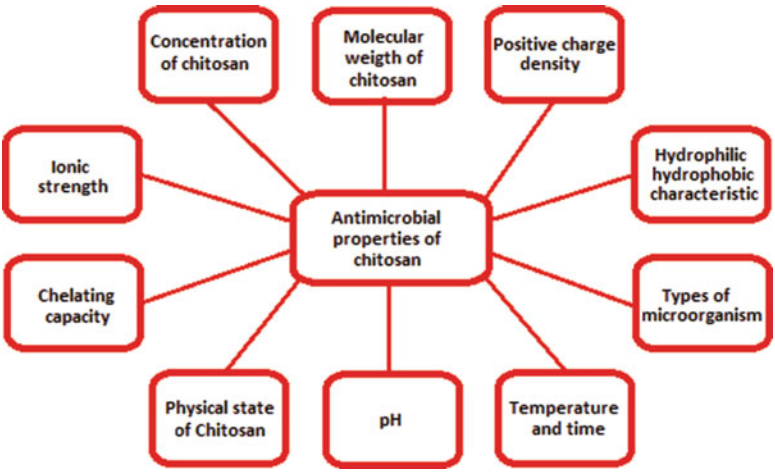


Fig. 15.2 Factors affecting antibacterial property of chitosan

by uniformly embedding 1, 3 and 5% chitosan (w/w) in low-density polyethylene (LDPE) matrix (Reesha et al. 2015). The antimicrobial assay against *E. coli* proved that LDPE/chitosan composite (LDPE/CS) films were highly efficient than virgin LDPE films. Virgin LDPE and 1%, 3% and 5% LDPE/CS films tested as packaging films for chill-stored tilapia showed that samples packed in LDPE films were rejected by seventh day, whereas fish packed in 1%, 3% and 5% LDPE/CS films remained acceptable up to 15 days. This study revealed that 3% LDPE/CS films had a better physical and antimicrobial property and enhanced the keeping quality of tilapia steaks during chilled storage when compared to the other films (Hosseinnejad and Jafari 2016).

15.4.2 Molecular Weight

Numerous studies on bactericidal activity of chitosan have generated equivocal results concerning correlation between bactericidal activity and chitosan Mw. Some studies reported increasing chitosan Mw leads to decreasing chitosan activity against *E. coli*, while in other studies high Mw (HMw) chitosan displayed greater activity than low Mw (LMw) chitosan. In addition, activities still were found to be equal against *E. coli* and *Bacillus subtilis* regardless of Mw (Tikhonov et al. 2006). Even though the limited available results on bactericidal activity of LMw chitosan were comparable depending on bacterial strains, conditions of biological testing and respective chitosan Mw, the results are not accordant with each other. For instance, 9.3 kDa chitosan inhibits growth of *E. coli*, while 2.2 kDa chitosan promotes its growth. Also, LMw chitosan (4.6 kDa) and its derivative showed better activity against bacteria, yeast and fungi (Kong et al. 2010).

15.4.3 Positive Charge Density

Tremendous literatures support the importance of polycationic structure in antimicrobial activity. A higher positive charge density leads to strong electrostatic interaction. Therein, the positive charge is associated with DD or degree of substitution (DS) of chitosan or its derivatives, which affect positive charge density. To some extent, chitosan microspheres with a high DD (97.5%) lead to higher positive charge density, which confers stronger antibacterial activity than moderate DD (83.7%) against *Staphylococcus aureus* at pH 5.5 (Kong et al. 2008). One study reported that a higher DD with more positive charge was especially successful in inhibiting the growth of *S. aureus*, suggesting that antibacterial activity of chitosan towards *S. aureus* was enhanced with increasing DD (Takahashia et al. 2008). Concerning chitosan derivatives, antimicrobial activity mostly depends on DS of the grafting groups. Investigation of the antibacterial activities of water-soluble N-alkylated disaccharide chitosan derivatives against *Escherichia coli* and *S. aureus* revealed that the antibacterial activity of chitosan derivatives is affected by the DS of disaccharides and the type of disaccharide present in the molecule (Yang et al. 2005). The same study suggested that, irrespective of the kind of disaccharide linked to the chitosan molecule, a DS of 30–40%, in general, produced the most pronounced antibacterial activity against *E. coli* and *S. aureus* and that both microorganisms were most susceptible to cellobiose chitosan derivative DS 30–40% and maltose chitosan derivative DS 30–40%, respectively, among the various chitosan derivatives examined (Kong et al. 2010).

15.4.4 Hydrophilic/Hydrophobic Characteristic

Irrespective of their form or quantity, antimicrobial agents typically require water for activity. Totally dry samples are virtually incapable of releasing their energy stored in chemical bonds to initiate interaction. Hydrophilicity and hydrophobicity are conceptions also based upon water ambience, upon which the manner of antimicrobial interaction of chitosan is determined. The hydrophilic characteristics of chitosan profoundly determine water solubility. The use of chitosan is limited by the compound's poor solubility in water (Dutta et al. 2004). Chemical modifications as an approach are efficient in improving the water solubility of chitosan and its derivatives and widening their applications (Xie et al. 2007). The creation of water-soluble chitosan and its derivatives has been a central goal of investigations of antimicrobial activity, which have included saccharization, alkylation, acylation, quaternization and metallization. As one example, quaternary ammonium chitosan can be prepared by introducing quaternary ammonium group on dissociative hydroxyl or amino group. For chitosan and its derivatives, the hydrophilic-lipophilic variation influences the antimicrobial properties. The hydrophobic characteristic of N-acylated chitosan can be favourable for the interaction of polymer molecule and bacterial cell, where the hydrophobicity of NHCS0.5 (N-hexanoyl chitosans, corresponding to a molar ratio of 0.5 compared with chitosan residue) is likely to

be a contributing factor for its enhanced inhibitory effect (Hu et al. 2007a, b). In another study, the presence of a long aliphatic chain facilitated the absorption and enhanced the effect of a substituted LMW chitosan, N−/2(3)-(dodec-2enyl) succinoyl/chitosans, onto cell walls via hydrophobic interaction with cell wall proteins (Kong et al. 2010).

15.4.5 Chelating Capacity

Chitosan possesses high chelating capacity for various metal ions (including Ni2+, Zn2+, Co2+, Fe2+, Mg2+ and Cu2+) in acid conditions, and it has been widely applied for the removal or recovery of metal ions in different industries (Kurita 1998). Metal ions that combine with cell wall molecules of microorganism are crucial for stability of the cell wall. Chitosan-mediated chelation of such metal ions has often been implicated as a possible mode of antimicrobial action (Rabea et al. 2003). Not only does chelation play a part in acid condition, it is also able to combine divalent metal ions in neutral condition. Additionally, via chelating capacity, chitosan metal complex is prepared and exerts strong antimicrobial activity (Kong et al. 2010).

15.4.6 pH

The antimicrobial activity of chitosan is pH dependent. Because chitosan is soluble in an acidic environment, and the molecule becomes polycationic as pH below the molecule's pKa (6.3–6.5) (Lim and Hudson 2004). It has been reported that chitosan displayed antibacterial activity only in an acid environment, as is not proven to be strictly correct. Chitosan definitely shows stronger inhibitory effect at lower pHs, with inhibitory activity weakening with increasing pH. The failure of chitosan to remain bactericidal at pH 7 may be due to the presence of a large majority of positively uncharged amino groups as well as poor solubility of chitosan (Sudarshan et al. 1992). However, chitosan and its derivatives completely lose their antimicrobial activities under neutral condition as reported by some workers which may not be totally correct. A novel approach of antibacterial research, chitosan microsphere (CM) in solid dispersing system, showed that CM sample with DD of 62.6% exerted inhibitory effect uniquely among the three DD (97.5, 83.5, 62.6%) under neutral condition (Kong et al. 2008). The CM samples in this experiment retained the properties of native chitosan without alteration. Another research observed that antibacterial activity of the N-alkylated chitosan derivatives (DS 30–40%) against *E. coli* increased as the pH increased from 5.0 and reached a maximum around the pH of 7.0–7.5 (Yang et al. 2005). These results also verify that positive charge on the amino groups is not the sole factor resulting in antimicrobial activities. However, little is known about the antimicrobial activity of chitosan under alkaline conditions (Kong et al. 2010).

15.4.7 Ionic Strength

Alternation of the ionic strength in a medium may disturb the inhibitory activity of chitosan, probably caused by two mechanisms. First, increase of metal ions, especially divalent ions, could attenuate the effective chelating capacity of chitosan. With the addition of 0.05 mol/L magnesium ions into a medium, the inhibitory ratio of chitosan samples decreased badly and resulted in abrogated antibacterial activity (Kong et al. 2008). In another study, 10 and 25 mM concentrations of divalent cations reduced the antibacterial activity of shrimp chitosan against *E. coli* in the order of Ba. Furthermore, the addition of Zn2+ and Ca2+ ions inhibited the antibacterial activity of chitosan most effectively compared with Ba2+, Ca2+, Mg2 + 2+ and Mg ions (Chung et al. 2005). Secondly, along with polycationic chitosan, existing cations in medium may interact competitively with the negative components dominating on the cell wall of bacterium, consequently weakening the antimicrobial activity. Addition of anion affected the antibacterial efficacy as well (Kong et al. 2010).

15.4.8 Physical State

Antimicrobial activity of chitosan is the result of series of reactions, rather than the cause of the reactions. The reactions take place between molecules of chitosan and cell wall. Morphology of molecules is responsible for the reactions effciency. Equally, physical state of chitosan, upon which the existing morphology of molecules depends, acts a decisive role in its antimicrobial activity. Nonetheless, scant focus has been paid to the influence of different physical state (Kong et al. 2010).

15.4.8.1 Antimicrobial Activity in Soluble State

Soluble chitosan existing as a disassociating form in solution has an extending conformation, which enables reaction with the counterparts to a sufficient degree and brings the potential to full play. This explains why soluble chitosan and its derivatives are more effective in inhibiting bacterial growth. According to the literatures (Chung et al. 2005; Xie et al. 2007), the minimal inhibitory concentration (MIC) of chitosan derivatives is significantly decreased against all tested bacteria than those of native chitosan. Meanwhile, owing to a sufficient touch with solution, soluble chitosan and its derivatives are readily affected by outer environmental factors as well as many intrinsic factors. In one study, chitosan derivatives (chitosan and maltose, glucose, fructose, glucosamine) produced through the Maillard reaction enhanced the solubility of the native chitosan. Among them, chitosan-glucosamine derivative appeared to be more effective than other chitosan or chitosan derivatives as a natural bactericidal agent (Chung et al. 2005). Quaternary ammonium chitosan is another major example to improve solubility of chitosan by introducing hydrophilic groups into molecule. After quaternization, derivatives exhibited better water solubility and stronger antibacterial activity as compared to chitosan (Xie et al. 2007; Kong et al. 2010).

15.4.8.2 Antimicrobial Activity in Solid State

Compared with soluble chitosan, rather than the extending conformation contact to solution, solid chitosan only gets into touch with solution through surface, such as fibres, membrane, hydrogels, microspheres and nanoparticles. Hydrogels can be formed by covalently cross-linking chitosan with itself. Recently, many attempts have been made to create chitosan particulate systems that could form dispersion in solution with considerable reactive surface area. The shift of physical state is sure to bring variation of its antimicrobial efficiency. Nanoparticles have less inhibition effect on *S. aureus* ATCC 29737 than the polymers in free soluble form since nanoparticles have less positive charge available to bind to the negative bacterial cell wall (Sadeghi et al. 2008). Conversely, another research reported that chitosan nanoparticles exhibit higher antibacterial activity than chitosan on account of the special character of the nanoparticles, likely the nanoparticle's larger surface area and higher affinity with bacterial cells, which yields a quantum-size effect (Kong et al. 2010).

15.4.9 Temperature and Time

For commercial applications, it would be practical to prepare chitosan solutions in bulk and to store them for further use. During storage, specific characteristics of chitosan, viscosity or Mw might be altered. Therefore, altered viscosity of a chitosan solution must be monitored since it may influence other functional properties of the solution. Stability of chitosan (Mw of 2025 and 1110 kDa) solutions and their antibacterial activity against Gram-positive (*Listeria monocytogenes* and *S. aureus*) and Gram-negative (*Salmonella enteritidis* and *E. coli*) bacteria were investigated at 4 °C and 25 °C after 15-week storage (No et al. 2006). Generally, chitosan solutions before storage showed higher antibacterial activity than those after 15-week storage. Chitosan solutions stored at 25 °C possessed parallel or weaker antibacterial activity compared with those at 4 °C. In one study, the susceptibility of *E. coli* to chitosan increased upon increasing temperature from 4 to 37 °C (Tsai and Su 1999), suggesting the low temperature stress was capable of changing the cell surface structure in a way that decreased the number of surface binding sites (or electronegativity) for chitosan derivatives (Kong et al. 2010).

15.4.10 Microbial Factors

15.4.10.1 Microbial Species

Although owning a broad spectrum of antimicrobial activity, chitosan exhibits differing inhibitory efficiency against different fungi, Gram-positive and Gram-negative bacteria. Chitosan exerts an antifungal effect by suppressing sporulation and spore germination (Hernandez-Lauzardo et al. 2008). In contrast, the mode of antibacterial activity is a complicating process that differs between Gram-positive and Gram-negative bacteria due to different cell surface characteristics. In several studies, stronger antibacterial activity was apparent against Gram-negative bacteria

than Gram-positive bacteria (Chung et al. 2004; No et al. 2002), while in another study Gram-positive bacteria were more susceptible, perhaps as a consequence of the Gram-negative outer membrane barrier. Still many workers demonstrated that there were no significant differences observed between the antibacterial activities and the bacterium. Various initial reaction materials and conditions contribute to the diverse consequences. Based on the available evidences, bacteria appear to be generally less sensitive to the antimicrobial action of chitosan than fungi. The antifungal activity of chitosan is greater at lower pH values (Roller and Covill 1999; Kong et al. 2010).

15.4.10.2 Part of Microorganism

Gram-negative bacteria possess an outer membrane (OM) that contains lipopolysaccharide (LPS), which provides the bacterium with a hydrophilic surface. The lipid components and the inner core of the LPS molecules contain anionic groups (phosphate, carboxyl), which contribute to the stability of the LPS layer through electrostatic interactions with divalent cations, demonstrated in Fig. 15.3 (Helander et al. 1997). Removal of these cations by chelating agents such as ethylenediaminetetraacetic acid results in destabilization of the OM through the release of LPS molecules. The OM serves as a penetration barrier against macromolecules and hydrophobic compounds; thus Gram-negative bacteria are relatively resistant to hydrophobic antibiotics and toxic drugs. Therefore, overcoming the OM is a prerequisite for any material to exert bactericidal activity towards Gram-negative bacteria (Kong et al. 2008).

The cell wall of Gram-positive bacteria comprises peptidoglycan (PG) and teichoic acid (TA) shown in Fig. 15.4. TA is an essential polyanionic polymer of the cell wall of Gram-positive bacteria, traversing the wall to contact with the PG layer. They can be either covalently linked to N-acetylmuramic acid of the peptidoglycan layer (wall teichoic acids) or anchored into the outer leaflet of the cytoplasmic membrane via a glycolipid (lipoteichoic acids, LTA) (Raafat et al. 2008). Poly

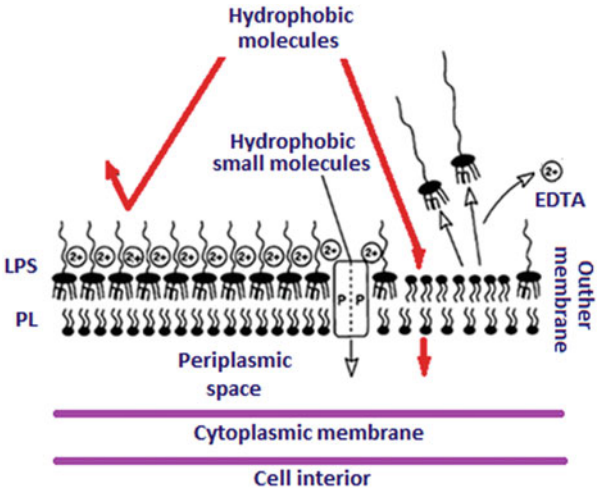


Fig. 15.3 Schematic view of the Gram-negative bacterial cell envelope. Data is based on Helander et al. (1997) and Kong et al. (2010)

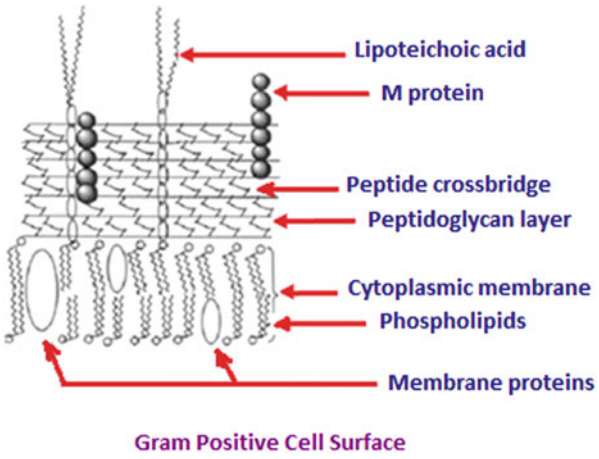


Fig. 15.4 Schematic view of Gram-positive bacterial cell wall (Kong et al. 2010)

(glycerol phosphate) anion groups make TA responsible for structural stability of cell wall. Besides, it is crucial for the function of various membrane-bound enzymes. Comparatively, TA's counterpart LPS acts similarly in the cell wall of Gram-negative bacteria (Kong et al. 2010).

15.4.10.3 Cell Age

For a given microbial species, age of the cell can influence antimicrobial efficiency. For example, *S. aureus* CCRC 12657 in late exponential phase are most susceptible to lactose chitosan derivative with no viability evident after 10 h of incubation. Meanwhile, a relatively less population reduction in viable cells of 3.75 and 3.96 log cfu/mL, respectively, was observed with cells in the mid-exponential and late stationary phases (Chen and Chou 2005).

It is suggested that the differences of cell surface electronic negativity vary with the phase of growth, which can lead to the differences in the susceptibility of cells towards chitosan. In contrast, *E. coli* O157:H7 in mid-exponential phase were the most susceptible, while stationary phase cells were the least susceptible to maltose chitosan derivative (Yang et al. 2007). The discrepancies were attributable to the different microorganisms examined, since the surface charge of microbial cells also varied with the microorganism (Kong et al. 2010).

15.5 Complexes of Chitosan with Certain Materials

In order to improve antimicrobial activity, complexes of chitosan with certain materials can be prepared. Incorporation of essential oils (EOs) in chitosan-based coatings has gained interest in the agricultural sciences owing to the bactericidal and fungicidal properties associated with these volatile compounds (Ali et al. 2015). Recently, different EOs, such as clove, carvacrol, oregano and lemongrass, have been successfully incorporated into chitosan showing strong antimicrobial activity

against a wide range of microorganisms. Also Ojagh et al. (Ojagh et al. 2010) showed that a unique compatibility can be achieved between chitosan and cinnamon EOs; their incorporation improved the antibacterial properties of chitosan. Films containing cinnamon EOs are useful for coating of highly perishable foods such as fish and poultry. In a further experiment by Gómez-Estaca et al. (2010), a complex of gelatin-chitosan film incorporating clove EOs was applied to fish during chilled storage. Results of this study revealed that clove film delayed or even prevented both the growth of microorganisms and the occurrence of total volatile nitrogen. Therefore film incorporating clove EOs could assure an extended shelf-life for chill-stored fish. Generally, the structure of chitosan/metal complexes depends on chitosan/metal ion molar ratio, type of metal ion, molecular weight and deacetylation of chitosan as well as the preparation conditions (Hosseinnejad and Jafari 2016).

15.5.1 Antimicrobial Activity of Chitosan Nanoparticles Loaded with Antibiotics or Other Microbicidal Substances

Chitosan was employed as nanocarrier for the delivery of both synthetic and natural substances in order to potentiate or modulate their antimicrobial activity. These substances depicted in Fig. 15.5 include antibiotics, antimicrobial peptides (AMP), natural compounds and proteins. In particular, chitosan nanoparticles were employed to improve the internalization of the antibiotics into cells infected by intracellular bacteria or to increase their efficacy against multiresistant microorganism. Zaki et al. demonstrated the cellular uptake of ceftriaxone sodium encapsulated in chitosan nanoparticles in Caco-2 and J774.2 (macrophages) cells and the higher intracellular antibacterial effect of these nanoparticles against *S. typhimurium* compared to the drug in solution (Zaki and Hafez 2012). A similar study was also performed using tetracycline-loaded O-carboxymethyl chitosan nanoparticles, and, in this case, the drug-loaded chitosan nanoparticles were found to enhance the efficacy of the antibiotics against intracellular infections caused by *S. aureus* (Maya et al. 2012). Jamil et al. evaluated the efficacy of cefazolin-loaded chitosan nanoparticles against multiresistant Gram-negative bacteria such as *E. coli*, *K. pneumoniae* and *P. aeruginosa*. The drug-loaded chitosan nanoparticles showed antibacterial activity against the three microorganisms, as determined by agar well discussion method and microdilution broth assay, greater than cefazolin in solution. Similarly, the efficacy of drug-loaded chitosan nanoparticles against antibiotic-resistant bacterial strains was also demonstrated for vancomycin against drug-resistant *S. aureus*. Recently, peptides and proteins with antimicrobial activity were encapsulated in chitosan nanoparticles. Among these molecules, lysozyme has received attention for its application as preservative in food products and pharmaceuticals (Wu et al. 2017), while the amphiphilic peptide temporin B has shown a strong and fast killing ability, especially against Gram-positive, multidrug-resistant nosocomial bacterial species (Mangoni et al. 2008).

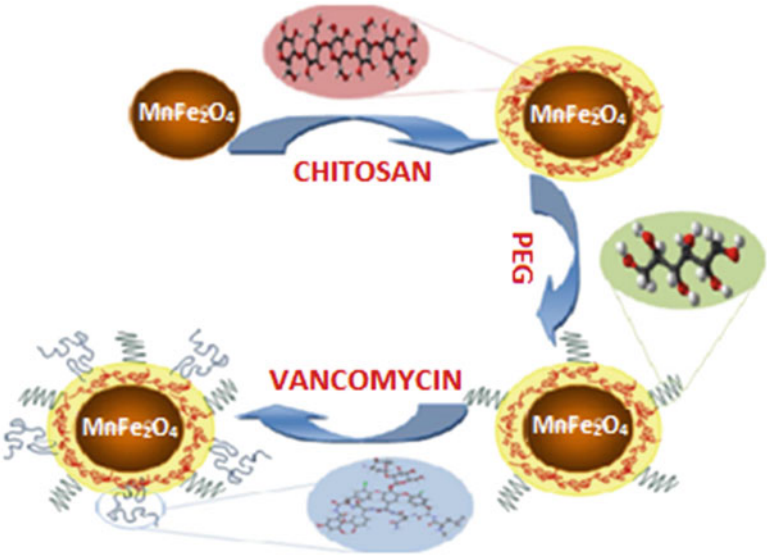


Fig. 15.5 Architecture of mixed chitosan/metals/antibiotic nanoparticles effective against Gram-negative bacteria. (Reprinted from Esmaeili and Ghobadianpour (2016) and Mangoni et al. (2008))

15.5.2 Antimicrobial Activity of Chitosan/Metal Nanocomposites

The combined antimicrobial effect of chitosan and metals was explored to prepare novel nanocomposite materials with improved microbicidal properties (Esmaeili and Ghobadianpour 2016). In particular, a broad spectrum of activities against both Gram-positive and Gram-negative bacteria were demonstrated for gold-, silver- or copper-loaded chitosan nanoparticles. Such nanoparticles were prepared by adding metal ion solutions into chitosan nanosuspension or by reducing a soluble salt of the metal in the presence of chitosan solutions (Rhim et al. 2006). The antibacterial activities by disk diffusion method were evaluated for nanoparticulate colloidal dispersion or in form of a thin film, in which the metal nanoparticles remained embedded inside the chitosan polymeric matrix. In all cases, a remarkable antimicrobial effect was observed for all the examined bacteria (*S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium*) with inhibition diameters ranging from 8 to 10 mm. Silver-based nanocomposites are the most frequently investigated metal/chitosan complexes. In order to investigate if the greater antibacterial effect of the silver-based chitosan composite material was exerted by the presence of silver as metallic nanoparticles or ions, Kumar-Krishnan et al. prepared chitosan films containing Ag nanoparticles or Ag + ions at different concentrations and tested them for their activity against *S. aureus* and *E. coli*. They found that the maximum bactericidal effect of the chitosan films was obtained with those containing 1% w/w of silver nanoparticles or 2% w/w of silver ions, concluding that Ag/chitosan nanoparticles have higher antibacterial effect than Ag + 2+ and Zn ions. The

maximum 2+ antibacterial effect was observed at a concentration close of that of the electrical percolation threshold (Kumar-Krishnan et al. 2015). It was also proposed that nanoparticles size could affect the antimicrobial effect. Indeed, smaller silver nanoparticles have a higher specific surface area and release Ag + ions at a faster rate (Perinelli et al. 2018).

15.5.3 Antimicrobial Activity of Chitosan Nanoparticles on Bacterial Biofilm

Biofilms are microbial communities embedded in a matrix of slimy extracellular polymers. Microorganisms in biofilms are significantly more resistant to antimicrobial agents. Natural biological molecules are currently being evaluated for their anti-biofilm activity in order to develop alternative preventive or therapeutic rationale. In this context, chitosan-streptomycin conjugates/gold nanoparticles were evaluated in terms of their anti-biofilm properties against Gram-negative *P. aeruginosa* and *S. typhimurium* and Gram-positive *Listeria monocytogenes* and *S. aureus* (Mu et al. 2016). In particular, the chitosan-streptomycin gold nanoparticles showed a disruption effect on biofilms formed by Gram-negative or Gram-positive bacteria and an inhibition effect on biofilm formation of Gram-negative bacteria. The conjugation of streptomycin to chitosan and gold nanoparticles facilitated its penetration into the biofilm matrix and improved the contact with the bacterial surface, thereby enhancing its bactericidal effect. Another application of chitosan nanoparticles is represented by photodynamic activation. Darabpour et al. investigated the effect of chitosan nanoparticles on the efficiency of methylene blue (MB)-mediated antimicrobial photodynamic inactivation (APDI) of *S. aureus* and *P. aeruginosa* biofilms. The authors observed that chitosan nanoparticles enhanced the efficacy of MB-APDI, causing the disruption of biofilm structure and subsequently a deeper and effective penetration of MB into *S. aureus* and *P. aeruginosa* biofilms (Darabpour et al. 2016). The use of chitosan nanoparticles has also been reported to combat biofilm formed by oral pathogens on tooth surfaces, which are associated with human caries, gingivitis and periodontitis. Chavez de Paz et al. investigated the antimicrobial activity of chitosan nanoparticles of different DA and MW on *S. mutans* biofilm. The low chitosan MW formulations (up to 150 kDa) disturbed the cell membrane integrity of *S. mutans* in a homogenous manner across the entire biofilm and the chitosan particles directly interacted with bacterial cell (> 95% of damaged cells) (Chávez de Paz et al. 2011) (Perinelli et al. 2018).

15.6 Applications of the Antimicrobial Activity of Chitosan-Based Nanosystems

Due to versatility, biocompatibility and biodegradability of chitosan, chitosan-based nanosystems have attracted a large interest in the last years especially for the formulation of mixed systems with improved properties. The antimicrobial activity

of chitosan has been exploited for a wide range of applications, ranging from agriculture to biomedical area (Perinelli et al. 2018). An ideal antimicrobial material should possess the following characteristics: (1) easily and inexpensively synthesized, (2) stable in long-term usage and storage at the temperature of its intended application, (3) not soluble in water for a water-disinfection application, (4) does not decompose to and/or emit toxic products, (5) should not be toxic or irritating to those who are handling it, (6) can be regenerated upon loss of activity, and (7) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact (Kenawy et al. 2007) (Kong et al. 2010). The following sections introduce the advances of chitosan-based nanomaterials in wound healing, textiles and food packaging fields.

15.6.1 Wound Healing

Skin wound treatment is an important research area. Poor wound management could lead to severe complications and loss of function. Wound healing is a complex process where numerous steps take place in order to re-establish the normal functionality of the skin. It includes an inflammatory, a proliferation and, finally, a remodelling phase. Many factors have to be considered when designing a wound material to provide an adequately moist environment and allow gas exchange in order to avoid dehydration and exudates accumulation. Moreover, wound-related infections represent a serious problem since bacteria can easily invade the tissues and proliferate, hampering the regeneration process. Hence, agents that are able to prevent infection and promote wound healing have been extensively explored. Chitosan has been widely applied as wound dressing due to its properties that include biocompatibility, biodegradability, haemostatic and antibacterial activities (Siafaka et al. 2016). As such, chitosan has been approved in commercial medical devices for topical applications in wound healing (e.g. HemCon bandages). Moreover, the gradual depolymerization of chitosan to N-acetyl glucosamine promotes fibroblast proliferation, thereby accelerating wound closure. In another study, a nanofibrous membrane made of chitosan and silk fibroin was fabricated. Its antibacterial activity against Gram-negative *Escherichia coli* was demonstrated to be directly dependent on the chitosan concentration in the composite nano-dressing, with a higher effect increasing chitosan proportion (Cai et al. 2010). A slightly different result was obtained by Sarhan and Azzazy that studied the combined antibacterial activity of honey, chitosan and polyvinyl alcohol electrospun nanofibrous wound dressing. These nanofibres showed a pronounced antibacterial effect against *S. aureus*, with an increased effect on chitosan concentration, while a weak antibacterial activity was observed against *E. coli* (Sarhan and Azzazy 2015). Recent studies reported the loading of different compounds such as antibiotics, antimicrobial agents and metal nanoparticles within the chitosan nanofibres in order to increase chitosan antibacterial activity and accelerate the wound-healing process (Perinelli et al. 2018).

15.6.2 Textile and Fabrics

Chitosan has been proposed to act as an antimicrobial agent in fabrics or textile in order to prevent microbial growth. In fact, textiles, especially those made of natural fibres such as proteins (silk) or cellulose (cotton), represent a favourable environment for the proliferation of different microorganisms including bacteria or fungi, due to extensive surface area, high porosity and ability to retain humidity. The research in the field of antimicrobial textiles has a great impact on many technological applications such as clothing, furnishing, filtering, medical devices, healthcare and hygienic products. With the increasing interest in the use of silk, collagen or cellulose for the production of membranes and supports for biomedical applications (as regenerative medicine), there is an enhanced demand for safe and biodegradable antimicrobial agents. Chitosan is a good candidate, with attractive characteristics in comparison to other commonly used organic antimicrobial agents (e.g. phenolic and formaldehyde derivatives), especially in terms of toxicological profile. The major drawbacks regarding the use of chitosan include its poor solubility in most of the solvents except acidic aqueous solution, the high viscosity of concentrated high MW chitosan solution for coating application and the thermal stability of chitosan. Nevertheless, some commercial textile products based on chitosan are available on market. Although the commercial products have reached the market more than 10 years ago, the research on the use of chitosan in textiles still has been flourishing. Different strategies have been applied in textiles and fabrics to improve the antimicrobial activity of chitosan. Microencapsulation is one of the most explored approaches (Ibrahim et al. 2017; Perinelli et al. 2018).

15.6.3 Food Packaging

Food industry is facing the problem of microbial contamination. Foodborne bacteria are responsible for many serious human infections, and they can also accelerate food spoilage with enormous economic losses. In this regard, food packaging represents a solution to prevent and retard bacterial invasion and proliferation. Different biopolymers, characterized by a good environmental profile, biodegradability and biocompatibility, have been screened in order to find alternatives to the conventional petroleum-derived materials for food packaging. Many research focused on the development of chitosan-based systems, as the intrinsic properties of chitosan could enhance the antimicrobial efficacy of the packaging. The film-forming properties of chitosan have led to the development of film packaging materials in combination with various natural polysaccharides such as starch, pectin and hydroxypropyl methylcellulose (HPMC). An example was given by Möller et al., who studied the antimicrobial activity of a chitosan-HPMC film against *Listeria monocytogenes*, demonstrating a complete growth inhibition. When chitosan-HPMC films were cross-linked with citric acid by the amino groups of chitosan, the antibacterial activity decreased drastically, thus demonstrating the critical role of the protonated amino groups of chitosan for the antimicrobial activity (Möller et al.

2004). Chitosan nanofibres have also been exploited as a packaging material by many research groups due to their numerous advantages such as biocompatibility, large surface area and good functional and antimicrobial properties. Nanocarrier systems have attracted an increasing attention in food packaging, being able to load different active compounds, including those with low solubility and stability. Chitosan nanoparticles have been intensively exploited for this purpose, and with their well-known antimicrobial properties, a potential combined effect with the loaded active compound could be achieved. As proof of concept study, Feyzioglu and Tornuk examined the antimicrobial activity of summer savoury essential oil-loaded chitosan nanoparticles against three different foodborne bacteria (*E. coli*, *L. monocytogenes* and *S. aureus*), showing the promising application in food packaging materials. Both chitosan nanoparticles and summer savoury essential oil-loaded chitosan nanoparticles displayed antibacterial activity, with the loaded nanoparticles demonstrating a higher effect (Feyzioglu and Tornuk 2016) (Perinelli et al. 2018).

15.6.4 Application in Medical Industry

In the area of healthcare and hygienic applications, biocidal polymers may be incorporated into fibres, membrane or hydrogel and used for contact disinfectants in many biomedical applications, including wound dressing, orthopaedic tissue engineering, drug delivery carrier and haemodialysis. Generally, an ideal wound dressing material must be capable of absorbing the exuded liquid from the wounded area and should permit water evaporation at a certain rate and allow no microbial transport (Yang and Lin 2004). As a key parameter regarding wound dressing, the antimicrobial property assessment is necessary for evaluating the eligibility and capability of the candidate. Polysaccharides, e.g. chitosan, owning hydrogel-forming properties have been considered to be advantageous in their application as a wound dressing materials (Chen et al. 2005). Chitosan-based materials have received much attention in this regard. Typically, there are four forms in which chitosan provides antimicrobial effect to wound dressing materials: fibre, membrane, sponge and hydrogel. The different approaches count on particular physicochemical characteristics of chitosan, which impart talent on specific displaying form. Majority of antimicrobial products perform their talent in fabric form. Micro- and nanofibre materials are suitable for preparing wound dressings. Among these, electrospinning is a favourable technique for producing continuous polymer fibres with diameters down to nanoscale range (Deitzel et al. 2001). Because of unique properties such as high surface-to-volume ratio, high porosity and diameters in the nanoscale, electrospun mats made from ultrafine polymer fibres have been drawing great attention. One study reported the cross-linked QCh/PVP (quaternized chitosan/polyvinylpyrrolidone) electrospun materials were efficient in inhibiting growth of Gram-positive bacteria and Gram-negative bacteria (Ignatova et al. 2007), while, in their previous work, the antibacterial activity of cross-linked electrospun QCh/PVA (polyvinyl alcohol) mats made of quaternized chitosan derivative against *S. aureus*

was observed to be bactericidal rather than bacteriostatic. PVP and PVA are both non-toxic, biocompatible and highly hydrophilic, possess good complexation properties and have good film-forming ability, which are crucial for wound-healing materials (Kong et al. 2010).

15.6.5 Antibacterial Coating

As it is explained above, chitosan has positively charged amino group which interacts with negatively charged microbial cell membranes. This leads to the leakage of proteinaceous and other intracellular constituents of the microorganisms. That may be one of the reasons of having antimicrobial feature of chitosan (Shahidi et al. 1999). Another reason is related to the moves of chitosan on the outer surface of bacteria. At a lower concentration (0.2 mg/mL), polycationic chitosan can be probably bound to the negatively charged bacterial surface for causing agglutination. The larger number of positive charges may impart a positive charge to the bacterial surfaces in order to keep them in suspension at a higher concentration (Dutta et al. 2009; Yılmaz Atay and Çelik 2017). In the UV absorption studies, it was detected that chitosan causes the considerable losses of proteinic material for *Pythium oaroecandrum* at pH 5.8 (H. Liu et al. 2004). Chitosan can bind tracing metals like a chelating agent and this can prevent the formation of toxic materials and the growth of microbes. By activating defensive processes in the host tissue, it can act as a water binding agent and also prevent various enzymes. Due to the penetration towards the nuclei of the microorganisms and the interference with the synthesis of mRNA and proteins, binding of chitosan with DNA and inhibition of mRNA synthesis take part (Sudarshan et al. 1992).

Some innate factors, such as structure of chitosan, its degree of polymerization, the host, the natural nutrient constituency, the chemical or nutrient composition of the substrates and the environmental conditions, can impress the antimicrobial activity of chitosan. *This will be discussed later in this chapter.* Coating materials including antimicrobial agents have been attractive areas for researchers due to preventing the growth of pathogenic bacteria. Chitosan has been still pointed out as an antimicrobial film or a forming agent because of its biodegradability, biocompatibility, cytotoxicity and antimicrobial activity (Dutta et al. 2009).

In contrast to neutral and alkaline conditions, acidic solutions lead to dye better grasping higher (Hasan et al. 2008). Yoshida et al. (1991) showed that at a lower pH, more protons are prosperous for protonation amino groups of chitosan molecules to form groups of –NH3+. Hence, it could be possible to see increasing electrostatic attractions between negatively charged dye anions and positively charged adsorption sites. Therefore, this will bring about an increase in dye adsorption. Chiou and Li (2003) presented similar explanations for the adsorption of RR 189 (reactive dye) on cross-linked chitosan beads. The adsorption was lower than acidic solution, for example, they can go down from pH 10.0 to 13.0. They expressed this action by the fact that chemical cross-linking reduces either the total number or the diameter of

the pores in chitosan beads. Thus, the transferring of the dye molecule was more difficult (Hasan et al. 2008; Yılmaz Atay and Çelik 2017).

In our study, an acrylic resin was converted to an antibacterial coating material by using chitosan. Different states of chitosan, solid state (powders) and colloid state, were inserted to the polymer matrix individually. For obtaining the powders, chitosan (poly-(D)-glucosamine, Sigma) was grounded in a grinding mill at 25 °C for 5 h in the air. The aim of the comminution is to obtain homogeneous distribution and to increase the effect of the particles by increasing contact points. Figure 15.6 shows SEM micrograph of pure chitosan.

Chitosan colloids were prepared as presented in Fig. 15.7. Different amounts of chitosan were dissolved in the acid solution. After mixing the solution thoroughly by using magnetic stirrer at 25 °C for 20 min, homogeneous chitosan colloids were obtained.

Acrylic composites were prepared by adding chitosan powders and colloids to the polymeric matrix. For the manufacture of the composites, acrylic resin (polymethyl acrylate) was used as a polymeric matrix supplied from DYO, Turkey. Chitosan powders and colloids were incorporated into the acrylic resin with different loading levels to assess the concentration dependence of material's antimicrobial effect. Glass substrates were coated with those polymeric composites. After the obtained

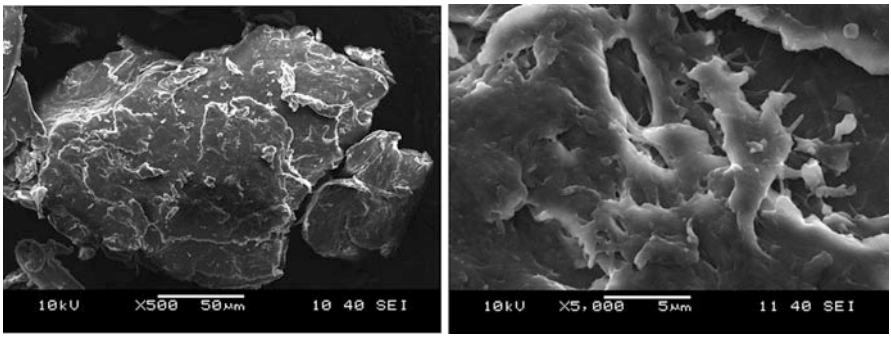


Fig. 15.6 SEM micrograph of pure chitosan

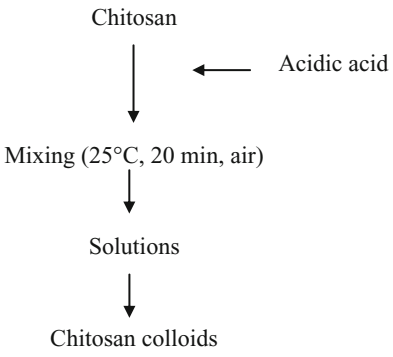


Fig. 15.7 Preparing method of chitosan colloids

Table 15.1 Description and sample codes of composite coatings

Sample codes	Chitosan	Chitosan percentage in the composites (%)
CH00	None	0
CHP1	Ground powder	1
CHP2	Ground powder	5
CHC1	Colloid	0.01
CHC2	Colloid	0.05
CHC3	Colloid	0.10

composite coatings were subsequently dried for 24 h at the room temperature in the air, no more curing process was performed. The sample codes and descriptions of coatings are indicated in Table 15.1.

SEM images of composite coatings are given in Fig. 15.8. It can be seen that chitosan particles look like sphere, not flake, and they were well blended with dye homogeneously. Smooth and rough areas could be seen in chitosan incorporated into the coatings. As characteristic property of chitosan, large crystals appeared. Rough surfaces and crystalline structures were raised as dominant features by increasing chitosan content. Concurrently, by increasing chitosan, the dissociation process occurred. In fact, the morphology of the coating samples was agreed well with this (Abdelrazek et al. 2010). Increasing with colloid amounts, formation of flocculation and cracks appeared.

Antimicrobial properties of the samples against *Staphylococcus aureus* were determined by inhibition zone test and percent decreasing test.

Inhibition Zone Test The agar disc diffusion method was employed for the determination of antimicrobial activities of chitosan-reinforced specimens with the size of 2.4 × 2.4 mm against *S. aureus* ATCC 6538P, a Gram-positive bacterium (NCCLS 1977). Briefly, test microorganisms were activated in Müller Hinton Broth (MHB) at 37 °C for 18 h, and a suspension of the test bacteria was spread on solid medium plates containing MHA. After 2 h, chitosan-reinforced polymer coatings were placed at the centre of inoculated plates and incubated at 37 °C for 24 h. At the end of the incubation period, the plates were inspected for growth on and under composite samples, as well as for the presence or absence of growth in a halo around the samples. The width of the halo was measured across the centre line of the sample, both horizontally and vertically. An average of these two values was then taken to give an estimate of the antimicrobial activity of the samples as shown in Eq. 15.1 (Fig. 15.9).

$$\text{Inhibition radius} : (r_1 + r_2)/2 \tag{15.1}$$

in which r_1 and r_2 are vertical and horizontal widths, respectively. Each experiment was repeated three times (NCCLS 1977).

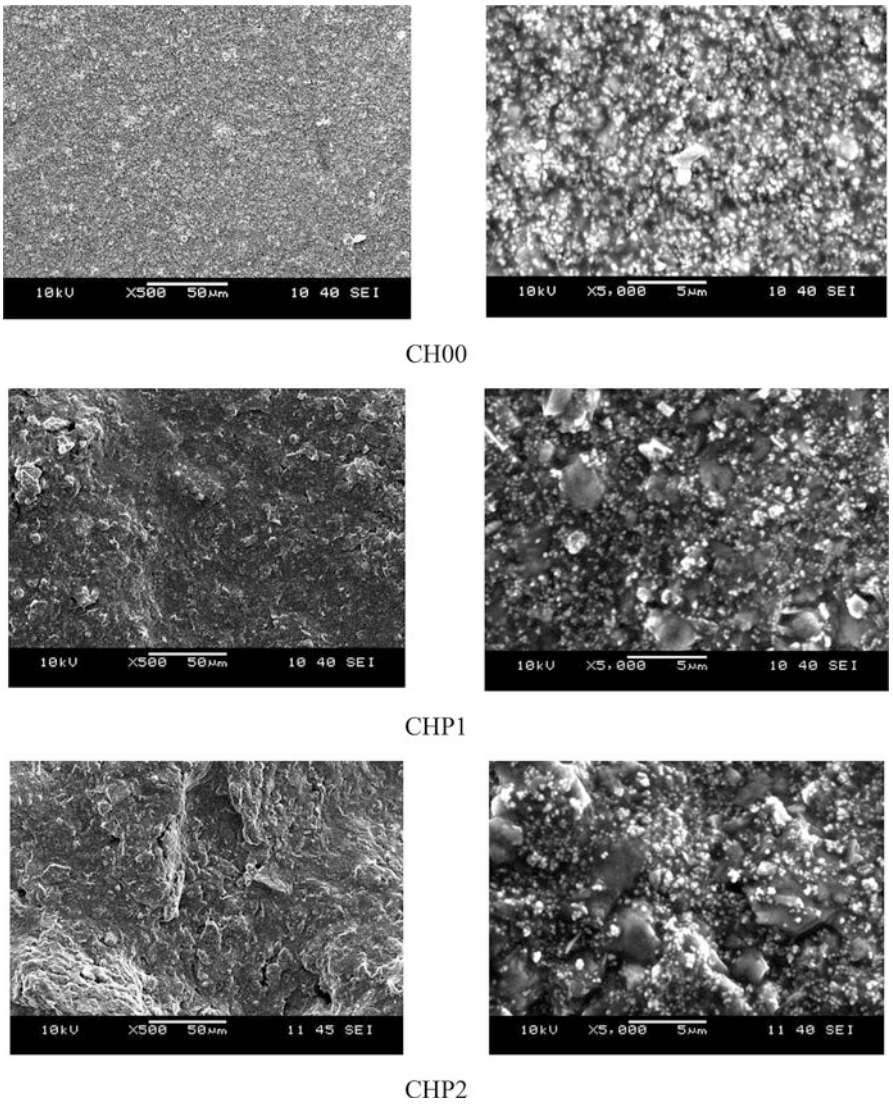
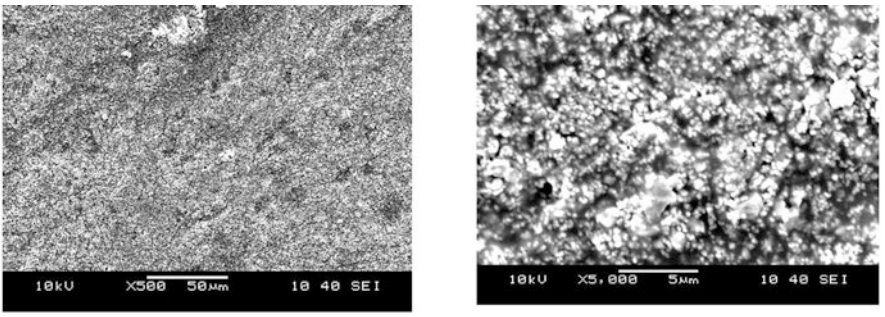
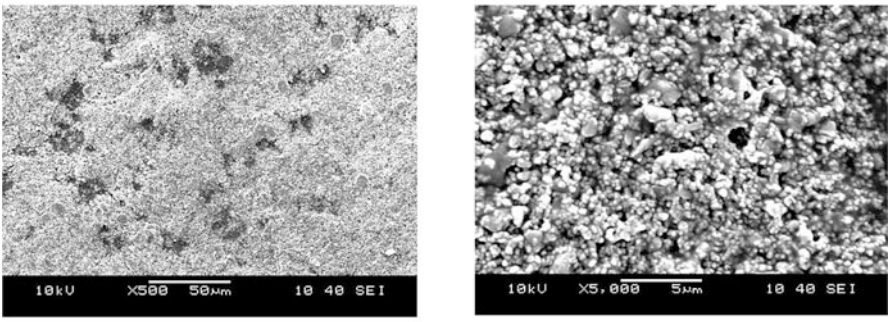


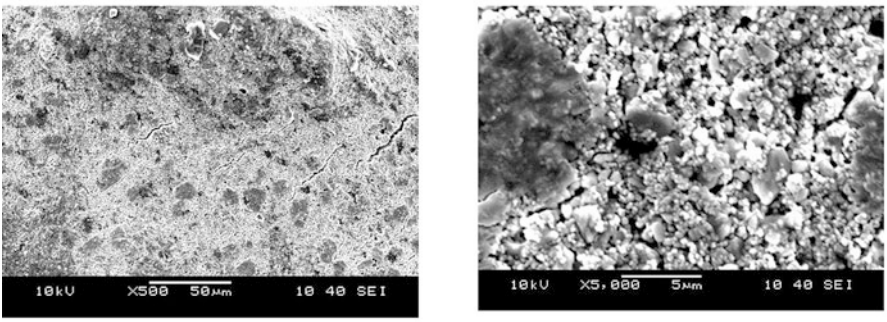
Fig. 15.8 SEM micrographs of chitosan-reinforced composites



CHC1



CHC2



CHC3

Fig. 15.8 (continued)

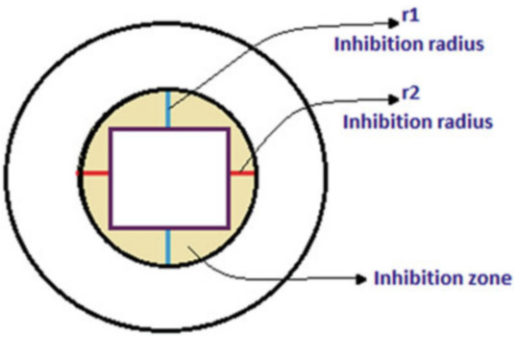


Fig. 15.9 Inhibition zone view (Yilmaz Atay et al. 2015)

Percent Decreasing Test A plate counter agar solid culture medium was poured into the plates that were subsequently incubated at 37 °C for 24 hours so that the v*ital* cells, eventually presented, could grow into colonies. The microorganisms' colonial presence was then evaluated, by counting the colony-forming units per Petri plate (CFU/mL). Used bacteria were *E. coli* (ATTC 11228). The difference between the number of the bacteria obtained at zeroth hour and the one obtained after 24 h will show the antibacterial performance (Eq. 15.2).

$$\% \text{ decrease} = \left[\frac{A - B}{A}\right] \times 100 \tag{15.2}$$

where A is the number of bacteria at zeroth hour and B is the number of bacteria after 24 h.

For the mentioned antibacterial tests, coated samples and agar discs are shown in Fig. 15.10 after the antibacterial tests. In the test sample, there is not any antibacterial activity. The inhibition zones around those specimens can be clearly seen in chitosan colloid-reinforced coatings. Increasing the loading level of the chitosan also increases the inhibition radius in those samples. Regarding the samples including chitosan powder, antimicrobial effects are seen on the surface of the coated region. It means the antimicrobial property works with direct contact of chitosan powders on the surface. To obtain the better results, particle size can be decreased to nanoscale if possible, as by this way the antibacterial effect of the particles can be increased due to increasing of contact surfaces. However, some researchers observed that nanoparticles can have less inhibition effect on *S. aureus* ATCC 29737 than in free soluble form polymers. The reason is that nanoparticles have less positive charge for binding to the negative bacterial cell wall (Sadeghi et al. 2008). On the contrary, in another research, it was recorded that due to the special character of the nanoparticles, the chitosan nanoparticles can present higher antibacterial activity against *S. aureus* (Qi et al. 2004). Similarly, larger surface area of the nanoparticle and affinity with bacterial cells, which yields a quantum-size effect, has influence in the antibacterial action (Kong et al. 2010).

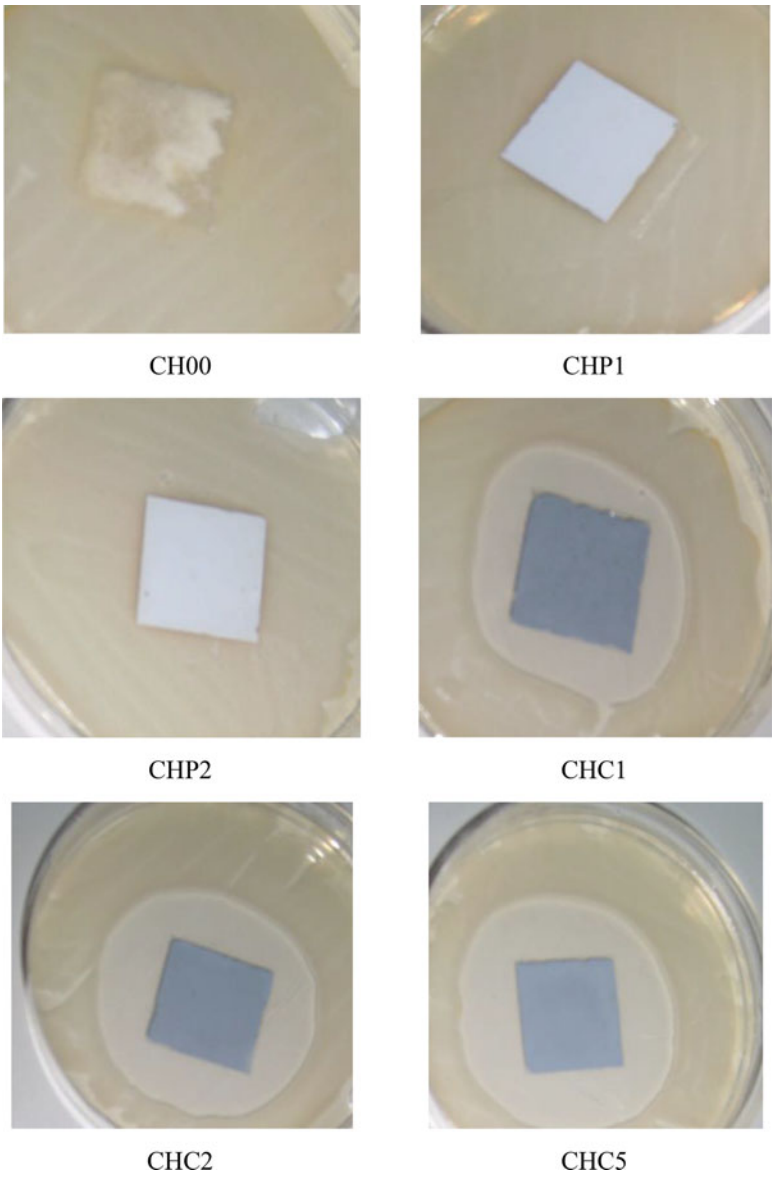


Fig. 15.10 Antibacterial test results: photographs of agar plates (Yılmaz Atay and Çelik 2017)

In addition, the prohibition activity of chitosan depends on different types of factors, such as solid surface characteristic and the morphology. Particle size, membrane and fibre thickness cause to occur different results. It was investigated the effect of particle size and shape of chitosan powder on *S. aureus*, and it was recorded that antibacterial activity was improved by decreasing the particle size. Conversely, the antibacterial property of chitosan

powders depends on the shape as well as specific surface area. The researchers showed that chitosan powders in the range of 74–500 μm looked like a flake or board, but they looked like spheres in the range of 37–63 μm (Kong et al. 2010; Phaechamud 2008; Yılmaz Atay and Çelik 2017).

Soluble chitosan and its derivatives are more efficient for preventing bacterial growth because soluble chitosan allows reaction with the counterparts to a sufficient degree by existing as a disassociating form in solution and an extending confirmation. Solid chitosan only gets into touch with solution through surface, such as fibres, membrane, hydrogels, microspheres and nanoparticles. However, by extending conformation contact to solution, hydrogels can be formed by covalently cross-linking chitosan with itself. Chitosan particulate systems can form dispersion in solution with the considerable reactive surface area (Kong et al. 2010).

Chitosan-reinforced composite samples were subjected to another type of an antibacterial test called "percent decreasing test". The initial number of bacteria was 2000, and they were counted again after 24 h. The results are shown in Table 15.2. It can be expressed that chitosan-reinforced coatings showed antibacterial property. The results support inhibition test results. As mentioned above, colloid chitosan demonstrated much better antimicrobial activity in the composite compared with powder chitosan-reinforced composites.

In this study, the antibacterial behaviour and effectiveness of solid and colloid chitosan in a polymer matrix were investigated. Chitosan can be considered as an effective antibacterial additive. Increasing loading level of the chitosan colloids in the polymer composites increased inhibition zone. Colloid chitosan demonstrated much better antimicrobial activity in the composites compared with powder chitosan-reinforced composites.

15.7 Conclusions and Future Perspectives

Considerable interest and attention have been focused on chitosan due to its potential application area and its unique advantages over the last decades. Investigations on its antimicrobial property are growing rapidly. Due to their multitude of application areas and people's environmental mindfulness, biodegradable, and non-toxic products from 'natural' sources are going to be more and more appealing for the replacement of synthetic compounds. Against other antibacterial materials, chitosan is a non-toxic, harmless and environmentally friendly vegetable material. Using this

Table 15.2 Decreasing test results of the chitosan-reinforced composite samples (Yılmaz Atay and Çelik 2017)

Code	Bacteria quantity after 24 h
CH00	1650
CHP1	1345
CHP2	1056
CHC1	0
CHC2	0
CHC3	0

material at the points of our life will be more healthful for people. Therefore, the investigations of this material for the antibacterial studies need to be improved which will incorporate a combination of disciplines involving chemistry, physics, informatics, nanotechnology and genetic engineering. This will be beneficial for the exploitation of new generation of antimicrobial agents and for the development of new biomedicine. On the other hand, future works can focus on the use of chitosan in the composites to avoid lower pH values, as soluble chitosan is used as a generally acidic environment. It will be beneficial to clarify the molecular circumstance of the underlying mechanisms and their involvement to the antimicrobial action of chitosan.

References

Abdelrazek M, Elashmawi IS, Labeeb S (2010) Chitosan filler effects on the experimental characterization, spectroscopic investigation and thermal studies of PVA/PVP blend films. Phys B Condens Matter 405:2021–2027

Ali A, Noh NM, Mustafa MA (2015) Antimicrobial activity of chitosan enrichedwith lemongrass oil against anthracnose of bell pepper, food Packag. Shelf Life 3:56–61

Allan CR, Hardwiger LA (1979) The fungicidal effect of chitosan on fungi of varying cell wall composition. Exp Mycol 3:285–287

Cai Z, Mo X, Zhang K, Fan L, Yin A, He C, Wang H (2010) Fabrication of chitosan/ silk fibroin composite nanofibers for wound-dressing applications. Int J Mol Sci 11:3529–3539. https://doi.org/10.3390/ijms11093529

Cha DS, Chinnan MS (2004) Biopolymer-based antimicrobial packaging: a review. Crit Rev Food Sci Nutr 44:223–237

Chapter 26: Microbial growth control (2018). https://quizlet.com/39912564/chapter-26-microbial-growth-control-flash-cards/. Accessed in 16.11.2018

Chávez de Paz LE, Resin A, Howard KA, Sutherland DS, Wejse PL (2011) Antimicrobial effect of chitosan nanoparticles on streptococcus mutans biofilms. Appl Environ Microbiol 77:3892–3895. https://doi.org/10.1128/AEM.02941-10

Chen YL, Chou CC (2005) Factors affecting the susceptibility of Staphylococcus aureus CCRC 12657 to water soluble lactose chitosan derivative. Food Microbiol 22:29–35

Chen S, Wu G, Zeng H (2005) Preparation of high antimicrobial activity thioureachitosan–Ag +complex. Carbohydr Polym 60:33–38

Chien RC, Yen MT, Mau JL (2015) Antimicrobial and antitumor activities of chitosan from shiitake stipes, compared to commercial chitosan from crabshells. Carbohydr Polym. https://doi.org/10.1016/j.carbpol.2015.11.061

Chiou MS, Li HY (2003) Adsorption behavior of reactive dye in aqueous solution on chemical cross-linked chitosan beads. Chemosphere 50:1095–1105

Chung YC, Wang HL, Chen YM, Li SL (2003) Effect of abiotic factors on theantibacterial activity of chitosan against waterborne pathogens. Bioresour Technol 88:179–184

Chung YC, Su CC, Chen Y, Jia G, Wang JCG, Wu H, Lin JG (2004) Relationship between antibacterial activity of chitosan and surfacecharacteristics of cell wall. Acta Pharmacol Sin 25:932–936

Chung YC, Kuo CL, Chen CC (2005) Preparation and important functional properties of water-soluble chitosan produced through Maillard reaction. Bioresour Technol 96:1473–1482

Claesson PM, Ninham BW (1992) Ph-dependent interactions between adsorbedchitosan layers. Langmuir 8:1406–1412

Darabpour E, Kashef N, Mashayekhan S (2016) Chitosan nanoparticles enhance the efficiency of methylene blue-mediated antimicrobial photodynamic inactivation of bacterial biofilms: an

in vitro study. Photodiagn Photodyn Ther 14:211–217. https://doi.org/10.1016/j.pdpdt.2016.04.009
Deitzel JM, Kleinmeyer J, Harris D, Beck TNC (2001) The effect of processing variables on the morphology of electrospun nanofibers and textiles. Polymer 42:261–272
Divya K, Vijayan S, Tijith KG, Jisha MS (2017) Antimicrobial properties of chitosan nanoparticles: mode of action and factors affecting activity. Fibers Polymers 18:221–230
Dutta PK, Dutta J, Tripathi VS (2004) Chitin and chitosan: chemistry, properties and applications. J Sci Ind Res 63:20–31
Dutta PK, Tripathi S, Mehrotra GK, Dutta J (2009) Perspectives for chitosan based antimicrobial films in food applications. Food Chem 114:1173–1182
El Ghaouth A, Arul J, Grenier J, Asselin A (1992) Antimicrobial effect of chitosan nanoparticles on Streptococcus. Phytopathology 82:398–402
Esmaeili A, Ghobadianpour S (2016) Vancomycin loaded superparamagnetic MnFe2O4 nanoparticles coated with PEGylated chitosan to enhance antibacterial activity. Int J Pharm 501:326–330. https://doi.org/10.1016/j.ijpharm.2016.02.013
Feyzioglu GC, Tornuk F (2016) Development of chitosan nanoparticles loaded with summer savory essential oil for antimicrobial and antioxidant delivery applications. LWT Food Sci Technol 70:104–110. https://doi.org/10.1016/j.lwt.2016.02.037
Gómez-Estaca J, Lacey AL, López-Caballero ME, Gómez-Guillén MC, Montero P (2010) Biodegradable gelatin-chitosan films incorporated with essential Oilsas antimicrobial agents for fish preservation. Food Microbiol 27:889–896
Goy RC, Britto D, Assis OBG (2009) A review of the antimicrobial activity of chitosan. Polímeros: Ciênc Tecnol 19:241–247
Hasan M, Ahmad AL, Hameed BH (2008) Adsorption of reactive dye onto cross-linked chitosan/oil palm ash composite beads. Chem Eng J 136:164–172
Helander IM, Wright AV, Mattila-Sandholm TM (1997) Potential of lactic acid bacteria and novel antimicrobials against gram-negative bacteria. Trends Food Sci Technol 8:146–150
Hernandez-Lauzardo AN, Bautista-Banos S, Velazquez-del Valle MG, Montealvo MG, Sanchez-Rivera MM, Bello-Perez LA (2008) Antifungal effects of chitosan with different molecular weights on in vitro development of Rhizopus stolonifer Vuill. Carbohydr Polym 73:541–547
Hosseinnejad M, Jafari SM (2016) Evaluation of different factors affecting antimicrobial properties of chitosan. Int J Biol Macromol 85:467–475
Hu Y, Du YM, Yang JH, Kennedy JF, Wang XH, Wang LS (2007a) Synthesis, characterization and antibacterial activity of guanidinylated chitosan. Carbohydr Polym 67:66–72
Hu Y, Du YM, Yang JH, Tang YF, Li J, Wang XY (2007b) Self-aggregation and antibacterial activity of N-acylated chitosan. Polymer 48:3098–3106
Ibrahim NA, El-Zairy EM, Eid BM, Emam E, Barkat SR (2017) A new approach for imparting durable multifunctional properties to linen-containing fabrics. Carbohydr Polym 157:1085–1093. https://doi.org/10.1016/j.carbpol.2016.10.074
Ignatova M, Manolova N, Rashkov I (2007) Novel antibacterial fibers of quaternized chitosan and poly(vinyl pyrrolidone) prepared by electrospinning. Eur Polym J 43:1112–1122
Jeihanipour A, Karimi K, Taherzadeh MJ (2007) Antimicrobial properties offungal chitosan, research. J Biol Sci 2:239–243
Jeon YJ, Park PJ, Kim SK (2001) Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr Polym 44:71–76
Kenawy ER, Worley SD, Broughton R (2007) The chemistry and applications of antimicrobial polymers: a state-of-the-art review. Biomacromolecules 8:1359–1384
Kong M, Chen XG, Liu CS, Yu LJ, Ji QX, Xue YP, Cha DS, Park HJ (2008) Preparation and antibacterial activity of chitosan microspheres in a solid dispersing system. Front Mater Sci China 2:214–220
Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: a state of the art review. Int J Food Microbiol 144:51–63

Kumar-Krishnan S, Prokhorov E, Hernández-Iturriaga M, Mota-Morales JD, Vázquez-Lepe M, Kovalenko Y, Sanchez IC, Luna-Bárcenas G (2015) Chitosan/silver nanocomposites: synergistic antibacterial action of silver nanoparticles and silver ions. Eur Polym J 67:242–251. https://doi.org/10.1016/j.eurpolymj.2015.03.066

Kurita K (1998) Chemistry and application of chitin and chitosan. Polym Degrad Stab 59:117–120

Leuba S, Stossel P (1985) Chitin in nature and technology. Plenum Press, New York, p 217

Lim SH, Hudson SM (2004) Synthesis and antimicrobial activity of a water-soluble chitosan derivative with a fiber-reactive group. Carbohydr Res 339:313–319

Lin HTV, Wu GJ, Tsai GJ (2015) PH effects on solubility, zetapotential, and correlation between antibacterial activity and molecularweight of chitosan. Carbohydr Polym 134:74–81

Liu H, Du Y, Wang X, Sun L (2004) Chitosan kills bacteria through cell membrane damage. Int J Food Microbiol 95:147–155

Mangoni ME, Nargeot J (2008) Genesis and regulation of the heart automaticity. Physiol Rev 88(3):919–982. https://doi.org/10.1152/physrev.00018.2007

Maya S, Indulekha S, Sukhithasri V, Smitha KT, Nair SV, Jayakumar R, Biswas R (2012) Efficacy of tetracycline encapsulated O-carboxymethyl chitosan nanoparticles against intracellular infections of Staphylococcus aureus. Int J Biol Macromol 51:392–399. https://doi.org/10.1016/j.ijbiomac.2012.06.009

Möller H, Grelier S, Pardon P, Coma V (2004) Antimicrobial and physicochemical properties of chitosan−HPMC-based films. J Agric Food Chem 52:6585–6591. https://doi.org/10.1021/jf0306690

Mu H, Liu Q, Niu H, Sun Y, Duan J (2016) Gold nanoparticles make chitosanstreptomycin conjugates effective towards gram-negative bacterial biofilm. RSC Adv 6:8714–8721. https://doi.org/10.1039/C5RA22803D

Nagy A, Harrison A, Sabbani S, Munson RS, Dutta PK Jr, Waldman WJ (2011) Int J Nanomedicine 6:1833

NCCLS (National Commitie for clinical Laboratory Standarts) (1977) Performance standards for antimicrobial susceptibility test, 6th ed. Approved standar. M2-A6, Wayne, PA

No HK, Park NY, Lee SH, Meyers SP (2002) Antibacterial activity of chitosansand chitosan oligomers with different molecular weights. Int J Food Microbiol 74:65–72

No HK, Kim SH, Lee SH, Park NY, Prinyawiwatkul W (2006) Stability and antibacterial activity of chitosan solutions affected by storage temperature and time. Carbohydr Polym 65:174–178

Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH (2010) Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chem 122:161–166

Perinelli DR, Fagioli L, Campana R, Lam JKW, Baffone W, Palmieri GF, Casettari L, Bonacucina G (2018) Chitosan-based nanosystems and their exploited antimicrobial activity. Eur J Pharm Sci 117:8–20

Phaechamud T (2008) Hydrophobically modified chitosans and their pharmaceutical applications. J Pharm Sci Technol 1:2–9

Qi LF, Xu ZR, Jiang X, Hu CH, Zou XF (2004) Preparation and antibacterial activity of chitosan nanoparticles. Carbohydr Res 339:2693–2700

Raafat D, Bargen KV, Haas A, Sahl HG (2008) Insights into the mode of action of chitosan as an antibacterial compound. Appl Environ Microbiol 74:3764–3773

Rabea EI, Badawy MET, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4:1457–1465

Reesha KV, Panda SK, Bindu J, Varghese TO (2015) Development and characterization of an LDPE/chitosan composite antimicrobial film for chilled fish storage. Int J Biol Macromol 79:934–942

Rhim JW, Hong SI, Park HM, Ng PKW (2006) Preparation and characterization of chitosan-based nanocomposite films with antimicrobial activity. J Agric Food Chem 54:5814–5822. https://doi.org/10.1021/jf060658h

Roller S, Covill N (1999) The antifungal properties of chitosan in laboratory media and apple juice. Int J Food Microbiol 47:67–77

Sadeghi AMM, Dorkoosh FA, Avadi MR, Saadat P, Rafiee-Tehrani M, Junginger HE (2008) Preparation, characterization and antibacterial activities of chitosan, N-trimethyl chitosan (TMC) and N-diethylmethyl chitosan (DEMC) nanoparticles loaded with insulin using both the ionotropic gelation and polyelectrolyte complexation methods. Int J Pharm 355:299–306

Sarhan WA, Azzazy HME (2015) High concentration honey chitosan electrospun nanofibers: biocompatibility and antibacterial effects. Carbohydr Polym 122:135–143. https://doi.org/10.1016/j.carbpol.2014.12.051

Severino R, Vu KD, Donsì F, Salmieri S, Ferrari G, Lacroix M (2014) Antimicrobialeffects of different combined non-thermal treatments against Listeria monocytogenes in broccoli florets. J Food Eng 124:1–10

Shahidi F, Arachchi JKV, Jeon YJ (1999) Food application of chitin and chitosans. Trends Food Sci Technol 10:37–51

Siafaka PI, Zisi AP, Exindari MK, Karantas ID, Bikiaris DN (2016) Porous dressings of modified chitosan with poly(2-hydroxyethyl acrylate) for topical wound delivery of levofloxacin. Carbohydr Polym 143:90–99. https://doi.org/10.1016/J.CARBPOL.2016.02.009

Sudarshan NR, Hoover DG, Knorr D (1992) Antibacterial action of chitosan. Food Biotechnol 6:257–272

Takahashia T, Imaia M, Suzukia I, Sawai J (2008) Growth inhibitory effect on bacteria of chitosan membranes regulated by the deacetylation degree. Biochem Eng J 40:485–491

Teli MD, Kale RD (2011) Polyester nanocomposite fibers with antibacterialproperties. Adv Appl Sci Res 2:491–502

Tikhonov VE, Stepnova EA, Babak VG, Yamskov IA, Palma-Guerrero J, Jansson HB, Lopez-Llorca LV, Salinas J, Gerasimenko DV, Avdienko ID, Varlamov VP (2006) Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2(3)-(dodec-2-enyl) succinoyl/−derivatives. Carbohydr Polym 64:66–72

Tsai GJ, Su WH (1999) Antibacterial activity of shrimp chitosan against Escherichia coli. J Food Prot 62:239–243

Tuncer M (2007) Effects of chloride ion and the types of oxides on the antibacterial activities of inorganic oxide supported Ag materials, in: MSc. Thesis, IzmirInstitute of Technology

Wu T, Wu C, Fu S, Wang L, Yuan C, Chen S, Hu Y (2017) Integration of lysozyme into chitosan nanoparticles for improving antibacterial activity. Carbohydr Polym 155:192–200. https://doi.org/10.1016/j.carbpol.2016.08.076

Xiao B, Wan Y, Zhao M, Liu Y, Zhang S (2011) Preparation and characterization of antimicrobial chitosan-N-arginine with different degrees of substitution. Carbohydr Polym 83:144–150

Xie YJ, Liu XF, Chen Q (2007) Synthesis and characterization of water-soluble chitosan derivate and its antibacterial activity. Carbohydr Polym 69:142–147

Xing K, Chen XG, Liu CS, Cha DS, Park HJ (2009) Oleoyl-chitosan nanoparticles inhibits Escherichia coli and Staphylococcus aureus by damaging the cell membrane and putative binding to extracellular or intracellular targets. Int J Food Microbiol 132:127–133

Yang JM, Lin HT (2004) Properties of chitosan containing PP-g-AA-g-NIPAAm bigraft nonwoven fabric for wound dressing. J Membr Sci 243:1–7

Yang TC, Chou CC, Li CF (2005) Antibacterial activity ofN-alkylated disaccharidechitosan derivatives. Int J Food Microbiol 97:237–245

Yang TC, Li CF, Chou CC (2007) Cell age, suspending medium and metal ion influence the susceptibility of Escherichia coli O157:H7 to water-soluble maltose chitosan derivative. Int J Food Microbiol 113:258–262

Yilmaz Atay (2013) The fabrication and technical applications of multifunctional materials. PhD Thesis, Dokuz Eylul University Fen Bilimleri Institute, İzmir Turkey

Yılmaz Atay H, Çelik E (2017) Investigations of antibacterial activity of chitosan in the polymeric composite coatings. Prog Org Coat. https://doi.org/10.1016/j.porgcoat.2016.10.013

Yılmaz Atay H, Yaşa I, Çelik E (2015) Antibacterial polymer composite coatings with synthesized silver nanoparticles. Synth React Inorg, Met-Org, Nano-Met Chem 45:784–798. https://doi.org/10.1080/15533174.2013.843561

Yoshida H, Fukuda S, Okamato A, Kataoka T (1991) Recovery of direct dye and acid dye by adsorption on chitosan fiber-equilibria. Water Sci Technol 23:1667–1676

Zaki NM, Hafez MM (2012) Enhanced antibacterial effect of ceftriaxone sodiumloaded chitosan nanoparticles against intracellular Salmonella typhimurium. AAPS PharmSciTech 13:411–421. https://doi.org/10.1208/s12249-012-9758-7

Zivanovic S, Basurto CC, Chi S, Davidson PM, Weiss J (2004) Molecular weight of chitosan influences antimicrobial activity in oil-in-water emulsions. J Food Prot 67:952–959

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