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Novel antimicrobial chitosan–cellulose composite films bioconjugated with silver nanoparticles

Shan Lin, Lihui Chen*, Liulian Huang, Shilin Cao, Xiaolin Luo, Kai Liu

College of Material Engineering, Fujian Agriculture and Forestry University, Fuzhou, Fujian 350002, PR China

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ABSTRACT

Cellulose-based membranes have emerged as an attractive alternative to non-biodegradable petrochemical materials. An important drawback, however, is that cellulose-based membranes are prone to biofouling. Silver nanoparticles (AgNPs) encapped with polyacrylic acid were conjugated with the chitosan/cellulose composite films to enhance the antimicrobial activities. Using the 1-ethyl-3-(3-dimethylaminopropyl) carbodii-mide hydrochloride and *N*-hydroxysuccinimide as biocoupling agents, AgNPs with an average size of 9 nm were distributed evenly in the film without agglomeration. The presence of AgNPs in the chitosan/cellulose–AgNPs composite films was further confirmed by X-ray diffraction measurements. Fourier transform infrared spectroscopy analysis supported the presence of amide bonds between the primary amino groups of chitosan and the carboxylic residues of coordination to silver nanoparticles. The antimicrobial properties of the chitosan/cellulose and chitosan/cellulose–AgNPs composite films were determined using the disk diffusion tests with *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). As compared to the chitosan/cellulose composite films, the chitosan/cellulose–AgNPs composite films showed significantly improved antimicrobial activities.

1. Introduction

Membrane separation technology has attracted substantial interests and demonstrated great potential for waste water reclamation, drinking water purification, sea water desalination, and separation. However, most membranes have been prepared with non-biodegradable petrochemical materials. Accumulation of these non-degradable plastic materials used as disposable items is becoming a significant burden to the ecosystem and has negative environmental and health impacts (Lu et al., 2006).

Cellulose can be used to prepare membranes for separation applications (Ruan et al., 2004; Jie et al., 2005; Madaeni and Heidary, 2011), and is attractive alternative to traditional petrochemical materials because it is biodegradable, biocompatible, reproducible, and inexpensive. However, the application of cellulose-based membranes has been limited by an important drawback, that biological matter can build up on the membrane surface and leads to biofouling (Worthley et al., 2011; Anitha et al., 2012). Hence, it is desirable to develop bio-membranes with antimicrobial activities (Liu et al., 2010; Zhu et al., 2010). Chitosan is non-toxic, has high antimicrobial activity, and has been widely used as wound dressing material (Hou et al., 2008; Alonso et al., 2009; Pandima Devi et al., 2012). Recently, chitosan/cellulose blend membranes were prepared to improve the antimicrobial properties of cellulose-based membranes (Shih et al., 2009; Morgado et al., 2011; Stefanescu et al., 2012). However, monocomponent antibacterial agents have been far from meeting requirements for some special conditions. Therefore, it is necessary to find composite antibacterial agents to solve this problem (Niu et al., 2009; Fu et al., 2011; Liu and Kim, 2012). In this work, we explore chemical modifications on chitosan to help overcome this major limitation and enhance the antimicrobial property.

It has been recognized that silver based compounds have antimicrobial activities. For example, silver is an effective biocide either bound to a solid surface or in solution (Kusnetsov et al., 2001; Ibrahim et al., 2012). Silver nanoparticles (AgNPs) have even stronger antimicrobial activities compared to the bulk metal, likely due to much larger surface-to-volume ratios. It has been shown that even nanomolar concentrations of AgNPs can be effectively against microbes (Vimala et al., 2010; Zhou et al., 2012). However, if simply composed with other materials (such as cellulose and chitosan), AgNPs can be easily leached and the composite/membrane will gradually lose its antimicrobial activity (Pinto et al., 2012; Tripathi et al., 2011). Thus, it is necessary to incorporate chemical connec-

* Corresponding author. Tel.: +86 591 83715175; fax: +86 591 83715175.
E-mail address: fafuclh@163.com (L. Chen).

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tion among chitosan, AgNPs and cellulose to eliminate leaching and extend the functional life cycle of the composite membrane. In particular, free $-NH_2$ groups available in chitosan provide tethering sites for chemical modifications in synthesis of composite AgNPs/chitosan/cellulose materials with enhanced antimicrobial properties.

In this work, we develop a new and green approach for synthesis of chitosan/cellulose–AgNPs composite films using water-soluble 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) as a conjugating agent. EDC can be used for conjugating biomaterials through a biocompatible and non-toxic process (Cabana et al., 2011). It can be directly added to the reaction buffer without prior organic solvent dissolution (Wang et al., 2003). Therefore, the carbodiimide reaction provides an efficient means to form amide bonds between the $-NH_2$ groups and the carboxylic residues. We first encapped the silver nanoparticles with polyacrylic acid (PAA) through a polyol process (Hu et al., 2008). The chitosan/cellulose–AgNPs composite films were then prepared by using the EDC and *N*-hydroxysuccinimide (NHS) as biocoupling agents. The developed chitosan/cellulose–AgNPs composite films were then characterized using a range of analytical methods and evaluated for their antibacterial activities.

2. Materials and methods

2.1. Materials

Chitosan ($M_W = 2 \times 10^5$ Da, degree of deacetylation = 90%) was obtained from Golden-Shell Biochemical Co., Ltd. (China). Bamboo cellulose was generously provided by Shaowu Bamboo Pulp Corporation (Fujian, China) and milled into powder (400 mesh screened) before used. The material was prepared by firstly kraft pulping and then ECF (elemental chlorine free, shorted as ECF) bleaching sequences. The mass average polymerization degree of the cellulose powder as prepared was determined to be 650. Then, both chitosan and cellulose were dried for 10 h at 60 °C and then used without any further purification. Zinc chloride ($ZnCl_2$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (China) and used as received. Silver nitrate ($AgNO_3$, 99.9%) was purchased from Boda Chemical Industry (Shanghai, China). Short-chain polyacrylicacid PAA powder (M_W = 1800, 99%), diethyleneglycol (DEG, 99%), EDC (99.9%) and NHS (99.9%) were purchased from Aladdin reagents Inc. All reagents were used without further purification. Ultrafiltration membranes (normal molecular weight limited (NMWL) = 14,000) were purchased from Jingke Hongda biotechnology (Beijing, China).

2.2. Preparation of chitosan/cellulose blend films

The preparation of the chitosan/cellulose blend was carried out in $ZnCl_2{\cdot}3H_2O$ solution according to a previously reported procedure (Lu and Shen, 2011). 7.2 g $ZnCl_2$ was dissolved in 2.8 mL deionized water to obtain $ZnCl_2{\cdot}3H_2O$ solution. Then, 0.4 g mixture of chitosan/cellulose (w/w = 1:6) was added to a 50 ml flask and further mixed with 10.0 g prepared $ZnCl_2{\cdot}3H_2O$ solution at 80 °C. During the process of dissolving, the mixture was heated and persistently stirred until a transparent homogeneous solution was obtained (Lin et al., 2012).

The chitosan/cellulose composite films were prepared on a coater (GBC-A4, GIST, Korea). 2 g chitosan/cellulose blend in $ZnCl_2{\cdot}3H_2O$ solution was firstly poured and then manually casted onto a glass slide. After the evaporation of water from blend, the gel sheet was formed. The gel sheet was then immediately immersed in water at ambient temperature for 15 min. The resulted fresh films were washed with running water and then deionized water to completely remove the solvent from the films. Finally, the films were air-dried at room temperature. The regenerated chitosan and cellulose were prepared by the same method.

2.3. Synthesis of AgNPs

AgNPs was synthesized by a modified polyol process (Hu et al., 2008). Briefly, $AgNO_3$ (0.2 g) was dissolved in DEG (6 mL) at room temperature. It was then quickly injected into a boiling solution of DEG (30 mL) and PAA (0.060 M) with vigorous stirring under a protective nitrogen atmosphere. Samples were cooled to room temperature by high pressure air flow. The final nanoparticles were harvested by washing with excessive ethanol, and redispersed in water for further purification with ultrafiltration membranes. Carboxylate contents in the AgNPs solution were determined by the electric conductivity titration method (Saito et al., 2006).

2.4. Synthesis of chitosan/cellulose–AgNPs composite film

EDC/NHS (10 mg/mL, the molar ratio of EDC to NHS = 1:1) was added to AgNPs solution with a carboxylate concentration of 0.01 mmol/mL, and the solution was incubated at 25 °C for 4 h with shaking (150 rpm). 5 mg of the chitosan/cellulose film was then submerged into the solution for 24 h with shaking (150 rpm). In the procedure, as depicted in Fig. 1, the carboxyl groups of AgNPs were activated by EDC and NHS and subsequently reacted with the amino groups of chitosan in the chitosan/cellulose film. Finally, the modified film was taken out, washed with the deionized water. Following this process, the chitosan/cellulose–AgNPs composite film was obtained.

2.5. Characterization

2.5.1. Transmission electron microscopy (TEM)

Morphology and size distribution of the AgNPs were characterized using a transmission electron microscope (Hitachi H7500) operating at 160 kV. For TEM measurements, samples were prepared by dropping 10–20 μL of hydrogel solution on a 400 mesh copper grid covered by an amorphous carbon supported film. The droplet was then allowed for full contact/spreading on the grid and dried at room temperature. The average diameter and size distribution were calculated from 50 pieces of AgNPs in the TEM image (Zhou et al., 2012).

2.5.2. Scanning electron microscopy (SEM)

Scanning electron micrographs (SEM) were taken on a scanning electron microscope (JEOL JSM-7500F). Working distance of 10 mm was maintained and the acceleration voltage used was 5 kV with different magnification. Before observation, the films were mounted on metal grids by using double-sided adhesive tape and the surface was coated with a thin layer of gold under vacuum.

2.5.3. The X-ray diffraction (XRD) measurement

The X-ray diffraction (XRD) measurement of the samples was conducted using a reflection method on a MiniFlex2 XRD diffractmeter (Japan Rigaku) using a monochromatized X-ray beam with a Cu K-radiation of 1.54 Å at 40 kV and 30 mA. Samples were gridded into powders so as to erase the effect of the crystalline orientation and freeze-dried. The patterns were collected in the region of 2θ from 5° to 85° at a scanning rate of 10° min.

2.5.4. Thermogravimetric analysis (TGA)

The changes of material mass loss with temperature were carried out in a TG-DTA instrument (Netzsch STA 449 F3) at a heating rate of 10 °C/min under nitrogen flow rate of 20 mL min^{-1}. Approx-