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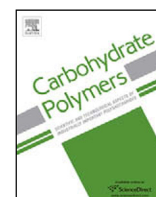

Antimicrobial application of nanofibrous mats self-assembled with quaternized chitosan and soy protein isolate

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ABSTRACT

Positively charged *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) and negatively charged soy protein isolate (SPI) were alternately assembled on cellulose acetate (CA) electrospun nanofibrous mats via electrostatic layer-by-layer self-assembly technique. CA nanofibrous mats coated with bilayers of HTCC and SPI possessed more orderly-arranged structure than uncoated CA mats according to the observation from scanning electron microscopy images. The average diameter of the nanofibers was enlarged by the increase of the bilayer number. X-ray photoelectron spectroscopy indicated that HTCC and SPI were coated on the surface of the CA mats successfully. The average diameters of inhibition zones of $(HTCC/SPI)_{10.5}$-films-coated nanofibrous mats against *Escherichia coli* and *Staphylococcus aureus* were 9.6 mm and 11.53 mm, respectively, which demonstrated the highest antimicrobial activity among samples in presented study.

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1. Introduction

The microorganism contamination on the surface of food products has led to safety and quality problems in food and consequent economic losses for food industry. Direct application of antibacterial substance on the surface of food has limited benefits (Quintavalla & Vicini, 2002), therefore research in developing biodegradable or edible films that are coated with antibacterial substances has been high-lighted (Appendini & Hotchkiss, 2002).

Chitosan, the second most abundant natural polymer found in nature, exists in a variety of living beings, such as crustaceans, fungi and insects (Bu, Wang, Fu, & Tian, 2012). This polysaccharide exhibits properties, such as nontoxicity, biocompatibility, biodegradability, antimicrobial activity and film forming, which make it a popular biopolymer for packaging applications (Bu et al., 2012). The positive charge of the amino group of chitosan at C-2 below its p*K*a (pH 6.5) creates a polycationic structure, which can interact with the anionic components at the outer membrane of bacteria, thereby disrupting the integrity of outer membrane and resulting in the inhibitory effects on bacterial growth (Alipour, Nouri, Mokhtari, & Bahrami, 2009; Helander, Nurmiaho-Lassila, Ahvenainen, Rhoades, & Roller, 2001; Tashiro, 2001). However, its poor water solubility at above pH 6.5 limits its applications in the field of bacterial inhibition due to the loss of cationic property (Qin et al., 2004). Therefore the development of water-soluble chitosan derivatives over a wide pH range has been intensively synthesized and studied (Lim & Hudson, 2004). *N*-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC), a water-soluble chitosan derivative with better antibacterial activity (Qin et al., 2004; Wang et al., 2009), is obtained by reacting chitosan with glycidyl trimethyl ammonium chloride (Ignatova, Starbova, Markova, Manolova, & Rashkov, 2006). The antibacterial activity of HTCC is resulted from damaging interaction of polycations with negatively charged surface of bacteria, leading to the loss of membrane permeability, the leakage of cell membrane and further the apoptosis of cells (Ignatova et al., 2006).

Soy protein isolate (SPI) is naturally derived from soybean by removing the carbohydrates and oil components (Kumar, Choudhary, Mishra, Varma, & Mattiason, 2002). It is edible. The byproduct of manufacture and the product of degradation are natural components, thus SPI is considered as a green adhesive

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(Kumar, Liu, & Zhang, 2008). Evidence has shown that incorporation of SPI into polymers (e.g. cellulose films) display biological safety to animals (Luo et al., 2015). In addition to the non-cytotoxicity, it has advantages in low price, non-animal origin, long-storage time and high stability. Thus, it has a significant potential in the field of food, agriculture and biomedical application (Kumar et al., 2008; Santin & Ambrosio, 2008; Silva, Vaz, Coutinho, Cunha, & Reis, 2003).

Cellulose acetate (CA) is a derivative of cellulose. It is cheap and can be easily fabricated to films with fine mechanical properties. CA nanofibrous mats produced by electrospinning technique have shown unique characteristics, such as ultrafine diameter, very large surface to volume ratio, small pore sizes, three dimensional (3D) nanofibrous structure (Deng et al., 2011a, 2011b). However, it has no property of microbial inhibition (Huang et al., 2012b).

Given the above mentioned advantage of SPI in the food application, HTCC in the field of bacteria inhibition and CA in the film fabrication, SPI and HTCC are employed in presented study and to be alternately deposited on CA mats via layer-by-layer (LBL) self-assembly technique to fabricate polymer films possessing antimicrobial activity. The LBL self-assembly technique introduced by Decher (Decher, 1997) has been widely used to prepare functional ultra-thin films in many fields. LBL deposition of those polyelectrolytes, nanoparticles and polyoxometalate on CA nanofiber has been well studied (Deng et al., 2010; Zhan et al., 2015; Zhang et al., 2015), however this approach for the deposition of SPI on CA substrates has never been studied to date. The antimicrobial effects of fabricated LBL-film-coated mats will be examined using two representative microorganisms *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*).

2. Experimental details

2.1. Materials

Cellulose acetate (CA, M_n 3×10^4) was purchased from Sigma Aldrich Co., USA. Soy protein isolated (SPI) was supported by Shandong Yuwang Industrial Co., ltd, China. Other chemical reagents used in this research were all of chemical grade. All aqueous solutions were prepared using purified water with a resistance of 18.2 MΩ cm. In addition, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) were obtained from State Key Laboratory of Agricultural Microbiology of Huazhong Agricultural University (Wuhan, China).

2.2. Fabrication of CA nanofibrous mats

CA nanofibrous mats were fabricated with electrospinning technique according to our previous reports (Deng et al., 2012; Huang et al., 2012a). Briefly, a 16 wt% of CA solution was prepared with 2/1 (w/w) acetone/*N*,*N*-dimethylacetamide mixture solvent. Then the prepared CA solution was loaded into a plastic syringe, which was connected to a metal needle tip. The syringe was driven with a syringe pump (LSP02-1B, Baoding Longer Precision Pump Co., Ltd., China). The positive electrode of a high voltage power supply (DW-P303-1ACD8, Tianjin Dongwen High Voltage Co., China) was clamped to the tip of the syringe. A grounded cylindrical collector covered with aluminum foil was rotated with a linear velocity of 100 m/min. The flow rate was set as 1 mL/h. The applied voltage was 16 kV and the tip-to-collector distance was 20 cm. The ambient temperature and relative humidity (KW-B1, Beijing Kawe Meters Co., Ltd, China) were maintained at 25 °C and 40%, respectively. During the electrospinning process, air conditioner (KFR-72LW/(72596)FNAa-A3, Gree Electric Appliances Inc. of Zhuhai, China) and dehumidifier (CH9488, SEN Electric, WGI Inc., USA) were running to maintain the consistence of temperature and relative humidity. Then the prepared fibrous mats were dried under vacuum to remove trace solvents.

2.3. Synthesis of N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride

N-[(2-hydroxy-3-trimethylammonium) propyl] chitosan chloride (HTCC) was obtained by reacting chitosan with glycidyl trimethyl ammonium chloride according to our previous studies (Deng et al., 2012; Huang et al., 2012a). Briefly, chitosan (5 g) was dissolved into 2% (w/v) of acetic acid (250 mL). After being soaked for 8 h, the solution was filtered to obtain purified chitosan. Then chitosan powder was put into boiling flask, additionally isopropanol (15 mL) and 2,3-epoxypropyl trimethylammonium chloride were added into chitosan powder under gentle agitation in the water bath of 80 °C. To obtain pure HTCC, the product was washed with ethanol. The obtained solid was subsequently dialyzed against deionized water for 3 days, and then lyophilized.

2.4. Zeta potential (ζ-potential) measurement

As-prepared CA mats (1 cm × 1 cm) were cut into pieces as small as possible and then dispersed in 200 mL of deionized water for 24 h under constant stirring. HTCC solution was prepared by adding 10 mg of powder in 200 mL of purified water at pH 5.0. SPI solution was prepared by dissolving 10 mg of SPI in 200 mL of purified water at pH 6.0. Both HTCC and SPI solution were kept under agitation for 24 h to obtain homogeneous solution. The prepared solutions were then filtered and taken to do ζ-potential measurement using Nano-25 zetasizer (Malven, England) at 20 °C.

2.5. Preparation of dipping solution for LBL process

Positively charged HTCC solution with a concentration at 1 mg/mL was prepared by dissolving HTCC in purified water. The pH value of the solution was adjusted to 5.0 (Huang et al., 2012a; Ruihua, Bingchao, Zheng, & Wang, 2012). The pH value of negatively charged SPI solution (1 mg/mL) was adjusted to 6.0 (Malhotra & Coupland, 2004; Tan, Ying-Yuan, & Gan, 2014). The ionic strength of all dipping solutions was regulated by the addition of NaCl at a concentration of 0.1 M.

2.6. Formation of LBL-structured films on CA nanofibrous mats

The schematic diagram of forming LBL-structured films on CA mats was shown in Fig. 1. First, CA nanofibrous mats were immersed into positively charged HTCC suspension for 20 min, followed by 2 min of rinsing with three pure water baths, respectively. Then the mats were immersed into the negatively charged SPI solution for 20 min followed by identical rinsing steps. The adsorption and rinsing steps were repeated until the desired number of deposition bilayers was obtained. All samples were firstly dried at room temperature and then dried at 30 °C for 2 h in vacuum prior to further characterizations. Here, $(HTCC/SPI)_n$ was used to label the LBL-structured films, where n was the number of HTCC/SPI bilayers. The outermost layer was HTCC when n equaled to 5.5 or 10.5. While n equaled to 5 or 10, the outermost layer was SPI.

2.7. Characterizations

The surface morphology of uncoated CA mats and LBL films coatings were observed with scanning electron microscopy (SEM, S-4800, Hitachi Ltd., Japan). The average diameter of fibers was measured using image analyzer (Adobe Photoshop 7.0). The surface elemental composition of the samples was identified with a Karato Axis Ultra Imaging X-ray photoelectron spectroscopy (XPS, Kratos

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