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Layer-by-layer immobilization of quaternized carboxymethyl chitosan/organic rectorite and alginate onto nanofibrous mats and their antibacterial application

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ABSTRACT

Quaternized carboxymethyl chitosan (QCM-chitosan) and organic rectorite (OREC) immobilized nanofibrous mats are fabricated via layer-by-layer (LBL) technique in a self-assembly manner. The negatively charged cellulose nanofibrous mats hydrolyzed from electrospun cellulose acetate (CEL) mats are alternately modified with the positively charged QCM-chitosan and OREC intercalated composites and the negatively charged sodium alginate (ALG) via LBL technique. The morphology and antibacterial activity of the resultant mats are studied by changing the number of deposition bilayers, the compositions of dipping solutions and outermost layer. X-ray photoelectron spectroscopy results imply that QCM-chitosan and OREC are coated on cellulose mats. Besides, wide angle X-ray diffraction and small angle X-ray diffraction are applied to investigate the crystalline of the composite mats and the interlayer distance of OREC, respectively. The antibacterial activity of the mats increases with the incorporation of OREC into LBL films.

1. Introduction

Electrostatic layer-by-layer (LBL) self-assembly technique, which is firstly reported by Decher (1997), is an efficient method to form multilayer ultra-thin films. It involves alternating adsorption of oppositely charged polyelectrolytes, particles, and ions on solid substrates (Lee, Kim, Chen, Shao-Horn, & Hammond, 2008; Song et al., 2013; Yang, Yang, Yang, Shen, & Yu, 2006). Natural biopolymers can be deposited on the surface of nanofibers to form composite materials via LBL coating process to endow a broad range of applications, including electrochromic devices (Sheng, Bai, Sun, Li, & Shi, 2011), fuel cells (Sun, Zhao, Yang, Song, & Xue, 2010), and electrochemical field (Li et al., 2011). Encouraged by our progress (Deng, Wang, et al., 2011), the cellulose mats with negatively charged fiber surface, which have low fiber density and good water insolubility, are considered as an ideal template for LBL deposition. It is difficult to obtain the cellulose mats via electrospinning technique. However, the template cellulose mats could be hydrolyzed from the electrospun cellulose acetate (CEL) mats in alkaline solution. Therefore, as the LBL template, the cellulose mats which are obtained by alkaline hydrolysis of CEL mats become popular.

CEL possesses good mechanical properties and relatively low cost. It is not only naturally abundant, but also widely applied in membranes for forward osmosis (Su, Yang, Teo, & Chung, 2010), chiral separation (Sueyoshi, Fukushima, & Yoshikawa, 2010) and water treatment (Tian et al., 2011). Additionally, CEL is easy to be electrospun into nanofibrous mats. The surface of cellulose mats can be modified by depositing other materials with LBL technique to obtain additional properties, such as antimicrobial activity.

In this study, alginate (ALG) and quaternized carboxymethyl chitosan (QCM-chitosan) were used as assembly objects for polyelectrolyte LBL assembling based on their electrical properties (Liu et al., 2012). ALG is a biodegradable linear polysaccharide made of (1 → 4) linked β-D-ManA and α-L-GulA units in varying proportions (Wang et al., 2014). ALG possesses properties of non-toxicity, low cost and hydrophilicity, and it is negatively charged from the carboxylate groups (Shalumon et al., 2011). Chitosan is one of the most abundant cationic polysaccharides with β-1,4-linked N-acetyl-D-glucosamine and D-glucosamine unit (Anitha et al., 2014; Pereda, Ponce, Marcovich, Ruseckaite, & Martucci, 2011), which possesses non-toxicity, biodegradability, and a broad antibacterial

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spectrum (Wang, Du, Luo, Lin, & Kennedy, 2007) (Sun, Du, Fan, Chen, & Yang, 2006). However, chitosan only shows the antimicrobial activity in acidic medium due to its poor solubility in neutral aqueous solution. In order to improve the solubility of chitosan and exploit its antimicrobial activity and biochemical significance, amphoteric derivatives are synthesized, such as N-carboxymethyl chitosan, methyl pyrrolidinone chitosan and N-carboxybutyl chitosan (Muzzarelli et al., 1990; Muzzarelli, 1988; Muzzarelli, Ilari, & Tomasetti, 1993; Muzzarelli, Tanfani, Emanuelli, & Mariotti, 1982). QCM-chitosan is a novel promising amphoteric polymer which is obtained by introducing carboxymethyl group and quaternary ammonium salt on chitosan (Ling, Luo, Luo, Wang, & Sun, 2013). The studies have showed many of its applications in antioxidant (Liu et al., 2009), drug encapsulation (Liang et al., 2008) and antibacterial activity (Sun et al., 2006). Interestingly, it has been reported that the antimicrobial activity is obviously enhanced when the chains of the QCM-chitosan is intercalated into the interlayer of organic rectorite (OREC) (Deng et al., 2012). It has also proved that the chitosan-OREC intercalated composites have better antibacterial activity than chitosan itself because OREC could absorb more bacteria and inhibit its growth (Wang et al., 2006).

OREC is modified by cation exchange from REC. OREC is a kind of layered silicate, which has larger interlayer distance, thicker layer and larger aspect ratio than REC or montmorillonite (Deng et al., 2012). It has many properties, including long expectancy of life, high resistance to heat and weak activity of inhibiting mold. In our previous report (Deng et al., 2012), the inhibiting effects of other antimicrobial reagents are remarkably enhanced by the addition of OREC. The positive potential and the enlarged interlayer distance of OREC exert the synergetic antimicrobial effect. As mentioned above, we speculate that the (QCM-chitosan/OREC)/ALG composite multilayers possess enhanced antimicrobial activity in water solution.

In the study, we developed a straightforward method for the fabrication of the nanofibrous mats containing OREC for bacterial inhibition via LBL self-assembling of QCM-chitosan and ALG. The cellulose template was obtained via hydrolyzing the electrospun CEL mats in alkaline solution. The Field Emission Scanning Electron Microscope (FE-SEM) was applied to investigate the morphology. The composition of the multilayer was investigated by Fourier transform infrared spectra (FT-IR) and X-ray photoelectron spectroscope (XPS). The Small angle X-ray diffraction (SAXRD) and Wide angle X-ray diffraction (WAXRD) were employed to study the layered structure of OREC and the crystallization of the mats. Finally the bacterial inhibition experiments were performed to examine the antimicrobial properties of the resultant samples.

2. Materials and methods

2.1. Materials

Cellulose acetate (CEL, $M_n = 3 \times 10^4$) was obtained from Sigma Aldrich Chemical Reagent Co., USA. Calcium rectorite (Ca^{2+}-REC) was supplied by Hubei Mingliu Co., China. Chitosan ($M_w = 2.1 \times 10^5$ Da, DD = 92%) was purchased from Yuhuan Ocean Biochemical Co., China. Sodium alginate (ALG, $M_w = 398.31$) and sodium dodecylsulfonate (SDS) was provided by Aladdin Chemical Reagent Co., China. The 2,3-epoxypropyl trimethylammonium chloride was obtained from Adamas Reagent Co. Ltd., China. Acetone, N,N-dimethylacetamide (DMAc), acetic acid, sodium hydroxide, hydrogen chloride, and sodium chloride were supplied by Aladdin Chemical Reagent Co., China. Chloroacetic acid was provided by Xi Ya Reagent Co., China. *Escherichia coli* and *Staphylococcus aureus* were obtained from State Key Laboratory of Agricultural Microbiology of Huazhong Agricultural University (Wuhan, China). Nutrient agar and nutrient broth were supplied by Qingdao Rishui Biological Technology Co. (Qingdao, China). Other chemicals were of analytical grade. And aqueous solutions were prepared using deionized water with a resistance of 18.2 MΩ cm.

2.2. Preparation of OREC

The OREC was synthesized by cation exchange reactions between REC and SDS. In brief, the REC was dispersed in deionized water to obtain the suspension with vigorous stirring for 72 h, then added SDS slowly into the obtained suspension at 90 °C with 5 h stirring. Additionally, the resultant sample was washed several times with deionized water and filtered to remove excessive SDS. Finally the product was dried at 90 °C to obtain OREC.

2.3. Preparation of quaternized carboxymethyl chitosan (QCM-chitosan)

QCM-chitosan was prepared according to the previous report (Wang et al., 2010). Five grams of chitosan was alkalified by 6 ml of 50% NaOH solution (w/w) and then frozen for 24 h at −20 °C. After 24 h, the frozen alkali chitosan was transferred to three-necked flask. Subsequently, 100 ml of isopropyl alcohol and 6 g of chloroacetic acid were added into frozen alkali chitosan in the three-necked flask. The mixture was kept under stirring at room temperature for 2 h. After that the temperature was elevated to 60 °C, and the mixture was continuously stirred for another 3 h. After 3 h, the mixture was cooled to room temperature and filtered. The residue was dissolved in 250 ml water. The pH value of obtained solution was adjusted to 7.0 with dilute hydrochloric acid solution. Then the solution was dialyzed in deionized water at 25 °C for 5 days followed by rotary evaporation at 65 °C for 90 min, and then lyophilized to obtain carboxymethyl chitosan (CM-chitosan). Three grams of CM-chitosan and 20 ml of water were transferred to a three-necked flask under stirring at 80 °C, then 100 ml of 2,3-epoxypropyl trimethylammonium chloride solution was added into the above mixture solution, and the reaction continued for 8 h. Finally the solution was dialyzed in deionized water at 25 °C for 5 days and lyophilized to obtain the spongy solid QCM-chitosan.

2.4. Preparation of template cellulose nanofibrous mats

Cellulose mats were fabricated based on our previous report (Huang et al., 2012). Sixteen percent of CEL solution was prepared by adding CEL powder into the mixed solution of acetone and DMAc (2:1 in wt/wt). The distance of tip-to-collector, applied voltage and delivery speed of solutions was 20 cm, 16 kV and 1 ml/h, respectively. The ambient temperature and relative humidity were kept at 25 °C and 45%, respectively. Then the as-prepared mats were dried in vacuum at 80 °C for 24 h in order to remove trace solvents. Finally the cellulose nanofibrous mats were obtained by hydrolysis of CEL mats in 0.05 M of NaOH solution at room temperature for 7 days.

2.5. Preparation of LBL structured nanofibrous mats

ALG, QCM-chitosan and QCM-chitosan/OREC solution were prepared as follows. To obtain 1 mg/ml of ALG solution and 1 mg/ml of QCM-chitosan solution, ALG powder and QCM-chitosan powder were dissolved, respectively, in the water under continuous stirring for 4 h at room temperature. The QCM-chitosan/OREC (the mass ratio of a QCM-chitosan and OREC was 6:1) solution with a concentration of 1 mg/ml were fabricated by dropwise adding the QCM-chitosan solution into OREC suspension under stirring for 12 h. The pH values of QCM-chitosan, QCM-chitosan/OREC and ALG solutions were adjusted to 5, 5 and 4, correspondingly. At last, the