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Facile approach to prepare drug-loading film from hemicelluloses and chitosan

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ABSTRACT

This study introduces a facile and green route to fabricate film from bio-based polymers. The film has been prepared by the cross-linking reaction of quaternized hemicelluloses (QH) and chitosan (CHO) with epichlorohydrin (ECH) as crosslinker. It exhibits an excellently mechanical performance as a result of its high tensile strength (up to 37 MPa). Importantly, the roughness of film was 2–5 nm in the area of 400 nm, and smooth surface with pores were presented on the film based on the results of scanning electron microscope (SEM) and atomic force microscope (AFM). Ciprofloxacin was utilized as a mode compound to investigate the loading behavior of the film, and the highest loading concentration was about 18%. The drug release was about 20% in film1 in comparison to only 15% in film3 within 48 h. Furthermore, the results of a 293T cell viability assay indicated its good biocompatibility and non-toxicity.

1. Introduction

Wound infection is the major problem among the wound care managements (Sinha, Banik, Haldar, & Maiti, 2013). A right approach to deal with the infection is the key point in the surgical department. Physiologically, wound repair normally involves systematic, coordinated and balanced activity of inflammatory, vascular, connective tissue, and epithelial cells (Boateng, Matthews, Stevens, & Eccleston, 2008). Therefore, the wound repairs often involve the use of an ideal material to cover the wound so as to protect the wound from any bacterial infection, inhibit bleeding and prevent water and electrolyte disturbances (Zahedi, Rezaeian, Ranaei-Siadat, Jafari, & Supaphol, 2010). Ciprofloxacin is a synthetic fluoroquinolone antibiotic that has been used for a variety of topical applications, such as skin, eye, nose, and ear infections (Campoli-Richards et al., 1988; Kevadiya et al., 2014). The ciprofloxacin possessed low price, good bactericidal effect, and high antibacterial activity. It is one of the most widely used antibiotics in wound healing because of its low minimal inhibitory concentration for both Gram-positive and Gram-negative bacteria that cause wound infections (Tsou et al., 2005; Yu et al., 2006).

Among various kinds of wound-dressings, composite films consisting of dense outer layer and porous sub-layer were benefit for skin-breathing and drug-loading. Many polymeric materials are investigated for the preparation of wound-dressing which include synthetic polymers made from petroleum (Mi et al., 2001; Ye, Wang, Liu, & Tong, 2005). But there are a few disadvantages of these synthetic polymers such as toxicity, slow biodegradability, non-renewable, environmental pollution, and immune response (Naira & Laurencin, 2007). Nowadays, biomass-based polymer is going to replace petroleum-based plastic due to the oil crisis and global environmental disruption (Boonkong, Petsom, & Thongchul, 2013). Hemicelluloses, representing about 20–35% of lignocellulosic biomass, have emerged as an immense renewable resource of biopolymers. In order to use hemicelluloses as novel biopolymers for functional biomaterials, chemical modification is necessary (Peng, Ren, & Sun, 2011; Sárossy et al., 2012). The chemical modification allows hemicelluloses to have special properties which can broaden their applications, such as acetylation, oleoylation, and lauroylation (Peng, Ren, Peng, Xu, & Sun, 2008; Ren, Sun, Liu, Cao, & Luo, 2007; Sun, Sun, & Sun, 2004). Quaternization of hemicelluloses could enhance their solubility and yield, cationic or ampholytic polymers which have similar chemical properties to quarternized derivatives obtained from starch, cellulose, and chitosan (Ren, Sun, Liu, Lin, & He, 2007). Meanwhile, quaternary ammonium ion itself

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has fairly good antibacterial property; the antibacterial mechanism of quaternized derivatives was also similar to that of chitosan.

Chitosan is a polysaccharide which is an N-deacetylated derivative of chitin. The unique structural feature of chitosan is the presence of primary amines at the C-2 position of the D-glucosamine residues (Wang & Zhang, 2014). These amine groups, in the first place, allow specific chemical reactions, and secondly confer important functional properties to chitosan (Ashori, Cordeiro, Faria, & Hamzeh, 2013). Chitosan has a great potential for a wide range of application fields due to its biodegradability, biocompatibility, non-toxicity, antimicrobial activity, and versatile chemical and physical properties (Rinaudo, 2006). It can be easily processed into films, gels, nanofibers, nanoparticles and scaffolds, thus it is used in advanced biomedical and pharmaceutical applications, tissue engineering, controlled drug delivery, biotechnology, and in the food industry (Jayakumar, Prabaharan, Nair, & Tamura, 2010; Park, Saravanakumar, Kim, & Kwon, 2010). Chitosan is easily processed into various forms which provide a wide variety of biomedical applications in tissue engineering, wound dressing, cancer drug delivery, and drug targeting in the area of nanobiotechnology (Nagarwal, Kant, Singh, Maiti, & Pandit, 2009).

The reports of films based on the hemicelluloses have been shown to possess good oxygen and grease barrier properties (Mikkonen et al., 2009; Mikkonen, Heikkilä, Helén, Hyvönen, & Tenkanen, 2010). However, because of its brittle, low moisture barrier property, and poor film-forming property, the hemicelluloses-based film was urged to be improved by adding other functional polymers (Mikkonen & Tenkanen, 2012). Usually, chemical cross-linking was an effective approach to obtain excellent properties of films such as high transparency, mechanical property, and homogeneous surfaces (Chang, Chen, & Zhang, 2011). Furthermore, epichlorohydrin has been used as a cross-linking agent of polymers by forming the glycidylether linkage through the reactions with hydroxyl groups of polysaccharide (Ahola, Österberg, & Laine, 2008). In this paper, a crosslink reaction was introduced to prepare QH/CHO composite films with epichlorohydrin as the crosslinker. The ciprofloxacin is selected as mode compounds to study the loading behavior of the composite film. The interactions among the macromolecules and the crosslinker are important, which would affect the loading properties of films. The structure of film was investigated by FT-IR and XRD analyses, and the AFM and SEM technologies were used to detect the morphology of the film. Finally, drug-loading and biocompatibility studies were performed to demonstrate the application of the wound-dressing.

2. Experimental

2.1. Materials

Quaternized hemicelluloses, which were prepared in the previous study (Guan et al., 2014), the average molecular weight (M_w) was 9 240 g mol^{-1} tested by gel permeation chromatography (GPC). The degree of substitution determined by the nitrogen content (DS_N) value of the modified hemicelluloses was 0.25, which was calculated from the ratio of the nitrogen to the carbon content according to the following equation: $DS_N = (60 \times \%N)/(14 \times \%C - 72 \times \%N)$) (Ren, Sun, Liu, Chao, & Luo, 2006). Chitosan (degree of acetylation of 80–95%, M_w = 200 000 g mol^{-1}, PDI = 1.5) was purchased from Sinopharm Chemical Reagent Co. Ltd. Epichlorohydrin (ECH, Chemical Agents Ltd. Co., Shanghai, China) (1.18 g mL^{-1}) was of analytical-grade, and was used without further purification. All the used reagents were of analytical grade.

Table 1
Films prepared with different conditons.

Number	ECH (mL)	Reaction time (h)	Reaction temperature (°C)
film1	0.2	1	25
film2	0.5	1	25
film3	0.2	1	60
film4	0.5	1	60

2.2. Preparation of QH/CHO films

To prepare the QH solution, a certain amount of quaternized hemicelluloses were dispersed into distilled water for 12 h, and then the obtained solution was stored at 4 °C after centrifugation. Chitosan solution was prepared by dissolving chitosan in 0.1% aqueous acetic acid at 60 °C for 30 min. After being purged with vacuum for 2 h to remove the oxygen from the system, the solution was heated to 60 °C, and then QH solution with the same concentration were added followed by adding ECH. The mixture was kept in water bath at 60 °C. Then, the solution was cast on a glass mould for air-drying. Finally, the film was vacuum-dried for 2 h. The different reaction conditions of QH/CHO films were shown and labeled in Table 1, and the quality ratio of QH/CHO was keep at 1:1.

2.3. Characterization

For the raw samples of QH and chitosan, the FT-IR spectra was recorded using a Thermo Scientific Nicolet iN 10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen cooled MCT detector. Dried samples were grounded and palletized using BaF_2 and their spectra were recorded from 4000 to 650 cm^{-1} at a resolution of 4 cm^{-1} and 128 scans per sample. The crystalline structure of the film samples were measured on a XRD-D8 Advance instrument (Bruker, Germany) with Ni-filtered Cu-Kα radiation generated at 40 kV and 30 mA. The X-ray diffraction (XRD) patterns were recorded from 5° to 60° (2θ) with a scanning speed of 2° min^{-1}. SEM of the film samples were carried out with a Hitachi S-3400N II (Hitachi, Japan) instrument at 15 kV. Prior to taking pictures, the samples were sputter-coated with a thin layer of gold. Images were obtained at magnifications ranging from 200× to 5000×, which was dependent on the feature to be traced. Thin film topography was analyzed using a Multimode8 Atomic Force Microscope (AFM) (Bruker, Germany). AFM images were obtained in phase contrast mode with vibration amplitude of 100 mV using an n-type silicon cantilever with a tip radius of curvature less than 10 nm and resonance frequency of approximately 300 kHz. UV–vis absorption spectra were obtained using an ultraviolet/visible spectrophotometer (Tech Comp, UV 2300) within the range of 200–800 nm. Before UV–vis measurements, the films were pasted on the surface of quartz pool. The tensile strength of the films was tested using a universal tensile tester (UTM6503, Shenzhen Suns Technology Stock CO. LTD. China) at room temperature. The film samples were 25 mm × 10 mm in dimension (length × width), and a tensile speed of 5 mm min^{-1} were employed for the test.

2.4. Cell viability assay

QH/CHO films were tailored to a disk with the diameter of 15 mm and sterilized in an ultraviolet irradiation for 60 min before the cells were cultured. 293T cells (5×10^4 cells per well) were seeded on the surface of the sterilized films with 10% FBS, and incubated at 37 °C. After incubation at 37 °C for 48 h, the medium was removed. Fresh medium (1 mL) and MTT (100 μL, 5 mg mL^{-1}) were added to each well, followed by 4 h of incubation at 37 °C. Subsequently, the supernatant was carefully removed, and 1 mL