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Development of silver sulfadiazine loaded bacterial cellulose/sodium alginate composite films with enhanced antibacterial property

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ABSTRACT

Sodium alginate (SA) and bacterial cellulose (BC) are widely used in many applications such as scaffolds and wound dressings due to its biocompatibility. Silver sulfadiazine (AgSD) is a topical antibacterial agents used as a topical cream on burns. In the study, novel BC/SA–AgSD composites were prepared and characterized by SEM, FTIR and TG analyses. These results indicate AgSD successfully impregnated into BC/SA matrix. The swelling behaviors in different pH were studied and the results showed pH-responsive swelling behaviors. The antibacterial performances of BC/SA–AgSD composites were evaluated with *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. Moreover, the cytotoxicity of BC/SA–AgSD composites was performed on HEK 293 cells. The experimental results showed BC/SA–AgSD composites have excellent antibacterial activities and good biocompatibility, thus confirming its utility as potential wound dressings.

1. Introduction

Development of novel wound dressing has attracted more and more attentions in recent years. Silver is known to be a powerful antibacterial agent with effective broad-spectrum against Gram-positive and Gram-negative microorganisms (Barud et al., 2011; Jung, Kim, Kim, & Jin, 2009). Silver-based materials with antimicrobial properties were studied by many researchers (Maneerung, Tokura, & Rujiravanit, 2008; Maria et al., 2010). Bacterial cellulose-silver nanocomposites were successfully prepared and they exhibited excellent antibacterial activity (Barud et al., 2008; Maria et al., 2009; Shao et al., 2015). Silver compounds such as silver sulfadiazine (AgSD), are used widespreadly in burn and wound treatment for its broad activity spectrum and wound healing promotion (Aguzzi et al., 2014). In particular, AgSD is considered to be the first choice for treatment in skin chronic lesions and burns (Atiyeh, Costagliola, Hayek, & Dibo, 2007). However, there may be some adverse effects of AgSD in clinical studies, such as cytotoxicity and allergic reactions, which lead to retard wound healing processes (Dellera et al., 2014; Sandri et al., 2014). Therefore, an alternative strategy is required to improve drug efficacy.

Sodium alginate (SA), a linear unbranched copolymer of 1,4-linked β-D-mannuronate (M) and α-D-guluronate (G), is isolated from marine algae (Seo et al., 2012). It is widely used in many applications such as scaffolds and wound dressings due to its biocompatibility, biodegradability under normal physiological conditions and capacity for bioresorption of the constituent materials (Becker, Kipke, & Brandon, 2001; Trandafilović, Božanić, Dimitrijević-Branković, Luyt, & Djoković, 2012). However, the rigid and fragile nature of the gelatinous SA may also be unfavorable in processing into non-spherical forms such as films and filaments *via* the gel state (Shalumon et al., 2011). A method to overcome this drawback is to blend SA with a compatible polysaccharide biopolymer.

Bacterial cellulose (BC) is another biopolymer of great potentials, which features a distinctive three-dimensional structure consisting of an ultrafine network of cellulose nanofibers (Czaja, Young, Kawecki, & Brown, 2007). This unique micromorphology enables it to have great water holding capacity, good conformability, high porosity, high crystallinity, excellent mechanical strength and large surface area, which determines its potential application as an excellent wound dressing material (Jonas & Farah, 1998; Yang, Xie, Hong, Cao, & Yang, 2012). A novel composite matrix which

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gained the beneficial properties of both BC and SA was developed. Previous studies have shown that SA and BC blends are biocompatible, the blend leading to an increase in the thermomechanical stability (Shi, Zheng, Wang, Lin, & Fan, 2014). However, both BC and SA are lack of antibacterial property, resulting in failing to provide a barrier against wound infection, which limits the possibilities of application in wound dressing areas.

To gain the beneficial properties of AgSD, BC and SA, a novel porous composites based AgSD loaded a blend of BC and SA as an efficient wound healing dressing was developed in this study. In our study, the ratio of BC:SA was chosen to be 4:1 since the tensile strength and elongation at break of BC/SA composite with this ratio were the higher than other ratios (3:2, 2:3, 1:4 and pure SA) (Phisalaphong, Suwanmajo, & Tammarate, 2008). Furthermore, the structure of BC/SA composites with alginate less than 30% was more stable after 2 h of immersion in PBS (Chiaoprakobkij, Sanchavanakit, Subbalekha, Pavasant, & Phisalaphong, 2011). BC/SA–AgSD composites were characterized by Scanning Electron Microscope (SEM), Fourier transform infrared spectra (FTIR) and thermogravimetric analyses (TG). The swelling behaviors of BC/SA–AgSD composites at different pH values were studied. The antibacterial activities of the obtained BC/SA–AgSD composites were investigated by Gram-negative *Escherichia coli* (*E. coli*) ATCC 25922, Gram-positive *Staphylococcus aureus* (*S. aureus*) ATCC 6538 and yeast *Candida albicans* (*C. albicans*) CMCC(F) 98001, respectively.

2. Materials and methods

2.1. BC preparation

BC was prepared in a static culture medium by *A. xylinum* GIM1.327, which was purchased from BNBio Tech Co., Ltd., China. The method of preparing BC was well-established and described in literature (Ge et al., 2011). Briefly, in a static culture system enriched with polysaccharides, bacterial strain was incubated and was able to produce a thin layer of BC in the interface of liquid/air (Shi et al., 2012). This layer was washed by de-ionized water and then boiled in a 0.1 M NaOH solution at 80 °C for 60 min to eliminate impurities such as medium components and attached cells. BC films were further washed thoroughly with de-ionized water until pH became neutral.

2.2. Production of BC/SA–AgSD composites

To prepare BC/SA–AgSD composite, the preparation procedure is separated into three stages. Firstly, 20 g obtained wet BC membranes were cut into small pieces and crushed by high speed homogenizer at 15,000 rpm for 30 min to form BC fiber slurry. Secondly, SA was dissolved in distilled water to achieve 2.0% (w/v) at room temperature to form a gel-like solution. To prepare BC/SA hybrid composite, BC slurry and SA solution, were mixed with the weight ratios of wet BC and SA solution at 4:1, to obtain homogenous BC/SA dispersions (marked as BS_0). Thirdly, AgSD was added into the BC/SA dispersions and mixed for 30 min. The weight ratio of AgSD to BC/SA was controlled to be 0.008 wt%, 0.016 wt%, 0.024 wt%, 0.06 wt% and 0.1 wt% (marked as BS_1, BS_2, BS_3, BS_4 and BS_5, respectively). Then the mixture was treated by ultrasonication for degassing at supersonic power of 500 W for 3 min under ice-water bath. 0.25 mL mixture was placed in a 48-well plate and cross-linked by an aqueous solution of 5% $CaCl_2$ for 3 h and rinsed by de-ionized water to remove the excess cross-linking agents. The homogeneous dispersions were freeze-dried at −40 °C for 10 h.

2.3. Characterization

A JSM-7600F Scanning Electron Microscope (SEM) operating at an accelerating voltage of 10–15 kV was used to investigate the surface morphologies of BC and BC-Ag nanocomposites. The samples were coated with a thin layer of gold under high vacuum conditions (20 mA, 100 s). Fourier transform infrared (FTIR) spectra were recorded on a Spectrum Two Spectrometer (Perkin Elmer, USA) with the wavenumber range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. Thermogravimetric analysis (TG) was carried out by using a TA Instruments model Q5000 TGA. The samples were heated from 20 to 800 °C with a heating rate of 10 °C/min under nitrogen atmosphere.

2.4. Porosity calculation

The bulk density of the composite (ρ_f) was calculated with Eq. (1):

$$\rho_f = \frac{W_0}{V_0} \tag{1}$$

where W_0 is the weight of the composite and V_0 is the volume of the composite, which was measured with the modified method of Lin et al. (2014). Briefly, the samples were infiltrated with 99% ethanol in a 25-mL beaker under −0.08 MPa for 5 min in a vacuum oven. Subsequently, the tested sample was weighed in a 5-mL test tube and recorded as W_1 and weighed again after ethanol was filled in the tube and recorded as W_2. V_0 was calculated from Eq. (2):

$$V_0 = 5 - \left[\frac{(W_2 - W_1)}{\rho_{\text{ethanol}}}\right] \tag{2}$$

where ρ_{ethanol} is the density of ethanol and is 0.79 g/cm^3 at room temperature. The density of the solid BC/SA material (ρ_{BS}) was calculated with Eq. (3):

$$\rho_{BS} = \frac{1}{[\mu_f/\rho_{BC} + (1-\mu_f)/\rho_{SA}]} \tag{3}$$

where μ_f is the weight fraction of BC in the BC/SA composite which is 80% in our study; ρ_{BC} and ρ_{SA} refer to the densities of BC and 2 wt% SA and were calculated to be 1.01 and 1.013 g/cm^3. The density of the solid BC/SA–AgSD composite (ρ_s) was calculated with Eq. (4):

$$\rho_s = \frac{1}{[(1-\mu_{AgSD})/\rho_{BS} + \mu_{AgSD}/\rho_{AgSD}]} \tag{4}$$

where μ_{AgSD} is the AgSD weight fraction in the BC/SA–AgSD composite; ρ_{AgSD} refer to the densities of AgSD and were 1.496 g/cm^3. The porosity of the composites was calculated from Eq. (5):

$$\text{Porosity} = \left(1 - \frac{\rho_f}{\rho_s}\right) \times 100\% \tag{5}$$

2.5. Swelling behavior assays

The swelling behaviors of the composites under different pH values were determined through a gravimetric method (Li et al., 2011). Initially, the tested films were cut into round pieces in diameter of 10 mm and their dry weights (W_0) were accurately measured. The dry samples were immersed in PBS solution with pH 7.4, and the solutions with pH 2.5 and 11.5 that prepared by dilution of 1 M NaOH and 1 M HCl at room temperature. After 15 h immersion to obtain equilibrium swelling, the swollen membranes were withdrawn. The wet weight of the swollen membranes (W_1) was measured after the removal of excess surface water by gently blotting with a filter paper. All testing was proceeded in triplicate; the