Journal of the Taiwan Institute of Chemical Engineers 63 (2016) 404–410

Contents lists available at ScienceDirect

Journal of the Taiwan Institute of Chemical Engineers

journal homepage: www.elsevier.com/locate/jtice

Preparation, antibacterial activity and pH-responsive release behavior of silver sulfadiazine loaded bacterial cellulose for wound dressing applications

Wei Shao[a,*], Hui Liu[a], Jimin Wu[a], Shuxia Wang[a], Xiufeng Liu[b], Min Huang[a], Peng Xu[a,*]

[a] College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, PR China
[b] Jiangsu Key Laboratory of TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing, Jiangsu 211198, PR China

ARTICLE INFO

Article history:
Received 2 November 2015
Revised 25 January 2016
Accepted 9 February 2016
Available online 4 March 2016

Keywords:
Bacterial cellulose
Silver sulfadiazine
pH-sensitive controlled release
Antibacterial activity
Biocompatibility

ABSTRACT

Bacterial cellulose (BC) has been extensively explored as some of the most promising biomaterials for biomedical applications. However, BC does not have intrinsically antibacterial property. In the present study, antibacterial BC-silver sulfadiazine (AgSD) composites were prepared by a simple blend method since AgSD is a topical antibacterial agents used as a topical cream on burns. The prepared composites were characterized by several techniques including SEM, FTIR, XRD and TG. These results indicate AgSD was successfully impregnated into BC matrix. The releases of Ag^+ and SD^- ions from BC-AgSD composites at different pH values were studied, which showed pH-sensitive controlled release behaviors. The antibacterial performances of BC-AgSD composites were evaluated with *Staphylococcus aureus* and *Candida albicans*. Moreover, the cytotoxicity of BC-AgSD composites was performed on HEK293 cells. The present work demonstrates a facile way to prepare BC-AgSD composites with excellent antibacterial activities and good biocompatibility, which can be used as potential wound dressings.

1. Introduction

Development of novel wound dressing has attracted more and more attentions in recent years, including alginates, chitosan and hyaluronic acid based biomaterials [1–3]. Bacterial cellulose (BC), a polysaccharide produced mainly by the acetic acid bacterium *Gluconacetobacter xylinus* (formerly *Acetobacter xylinus*), is chemically the same as plant cellulose and features a distinctive three-dimensional structure consisting of an ultrafine network of cellulose nanofibers [4]. This unique micromorphology determines its potential application as an excellent wound dressing with its distinguished physical and mechanical properties, *e.g.* high porosity, high crystallinity, excellent mechanical strength and large surface area because its 3-D network structure enables it to have great water holding capacity, good conformability and excellent wet strength [5,6]. However, BC does not have intrinsically antibacterial property, resulting in failing to provide a barrier against wound infection, which limits the possibilities of application in the wound dressing areas. One suitable revolution is to load antibacterial agents into BC matrix.

Silver is known to be a powerful antibacterial agent with effective broad-spectrum against Gram-positive and Gram-negative microorganisms, many aerobes and anaerobes, and several antibiotic resistant strains that has been used since ancient times [6, 7]. Recent technical innovations facilitate the incorporation of silver-based materials to commercial formulations with antimicrobial properties [8]. Bacterial cellulose-silver nanocomposites were successfully prepared and they exhibited excellent antibacterial activity [6,9]. Silver compounds such as silver nitrate and silver sulfadiazine (AgSD) are still largely used in burn and wound treatment for their broad activity spectrum [10]. In particular, AgSD is considered to be the first choice for treatment in skin chronic lesions and burns [11]. The chemical structure of AgSD is displayed in Fig. 1, which consists of both silver and SD. Therefore, AgSD has not only excellent antibacterial property, but also wound healing activity due to the existence of SD. However, there are still some controversies on the employment of AgSD in clinical studies. Storm-Versloot et al. [12] pointed out there exists delayed wound healing which is mainly due to the demonstrated cytotoxicity of AgSD toward fibroblasts and keratinocytes *in vitro* and consequently to retard wound healing *in vivo* [13–15]. In order to control the release behavior and decrease the cytotoxic action of AgSD, BC was considered to be ideal matrix act as the drug carrier. AgSD had been loaded into BC by impregnation method with limited and acceptable AgSD loading [16].

* Corresponding authors. Tel.: +86 25 85427024; fax: +86 25 85418873.
E-mail addresses: w.shao@njfu.edu.cn (W. Shao), xupeng@njfu.edu.cn (P. Xu).

http://dx.doi.org/10.1016/j.jtice.2016.02.019
1876-1070/

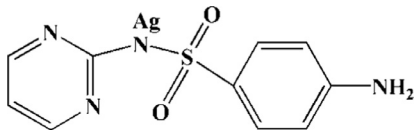

Fig. 1. Chemical structure of AgSD.

In the present work, BC-AgSD composites were prepared by loading AgSD particles into BC matrix via a simple blending method. BC-AgSD composites were characterized by Scanning Electron Microscope (SEM), Fourier transform infrared spectra (FTIR), X-ray diffraction (XRD) and thermogravimetric analyses (TG). The release behaviors of Ag and SD ions from BC-AgSD composites at different pH values were studied. The antibacterial activities of the obtained BC-AgSD composites were investigated by *Staphylococcus aureus* (*S. aureus*) ATCC 6538 and fungal *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 [17]. Briefly, in a static culture system enriched with polysaccharides (5 g/L peptone, 5 g/L yeast, 5 g/L glucose, 5 g/L mannitol and 1 g/L $MgSO_4{\cdot}7H_2O$), bacterial strain was incubated at 30 °C for 5 days and was able to produce a thin layer of BC in the interface of liquid/air [6]. 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 membranes were further washed thoroughly with de-ionized water until pH became neutral.

2.2. Production of BC-AgSD composites

5 g obtained wet BC membranes were cut into small pieces and crushed by high speed homogenizer (XHF-D, Ningbo Xingzhi Biotechnology, China) in 50 mL de-ionized water at 15,000 rpm for 30 min to achieve BC fiber slurry. AgSD (Aladdin, China) was added into the BC homogenate and treated by ultrasonication at supersonic power of 500 W for 30 min under ice-water bath. The weight ratios of AgSD to wet BC membrane were controlled to be 0.008 wt%, 0.016 wt%, 0.024 wt%, 0.06 wt% and 0.1 wt% (marked as BC_1, BC_2, BC_3, BC_4 and BC_5, respectively). The homogeneous dispersions were filtered through cellulose acetate membrane filter (0.22 mm pore size, 47 mm in diameter) by filtration under negative pressure at −0.1 MPa. Finally, BC-AgSD composites 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-AgSD nanocomposites. The samples were coated with a thin layer of platinum 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}. XRD patterns of the samples were recorded using a Rigaku Ultima III X-ray powder diffractometer, using a Cu Kα X-ray tube with a wavelength of 1.5406 Å, running at 40 kV and 30 mA, respectively. The diffraction angle (2θ) ranged from 5° to 60° with a step size of 0. 02°. Thermogravimetric analysis (TG) was carried out by using a TA Instruments model Q5000 TGA. The samples were heated from 20 to 600 °C with a heating rate of 10 °C /min under nitrogen atmosphere.

2.4. In vitro release assays

The release behaviors of silver and SD ions from the prepared BC-AgSD composite (BC_5) were studied. The tested composites were cut into round pieces in diameter of 10 mm. In order to determine the effect of pH on the release profiles of the composites, HEPES (Sigma) buffers with different pH values at 5.5, 7 and 8.5 were used, respectively. Free AgSD powders with the same amount of BC_5 were used as control. The prepared samples were placed in dialysis bags (MW 3500 KDa) and immersed in a beaker containing 100 mL 10 mM HEPES at 37 °C and sealed using PARAFILM®M without any agitation. The concentrations of Ag^+ in solution withdrawn from the test medium at fixed time intervals were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and SD ion concentrations were analyzed by a SHIMADZU UV 2450 spectrophotometer. An equivalent volume of fresh HEPES buffer was replaced into the system after each sampling to maintain constant medium volume.

2.5. Antibacterial activity

The antibacterial activities of BC-AgSD composites were investigated against *S. aureus* ATCC 6538 and *C. albicans* CMCC(F) 98001 by disk diffusion method. BC-AgSD composites and BC (the control) were cut into round shapes with 10 mm diameter and sterilized by ultraviolet lamp for 60 min.

Lawns of test bacteria (about 0.8×10^5 CFU/plate) were prepared on TSA. The sterilized samples were then carefully placed upon the lawns and BC was used as control. The plates were placed in a 37 °C incubator for 24 h. Then inhibitory action of tested samples on the growth of the bacteria was determined by measuring diameter of inhibition zone.

2.6. Cytotoxicity tests

The HEK293 cell line was cultured in RPMI medium supplemented with 10% FBS, 100 μg/mL penicillin and 100 μg/mL streptomycin. The cells were then incubated for 3 days in a humidified 5% CO_2-containing balanced-air incubator at 37 °C.

The cytotoxicity was measured using the MTT assay method. 200 μL of HEK293 cells, at a density of 1×10^5, were placed in each well of a 48-well plate. Then the cells were incubated over night at 37°C in a humidified 5% CO_2-containing atmosphere. After that, media was discarded. BC-AgSD composites with same size (5 mm × 5 mm) were placed slightly on the top of cells and then fresh media was added. Wells containing only the cells were used as control. The cells were treated for another 24 h. Then the media containing sample was changed with fresh media and 20 μL of dimethyl thiazolyl diphenyl (MTT) was added and the incubation continued for 6 h. Medium was removed, and 200 μL DMSO was added to each well to dissolve the formazan. The absorbance was measured with a test wavelength of 570 nm and a reference wavelength of 630 nm. Empty wells (DMSO alone) were used as blanks. The relative cell viability was measured by comparison with the control well containing only the cells.

3. Results and discussion

3.1. Surface morphology

In this study, AgSD particles with different loadings were incorporated into the BC matrix by a simple blend method. When BC