Materials Science and Engineering C 59 (2016) 86–91

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Release behavior of tetracycline hydrochloride loaded chitosan/poly(lactic acid) antimicrobial nanofibrous membranes

Suwei Jiang [a], Jian Lv [a], Man Ding [a], Yanan Li [a], Hualin Wang [a,c,*], Shaotong Jiang [b,c]

[a] School of Chemistry and Chemical Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China
[b] School of Biotechnology and Food Engineering, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China
[c] Anhui Institute of Agro-Products Intensive Processing Technology, Hefei, Anhui 230009, People's Republic of China

ARTICLE INFO

Article history:
Received 10 July 2015
Received in revised form 14 September 2015
Accepted 1 October 2015
Available online 3 October 2015

Keywords:
Release
Tetracycline hydrochloride
Chitosan/poly(lactic acid)
Nanofibrous membrane
Electrospinning technique

ABSTRACT

The present work aimed to evaluate the release behavior of tetracycline hydrochloride loaded chitosan/poly(lactic acid) (Tet–CS/PLA) antimicrobial nanofibrous membranes fabricated *via* electrospinning technique. The electrospinning solution was a blend of Tet, CS formic acid solution and PLA chloroform/ethanol solution. The interaction between CS and PLA in CS/PLA nanofibers was confirmed to be hydrogen bond. The incorporation of Tet caused a slight decrease in the diameter of nanofibers with Tet content below 30%. Tet–CS/PLA nanofibrous membrane showed a slight initial burst within the first 4 h before a gradual increase in cumulative release, and the release percentage increased with increasing Tet contents. Tet release ($M_t/M_\infty < 0.6$) from the medicated nanofibers could be described by Fickian diffusion model and the release profiles showed two sequential stages. Tet–CS/PLA nanofibrous membranes exhibited an effective and sustainable inhabitance on the growth of *Staphylococcus aureus*, and the antimicrobial activity increased rapidly with increasing Tet contents below 20%. Furthermore, the incorporation of Tet promoted the degradation of nanofibrous membranes.

1. Introduction

Controlled delivery systems are used to improve therapeutic efficacy and safety of drugs by delivering them at a rate dictated by the need of physiological environment over a period of treatment to the site of action [1,2]. In recent years, drug delivery with polymer nanofibers has gained widespread interest [3–6]. Electrospinning has been recognized as an important method for the fabrication of nanofibers, owing to its relative ease of use, adaptability, and the ability to fabricate with diameters on the nanometer size scale [7–9].

Natural polymers are ideal drug carriers because of their biocompatibility, biodegradability, convenient processing and repeatability [10,11]. As a nature polymer, chitosan (poly-N-acetyl glucosamine, CS) is the deacetylated derivative of chitin which is the second most abundant polysaccharide found in crab, shrimp shells, and fungal mycelia [12,13]. CS has exhibited a promise for its application in drug delivery by the virtue of biocompatibility, biodegradability and antimicrobial activity [14–16]. In addition, CS is a polycationic polymer with a large amount of primary amines, which allow it to be easily modified by blending with some functional polymers. Many achievements have been reported on the fabrication of CS-based electrospun nanofibers, such as cellulose/chitosan hybrid nanofibers [17], PET/chitosan nanofibrous mats [18], poly(vinyl alcohol)/chitosan blend nanofibers [19], chitosan/PCL fibers [20], chitosan/PEO nanofibers [21], chitosan/P(LLA-CL) nanofibers [22] and chitosan/PLA blend micro/nanofibers [23]. Poly(lactic acid) (PLA) is a biodegradable and biocompatible polyester derived from renewable resources. On the basis of its excellent mechanical properties, biocompatibility and biodegradability, PLA nanofibers [24,25] or PLA based nanofibers such as poly(lactide-co-glycolide) nanofibers [26], PEG–PLA nanofibers [27] and poly(lactic acid)/poly(ε-caprolactone) [28] have been widely used in drug carriers for a sustained release. Encouraged by these results, CS/PLA electrospun nanofibers were used as drug carriers for tetracycline hydrochloride (Tet). Some valuable research was reported on the fabrication and characterization of CS/PLA nanofibers [19,29,30]. However, few reports were found in the literatures describing CS/PLA nanofibers as drug carriers in controlled release at present to the best of our knowledge.

The objective of this work is to assay the release behavior of Tet-loaded CS/PLA (Tet–CS/PLA) antimicrobial nanofibrous membranes fabricated *via* electrospinning technique. Herein, CS/PLA nanofibers were used as the drug carrier, Tet as a model antimicrobial drug and a representative Gram-positive bacterium *Staphylococcus aureus* (*S. aureus*, ATCC6538) as the test bacterium. Attentions were focused on the release behavior, antimicrobial activity and *in vitro* degradation of Tet–CS/PLA nanofibrous membranes.

* Corresponding author at: School of Chemistry and Chemical Technology, Hefei University of Technology, Hefei, Anhui 230009, People's Republic of China.
E-mail address: hlwang@hfut.edu.cn (H. Wang).

http://dx.doi.org/10.1016/j.msec.2015.10.005
0928-4931/

2. Materials and methods

2.1. Materials

PLA (Mw, 35,000, purity > 99%) was prepared by two-steps (preparing lactide and ring opening polymerization) in our laboratory. Chitosan (CS, Mw 300 kDa, DD 95%) was purchased from Shanghai Yuanju Reagent Biotechnology Co. Ltd. (Shanghai, China). Tetracycline hydrochloride was obtained from Sigma (St. Louis, MO, USA). *Staphylococcus aureus* (*S. aureus*, ATCC6538) was provided by China Center of Industrial Culture Collection (Beijing, China). All the other chemical solvents were of analytical grade available from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Preparation of electrospinning solution

A PLA solution (15.0%, *w*/*w*) was prepared by dissolving PLA in chloroform/ethanol (1:1, *v*/*v*) medium. A CS solution (1.6%, *w*/*w*) was obtained by dissolving CS in formic acid at room temperature. On the basis of the total weight of CS and PLA, the required Tet was added into CS solutions (0, 3, 5, 10, 20 and 30%, *w*/*w*), respectively. Subsequently, the as-prepared PLA solution was titrated slowly into CS–Tet solution to obtain electrospinning solution (the employed ratio of PLA solution to CS–Tet solution was at 1:1, *v*/*v*). The above experiments were performed under room temperature assisted with magnetic stirring.

2.3. Electrospinning procedures

Electrospinning setup consisted of a syringe and needle (0.50 mm internal diameter), a ground electrode, a high voltage supply (DW-P403-1ACCC, Tianjin Dongwen, China), and a copper sheet was used as a collector. Based on our experimental results, the applied voltage was kept at 15 kV and the tip-to-collector distance (TCD) was kept at 15 cm. Electrospinning processing was carried out at room temperature. The resulting nanofibrous membranes were dried at 80 °C under vacuum condition for 24 h.

2.4. Measurement and characterization

2.4.1. Characterization

The morphologies of nanofibrous membranes were observed using a field scanning electron microscopy (SEM, Jeol JSM-6700F, Japan) and all of the specimens were sputter-coated with a layer of gold. The obtained images were analyzed by Image Tool Software, and a total of 50 counts were used to calculate the average diameter of fibers [31]. Fourier transform infrared spectroscopy (FTIR) was conducted with a Nicolet 6700 spectrometer (Thermo Nicolet, USA) using KBr pellets.

2.4.2. Tet release behavior

To obtain the calibration curve between concentration and absorbance, Tet mother solution (5.0×10^{-3} g/ml) was prepared by dissolving 0.05 g Tet in 100 ml phosphate buffered saline solution (PBS, pH 7.2), and then a series of Tet solutions (0.625×10^{-5}, 0.75×10^{-5}, 1.0×10^{-5}, 1.25×10^{-5}, 1.5×10^{-5}, 2×10^{-5} and 2.5×10^{-5} g/ml) were prepared by diluting 1.25, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 ml of the mother solution in 100 ml PBS at 37 °C, respectively. The absorbance at 360 nm for each solution was measured by a spectrophotometer (UV-754PC, Shanghai Jinghua Technology Instruments Co., Ltd., Shanghai, China, PBS solution as reference). On the basis of experimental data, the linear regression equation from the calibration curve was determined as follows:

$$Y = 0.0327X - 0.0424 \quad \left(R^2 = 0.9990\right) \tag{1}$$

where X is the concentration of Tet (μg/ml) and Y is the absorbance.

The release behavior of nanofibrous membranes was studied in PBS (pH 7.2, 37 °C). 100 mg sample was placed in an Erlenmeyer flask (50 ml) containing 10 ml PBS. After being sealed, the flask was put on a shaker platform (100 rpm, 37 °C). At designated time intervals, 5 ml supernatant was taken from the release medium and 5 ml fresh PBS (pH 7.2, 37 °C) was immediately added to maintain the volume. The absorbance was measured at 360 nm and the cumulative release amount of Tet was calculated by Eq. (1). The cumulative release percentage of Tet was calculated and plotted *versus* time based on Eq. (2):

$$\text{Cumulative release } (\%) = \frac{W_r(t)}{W_0} \times 100\% \tag{2}$$

where $W_r(t)$ is the released Tet at time t and W_0 is the total entrapped Tet.

2.4.3. Antimicrobial activity in liquid culture medium

Spawn rejuvenation was performed by transferring *S. aureus* from stock culture to KMB (Kings Medium B Agar) broth and propagated for 24 h on a shaker platform (37 °C, 200 rpm). To obtain the calibration curve, the concentration of *S. aureus* after rejuvenation was identified as 1.0. The absorbance was measured at 600 nm for each sample with relative concentrations of *S. aureus* at 0.05, 0.1, 0.2, 0.3 and 0.4 by the spectrophotometer. On the basis of experimental data, the linear regression equation from the calibration curve was determined as follows:

$$Y = 2.1309X - 0.0229 \quad \left(R^2 = 0.9990\right) \tag{3}$$

where X is the relative concentration of *S. aureus* and Y is the absorbance.

Nanofibrous membranes with different Tet contents were weighed to 10 mg (±0.1 mg) and sterilized by ultraviolet light for 30 min. Then, each specimen was added into a liquid culture medium containing 2 ml rejuvenated bacterial suspension and 8 ml KMB broth. After being propagated for 24 h on a shaker platform (200 rpm, 37 °C), the relative absorbance was measured by the spectrophotometer at 600 nm and the relative concentration of *S. aureus* was calculated based on Eq. (3).

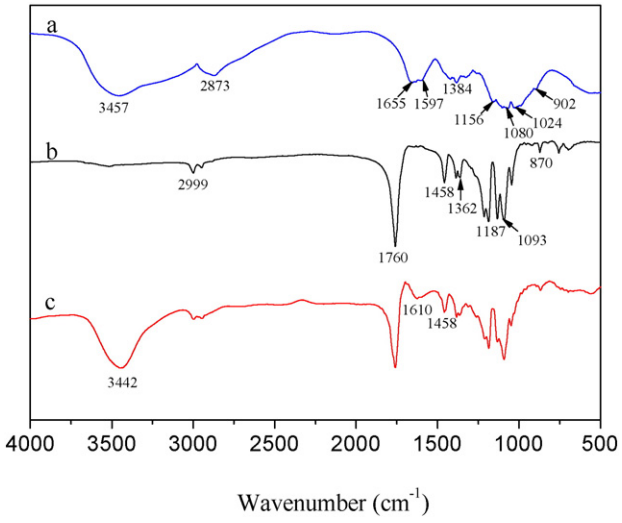

Fig. 1. FTIR spectra of (a) CS, (b) PLA and (c) CS/PLA nanofibers.