Materials Science and Engineering C 69 (2016) 726–732

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

A simple green route to obtain poly(vinyl alcohol) electrospun mats with improved water stability for use as potential carriers of drugs

Alex López-Córdoba [a,c], Guillermo R. Castro [b,c], Silvia Goyanes [a,c,*]

[a] Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Física de Buenos Aires (IFIBA). Facultad de Ciencias Exactas y Naturales. Buenos Aires 1428, Argentina
[b] Laboratorio de Nanobiomateriales, CINDEFI, Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata-CONICET (CCT La Plata), La Plata 1900, Argentina
[c] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ARTICLE INFO

Article history:
Received 3 April 2016
Received in revised form 22 June 2016
Accepted 20 July 2016
Available online 21 July 2016

Keywords:
Poly(vinyl alcohol)
Electrospinning
Nanofibers
Tetracycline hydrochloride
Water-insoluble mats

ABSTRACT

Poly(vinyl alcohol) (PVA) is a hydrophilic, biocompatible and nontoxic polymer. However, because of its low water-resistance, some applications for PVA-based materials are limited (e.g., drug delivery systems and wound dressings). In the current work, PVA mats containing tetracycline hydrochloride (TC) were successfully developed by electrospinning. In order to improve the water stability of the systems, the cross-linking of the PVA matrix was induced by citric acid (CA) addition together with heating treatments (150 °C or 190 °C for 3 min). TC presence led to a strong increase in the electrical conductivity of the blends and as a result, fibers with about 44% lower diameter (270 nm) than that of the corresponding unloaded mats (485 nm) were obtained. Laser scanning confocal microscopy images indicated that TC was well distributed along the PVA nanofibers. The mats were evaluated by FTIR, which revealed chemical interactions between PVA hydroxyl groups and CA carboxylic ones. The treatment at 150 °C for 3 min proved to be the more suitable for the preparation of TC-containing mats with improved water resistance, maintaining the TC antimicrobial activity against both *Escherichia coli* and *Staphylococcus aureus* almost unaltered. These mats showed a burst release of TC, giving around 95% of the drug within the first hour of immersion in water.

1. Introduction

Over the past decades, the growing interest in sustainability and Green Chemistry has led to increase the use of ecofriendly materials and processes. Poly(vinyl alcohol) (PVA) is a hydrophilic semicrystalline polymer produced by polymerization of vinyl acetate to poly(vinyl acetate) (PVAc), and the subsequent hydrolysis of PVAc to PVA. This polymer is widely used industrially and also has the advantages of being biocompatible, biodegradable and nontoxic [1].

Electrospinning is an emerging technology for the production of mats of continuous micro- or nanofibers by an electrostatically driven jet of polymer solution (or polymer melt) [2]. This electrohydrodynamic technology has gained high impact for polymer processing, mainly because it allows the control of fiber morphology, diameter and even porosity. Moreover, the production of fibers for biomedical applications by electrospinning is an attractive alternative for the entrapment or encapsulation of bioactive molecules [1,3–6]. A wide variety of micro- and nanofibers have been fabricated by electrospinning using polymers from natural sources (e.g., collagen, elastin, fibrinogen and silk fibroin) and synthetic polymers (e.g., poly(vinyl alcohol), poly(ε-caprolactone) and poly(lactic acid)) [7–10]. Among them, PVA produces a stable jet during electrospinning and allows obtaining bead-free nanofibers [1, 11–13].

Recently, PVA-based materials have been gaining attention globally because of the advantage of being used as environmentally friendly water-soluble packaging (e.g., detergent and agrochemical packaging, and laundry bags for hospitals). However, the use of PVA in other applications (e.g., semipermeable membranes, wound dressing and tissue substitute) is limited due to its low water stability [12]. Therefore, PVA-based materials are often treated to ensure prolonged structural integrity and to enhance their performance. For this purpose, several strategies have been developed, including freeze–thaw inducement of crystallization, heat treatment, acid-catalyzed dehydration, irradiation and radical production [14]. Bifunctional aldehydes have also been used in the cross-linking of PVA (e.g., glutaraldehyde) [14]. However, some of them are toxic and expensive [15,16]. Citric acid (CA) constitutes a green route for PVA cross-linking. This natural compound has the advantages of being abundant and of low cost. Moreover, CA is currently used as preservative, food additive and cleaning agent [17]. Other important advantages of the use of CA as cross-linker are its low toxicity and high biocompatibility, extending the applications of cross-linked materials to the biomedical field. Being a polycarboxylic acid, CA may react with the hydroxyl groups of PVA chains and lead to

* Corresponding author at: Universidad de Buenos Aires. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Física de Buenos Aires (IFIBA). Facultad de Ciencias Exactas y Naturales. Buenos Aires, Argentina.
E-mail address: goyanes@df.uba.ar (S. Goyanes).

http://dx.doi.org/10.1016/j.msec.2016.07.058
0928-4931/

intermolecular-type and intramolecular-type ester linkages among them [17–19]. Commonly, the cross-linking reaction between PVA and CA is induced by heating at temperatures ranging between 130 and 220 °C for long periods of time (*c.a.* 2 h) [17]. This approach can lead from the partial to the total degradation of heat-sensitive bioactive compounds present in the polymer.

Tetracycline hydrochloride (TC) is a broad-spectrum and inexpensive antibiotic, highly effective in the inhibition of the synthesis of bacterial proteins, which has been used extensively in the treatment of human and animal infections [20].

The aim of the current work was to fabricate PVA electrospun mats to carry TC, using a green route. Citric acid (CA) (a natural cross-linker) was added to the mats, which were then heat-treated (150 °C or 190 °C for 3 min) in order to improve their water stability. The morphology, the size, the chemical conformation, the thermal behavior, the water solubility and the antimicrobial activity of the TC-PVA/CA electrospun mats were evaluated before and after the heat treatments. To the best of our knowledge, this is the first time that TC-containing PVA electrospun mats with improved water stability have been produced using this approach and preserving their antimicrobial activity against typical Gram-positive and Gram-negative microorganisms, *Escherichia coli* and *Staphylococcus aureus*, respectively.

2. Materials and methods

2.1. Materials

Fully hydrolyzed polyvinyl alcohol (PVA, ELVANOL® T25) was supplied by Diatex (Buenos Aires, Argentina). The PVA molecular weight (M_w) is in the range of 50 to 55 kDa (E. I. DuPont Co., USA).

Tetracycline hydrochloride (TC) (Parafarm) was purchased from Droguería Saporiti (Argentina). Citric acid (CA) was supplied by Sigma-Aldrich (USA).

2.2. Preparation of the electrospun mats with and without drug

The stock solutions to prepare tetracycline hydrochloride-loaded electrospun mats (TC-PVA/CA) were prepared by dissolving 12.0 g of PVA in 100 mL of distilled water, under heating at 75 °C until complete dissolution. CA was added to the PVA solution at 5.0% (wt./PVA weight) under constant stirring for 30 min. After total dissolution, the blends were cooled down to room temperature and then TC was added at 5.0% (wt./PVA weight) under constant stirring for 30 min. Finally, the solutions were sonicated for 5 min to remove bubbles. PVA/CA blends without TC were also prepared under identical conditions.

The viscosity and the electrical conductivity of the electrospinning solutions were measured in a rheometer (RS 600, Haake, Germany) and a conductivity meter (Mettler Toledo, USA), respectively.

The electrospinning process was carried out using a horizontal setup as shown in Fig. 1. The solutions contained in 10-mL syringes were pushed employing a syringe pump (Apema PC11U, Argentina) with controlled feed rate (0.5 mL h^{-1}) through a 21G needle (0.8 mm).

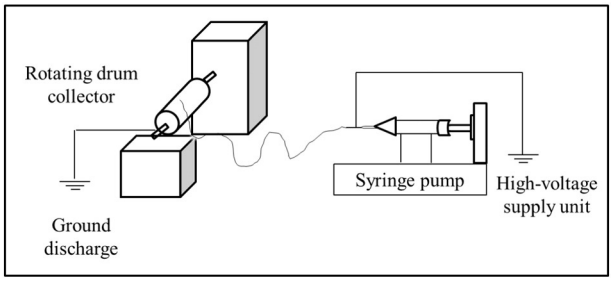

Fig. 1. Schematic representation of the electrospinning process.

Electrical voltages in the range of 20 to 35 kV were applied to the needle using a high-voltage supply unit (50 kV, 20 mA).

The polymer ejected from the needle tip was collected on a rotating drum of 6-cm diameter covered by aluminum foil, located at 20 cm from the tip. The collector was connected to the negative electrode of the power supply (ground), while the spinneret filled with the polymer solution was connected to the positive terminal (Fig. 1). For the morphological study, the collection time was about 5 min; while for the rest of the experiments the collection time was about 5 h. The thickness of the resulting electrospun mats collected during 5 h was between 20 and 30 μm. The electrospun mats were treated at 150 °C or 190 °C for 3 min, in order to improve their water stability. These conditions were chosen based on both literature reports [14,17] and the results of preliminary experiments on the water stability of the mats and the thermal degradation of the drug.

2.3. Scanning electron microscopy and confocal laser microscopy

The morphology of the resulting fibers was examined by scanning electron microscopy using a field emission gun (FE-SEM) (SUPRA 40, Carl Zeiss NTS, Germany). The electrospun mats were sputtered with a thin layer of gold prior to SEM observation. The fiber sizes were determined from the micrographs using the ImageJ free software [21]. The results were reported as average values from at least 100 measurements. In order to evaluate the distribution of TC in the electrospun nanofibers, a confocal laser scanning microscope (CLSM, FV 300, Olympus, Japan) equipped with an Argon ion laser (488 nm) was used. The recording was done focusing with a 20× objective (NA = 1.40). The emission of TC was detected in the range of 500–560 nm [22].

2.4. Water solubility of the electrospun mats

Samples cut into 5 × 5 cm were weighed and submerged in 20 mL of distilled water (pH = 6.0) at room temperature (22–25 °C) for 24 h. Later, the water was removed and the samples were dried at 50 °C until constant weight (around 2 h). The mass loss during dissolution in water was calculated with the following equation:

$$S(\%) = \left(\frac{W_0 - W_1}{W_0}\right) \times 100$$

where W_0 is the initial dry weight of the sample and W_1 is the dry weight after dissolution in water.

2.5. Determination of the drug content of electrospun mats

Samples of untreated and heat-treated TC-PVA/CA mats were weighed and immersed in an exact amount of distilled water at room temperature. After 24 h, aliquots of the solutions were removed and their UV–vis spectra were spectrophotometrically measured in a range from 200 to 500 nm (SHIMADZU UV-1800, Japan). A TC standard solution was prepared and its UV-VIS spectrum was also recorded for comparison. The concentrations of drug released from the untreated and heat-treated TC-PVA/CA mats were determined at 357 nm with an appropriate calibration curve. The results were expressed as mg of TC per gram of electrospun mat.

2.6. Release studies

Samples of TC-PVA/CA mats were carefully removed from the aluminum foil, weighed and immersed in an exact amount of distilled water at room temperature. Aliquots of supernatant were removed at different times for 24 h, and the amount of the drug dissolved in the withdrawn solution was quantified as described above (Section 2.5). Finally, the cumulative amount of drug released from the specimens for each specified immersion period was calculated.