Neomycin-loaded poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA)/polyvinyl alcohol (PVA) ion exchange nanofibers for wound dressing materials

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ARTICLE INFO

ABSTRACT

In this study, poly(styrene sulfonic acid-co-maleic acid) (PSSA-MA) blended with polyvinyl alcohol (PVA) was electrospun and then subjected to thermal crosslinking to produce PSSA-MA/PVA ion exchange nanofiber mats. The cationic drug neomycin (0.001, 0.01, and 0.1%, w/v) was loaded onto the cationic exchange fibers. The amount of neomycin loaded and released and the cytotoxicity of the fiber mats were analyzed. In vivo wound healing tests were also performed in Wistar rats. The results indicated that the diameters of the fibers were on the nanoscale (250 ± 21 nm). The ion exchange capacity (IEC) value and the percentage of water uptake were 2.19 ± 0.1 mequiv./g-dry fibers and $268 \pm 15\%$, respectively. The loading capacity was increased upon increasing the neomycin concentration. An initial concentration of 0.1% (w/v) neomycin (F3) showed the highest loading capacity (65.7 mg/g-dry fibers). The neomycin-loaded nanofiber mats demonstrated satisfactory antibacterial activity against both Gram-positive and Gram-negative bacteria, and an in vivo wound healing test revealed that these mats performed better than gauze and blank nanofiber mats in decreasing acute wound size during the first week after tissue damage. In conclusion, the antibacterial neomycin-loaded PSSA-MA/PVA cationic exchange nanofiber mats have the potential for use as wound dressing materials.

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1. Introduction

Pharmaceutical applications of ion exchange resins are extensively focused in drug delivery systems, such as controlled release, taste masking, site specific and topical delivery systems [Anand et al., 2001]. Recently, ion exchange fibers have been increasingly studied due to the many advantages of these mats over ion exchange resins, such as the easier incorporation of large molecules, more efficient drug loading onto and release from the ion exchange fibers and more rapid and efficient ion-exchange performance [Hänninen, 2008]. There are only a few studies reporting the pharmaceutical use of ion exchange fibers, such as in transdermal delivery systems [Jaskari et al., 2000] and gastro-mucoadhesive delivery systems [Yao et al., 2008] to control drug release and permeation and to improve drug absorption and stability.

Ion exchange fibers consist of polymeric frameworks and ion exchange groups. Polymeric frameworks are produced primarily in nanofiber form due to the notable characteristics of nanofibers, which include a very large surface-to-volume ratio, a high porosity

with a small pore size and good mechanical properties. The addition of ionic functional groups into the nanofibers is a promising option to produce novel ion exchangers with high exchange capacities [Matsumoto and Tanioka, 2011]. Electrospinning is a popular technique used to create nanofibers because it is a simple and easy way to control the morphology of ultrafine fibers [Huang et al., 2003; Bhardwaj and Kundu, 2010; Sill and von Recum, 2008]. Two approaches are applied to produce ion-exchange nanofibers. In one approach, electrospinnable polymers, such as polyvinyl alcohol (PVA) [Matsumoto et al., 2007] and polyethylene oxide (PEO) [Seo et al., 2005], are added to the spinning solution of an ionic polymer with a high electric conductivity that acts as the carrier because of the low electrospinnability of ionic polymer solutions. In the second approach, nonionic polymers are electrospun and subjected to successive chemical modification (sulfonation [Matsumoto et al., 2006] or amination [Park and Na, 2006]) to create ion exchange groups. Recently, ionic polymers such as chitosan [Matsumoto et al., 2007] and polysaccharide [Seo et al., 2005] have been used in the preparation of ion exchange fibers, whereas poly(styrene sulfonic acid) (PSSH), poly(sodium styrene sulfonate) (PSSNa), poly(acrylic acid) (PAA), poly(dimethyl dimethylenepiperidinum chloride) (PDMeDMPCl) [Matsuyama et al., 2001], poly(acrylic acid-co-maleic acid) (PAM) [Kim et al., 2005] and poly(styrene sulfonic

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acid-co-maleic acid) (PSSA-MA) are used to prepare ion exchange membranes [Kim et al., 2006; Kang et al., 2002, 2005].

PSSA-MA is an ionic polymer with both strong (sulfonic acid) and weak (maleic acid) ion-exchangeable groups in its structure. However, PSSA-MA cannot be used to produce ion exchange fibers via electrospinning. Therefore, PVA, a water-soluble, non-toxic, biodegradable, and biocompatible synthetic polymer with enhanced fiber-forming ability, is blended with PSSA-MA. In our previous study, the PSSA-MA/PVA ion exchange fibers were successfully electrospun. However, PSSA-MA/PVA ion exchange fibers are completely water soluble; therefore, crosslinking the ion exchange fibers is required to improve the properties of these fibers. A precise temperature is selected to promote the crosslinking of the PSSA-MA/PVA ion exchange fibers.

The use of dressings to deliver antibiotics to wound sites is useful because of this technique's ability to provide tissue compatibility, a low occurrence of bacterial resistance and reduced interference with wound healing [Boateng et al., 2008]. In this study, the cationic drug neomycin was selected to load onto PSSA-MA/PVA ion exchange fiber mats. Neomycin is an aminoglycoside antibiotic that has excellent activity against Gram-negative bacteria and partial activity against Gram-positive bacteria. It is widely used in many topical medications, such as creams, ointments, and eye-drops. The aim of this study was to fabricate PSSA-MA/PVA ion exchange fiber mats loaded with neomycin to enhance the efficacy of this antibiotic as an antibacterial agent for enhanced wound healing. The ion exchange capacity (IEC) value and water uptake of the fiber mats were investigated. The morphology and diameter of the electrospun fiber mats were analyzed using a scanning electron microscope (SEM). The amount of neomycin loaded, the amount released, and the cytotoxicity of the fiber mats were analyzed. The in vivo wound healing effects of the nanofiber mats were investigated using an animal model.

2. Experimental methods

2.1. Materials

The PSSA-MA (sodium salt, styrene sulfonic acid:maleic acid = 3:1, average Mw = 20,000 g/mol), neomycin sulfate were purchased from Sigma-Aldrich Chemical Company, USA. Polyvinyl alcohol (PVA; degree of polymerization ≈ 1600, degree of hydrolysis ≈ 97.5–99.5 mol%, average Mw = 77,000–82,000 g/mol) was purchased from Fluka, Switzerland. Normal human foreskin fibroblast (NHF) cells were obtained from the American Type Culture Collection (ATCC) in Rockville, MD, USA. Dimethyl sulfoxide (DMSO) was obtained from BDH Laboratories, UK. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), trypsin, and penicillin–streptomycin were purchased from Gibco BRL, Rockville, MD, USA. All other reagents and solvents were commercially available.

2.2. Preparation of PSSA-MA/PVA ion exchange nanofibers

2.2.1. Electrospinning

The PSSA-MA (sodium salt) was transformed prior to use from the Na$^+$- to the H$^+$-form by the dialysis method. Briefly, 50% (w/w) PSSA-MA (sodium salt) solution was prepared by adding an excess concentrate hydrochloric acid. The solution was placed in a dialysis bag (membrane: Spectra/Por® 6000 MWCO, Spectrum Laboratories, USA), dialyzed against distilled water for 2–3 days and then lyophilized in a freeze dryer (LABCONCO, Freezone 2.5, USA). The PSSA-MA and PVA aqueous solutions were prepared at 20% and 10% (w/w). The PSSA-MA and PVA aqueous solutions were mixed at a solid polymer weight ratio of 0.4/1. The viscosity and conductivity

of these mixed solutions were determined using a Brookfield viscometer (DV-III ultra, Brookfield Engineering Laboratories, USA) and conductivity meter (Eutech Instruments Pte. Ltd., Singapore), respectively. The PSSA-MA/PVA solution was contained in a glass syringe with a plane tipped stainless steel needle with an inner diameter of 0.5 mm. The electrospinning process was conducted at 25 °C with the fixed applied voltage, the distance between the tip and the collector, and the feeding rate set at 15 kV, 15 cm and 0.4 ml/h, respectively. The electrospun PSSA-MA nanofibers were collected on aluminum foil that was covering the rotating collector.

2.2.2. Crosslinking process

To improve the chemical, thermal and mechanical stability, the PSSA-MA/PVA nanofibers were thermally crosslinked using a hot air oven at 130 °C for 5 h.

2.3. Characterization of the fibers

2.3.1. Morphology and diameter

The morphologies of the electrospun PSSA-MA/PVA nanofibers and the crosslinked PSSA-MA/PVA nanofibers were investigated using a scanning electron microscope (SEM, Camscan Mx2000, England). For this process, a small section of each fiber was sputtered with a thin layer of gold prior to the SEM observation. The average diameter of the fibers was determined using image analysis software (JMicroVision V.1.2.7, Switzerland). The chemical structure of the fibers was characterized using a Fourier transform infrared spectrophotometer (FTIR, Nicolet 4700, USA). The fiber samples were ground and pressed into KBr plates prior to the FTIR analysis with a wavenumber range of 400–4000 cm^{-1}. The texture analyzer (XT plus) was used to determine the tensile stress, tensile strain and Young's modulus values of crosslinked PSSA-MA/PVA nanofibers.

2.3.2. Water insolubilization

To confirm the crosslinking of the PSSA-MA/PVA nanofibers, the water insolubilization of crosslinked nanofibers was determined. The crosslinked PSSA-MA/PVA nanofibers were cut into small pieces and accurately weighed (W_1). Then, the cut fibers were immersed in water for 24 h, dried at 60 °C until they obtained a constant weight and accurately weighed (W_2). The water insolubilization of the crosslinked nanofibers was calculated according to Eq. (1):

$$\text{water insolubilization}(\%) = \frac{W_2}{W_1} \times 100 \qquad (1)$$

2.3.3. Ion exchange capacity (IEC)

The IEC of the crosslinked PSSA-MA/PVA nanofibers was performed by the back titration. Briefly, the H$^+$ sites on the crosslinked nanofibers were converted into Na$^+$ by immersing the crosslinked nanofibers in 25 ml of 0.01 N NaOH solution for 12 h. The residue NaOH solution was titrated with 0.01 N standardized HCl solution to a phenolphthalein end-point. The IEC of the crosslinked nanofibers was calculated according to Eq. (2):

$$\text{IEC} = \frac{C_{\text{NaOH}} V_{\text{NaOH}} - C_{\text{HCl}} V_{\text{HCl}}}{W} \qquad (2)$$

where C_{NaOH} is the concentration (N) of the NaOH solution, C_{HCl} is the standardized concentration (N) of the HCl solution, V_{NaOH} is the volume (ml) of the NaOH solution at the starting point, V_{HCl} is the volume (ml) of the HCl solution at the endpoint and W is the mass (g) of the dry crosslinked PSSA-MA/PVA nanofibers.

2.3.4. Water uptake

The quantification of water uptake was measured by drying the crosslinked PSSA-MA/PVA nanofibers for 24 h and recording the weight (W_1). The crosslinked PSSA-MA/PVA nanofibers were