Journal of Industrial and Engineering Chemistry 39 (2016) 127–135

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Sulfanilamide and silver nanoparticles-loaded polyvinyl alcohol-chitosan composite electrospun nanofibers: Synthesis and evaluation on synergism in wound healing

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ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form 19 May 2016
Accepted 20 May 2016
Available online 30 May 2016

Keywords:
Nanofiber
Sulfanilamide
AgNP's
Wound healing
Antimicrobial

ABSTRACT

Novel silver nanoparticles-decorated chitosan (CS)-polyvinyl alcohol (PVA) composite electrospun nanofibers, loaded with sulfanilamide for enhanced wound healing have been developed. Herein, formic acid was used as a reducing agent to produce *in situ* colloidal silver nanoparticles (AgNPs) in the composite polymeric solution with the active agent sulfanilamide. The prepared electrospun fibers were characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray diffraction, ultraviolet spectroscopy, and thermal gravimetric analysis (TG). Further, *in vitro* release, antimicrobial properties and *in vivo* wound healing activity were evaluated. The results revealed that the composite fibers displayed a synergistic antibacterial and wound healing activities.

Introduction

Ultra-fine structures including microspheres and microporous and mesoporous nanostructures such as nanoparticles, nanospheres, and nanomachines, have been shown to play pivotal roles in the fields of gas sorption, catalysis, and environmental and medical science. Most importantly, mesoporous nanoparticles including those made from silica and hydroxyapatite, and microporous nanostructures such as bio-MOF have found applications in medical imaging, drug delivery, and bone and organ replacements [1–8]. Over the last two decades, their tunable high surface area, surface to volume ratio, softness, surface coverage, high porosity, and high drug loading and delivery capacity to their target tissues, has made them attractive in drug delivery research. Nanofibers are among the ultra-fine nanostructures that are produced using the principles of drawing [9], template synthesis [10], phase separation [11], self-assembly [12], and electrospinning [13]. A detailed discussion regarding these procedures can found in the review by Huang et al. [14].

Electrospinning is the most versatile among the techniques listed above, and can produce nanofibers of different diameters with well-ordered surface morphologies. Herein, the nanofibers were produced by applying a high electrical voltage to polymers spun over the target electrode. The era of electrospinning began in 1934, when a series of patents were released describing the technique [15–17]. Due its reliability, simplicity, versatility, and potential uses in diverse fields, electrospinning has gained increasing applications including fiber reinforcement [18], filtration [19,20], protective clothing [21,22], nanosensors [23], and cosmetics [24].

In addition to the above exciting applications by their high encapsulation efficiency, high loading capacity, simultaneous delivery of diverse therapeutics, ease of preparation, and low

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http://dx.doi.org/10.1016/j.jiec.2016.05.021
1226-086X/

cost, polymeric electrospun nanofibers using synthetic and biological polymers have found applications in drug-loaded wound dressing preparation, drug [25–30] and protein delivery [31], and in tissue engineering scaffolds [32,33]. Electrospun nanofibers have been reported to possess great potential for wound dressings as a result of special characteristics such as the high porosity of the nanofibrous membrane effectively contributing to air permeability, providing the required oxygen for cell respiration, and having a relatively small pore size that can preserve the wound from bacterial infections. Moreover, the fibrous surface structure displays strong adhesiveness to mucous layers since the nanoporous structure instantly absorbs moisture into the voids.

Chitosan [poly[(1,4)-2-amino-2-deoxy-D-glucose], is an amino polysaccharide obtained from chitin through the deacetylation process. Chitin is the principal structural polysaccharide in arthropods (crabs and insects), and the second most abundant natural polysaccharide next to cellulose. It possesses antimicrobial properties that are beneficial for wound dressing development. Many authors have reported that the electrospinning of the CS polymer has major disadvantages due to the use of solvents such as trifluoroacetic acid (TFA), which is environmentally harmful, and very toxic and corrosive, which has led to limited use in biomedical applications. However, chitosan is not electrospinnable when other solvents, such as acetic acid, are used. A common approach to improve the electrospinnability of CS is to blend it with other easily-electrospinnable polymers such as polyethylene oxide (PEO), polyvinyl alcohol (PVA), and polylactic acid (PLA) [34].

The present study shows the preparation of *in situ* synthesized silver nanoparticles-decorated composite nanofibers of CS and PVA, embedded with sulfanilamide (SN) as a small molecule to improve the antimicrobial and wound healing properties. The effect of weight ratio and process parameters on the morphology of the blended nanofibers were investigated using SEM. Nanofibers were characterized using FTIR and DSC, and the release rate of sulfanilamide and silver from nanofibers were also determined.

Experimental

Materials and methods

Polymers such as high molecular weight polyvinyl alcohol (PVA, Molviol) low molecular weight chitosan (CS), and silver nitrate ($AgNO_3$), were obtained from Sigma–Aldrich (GmbH, Germany). The model drug sulfanilamide and commercial grade solvents such as acetic acid (99%), formic acid (85%), and isopropyl alcohol (IPA) were purchased from Daejung Chemicals and Metals (Korea). DD water used in the studies was obtained from an in-house deionizing and distillation system. All other chemicals were of commercial grade unless otherwise specified.

Preparation of the polymer solution

12% w/v PVA was prepared in the solvent (A) system consisting of DD water and IPA (4:1). The mixture was heated to 90 °C for 1 h to obtain a transparent homogenous solution. 3% CS (w/v) was prepared in the solvent (B) system consisting of 60 ml 2% acetic acid and 40 ml 85% formic acid (reducing agent for $AgNO_3$) [35].

Preparation of the electrospun nanofibers

Various weight ratios (9:1, 8:2, 7:3, 6:4, and 5:5) of the above polymer solutions (A and B) were mixed thoroughly for 1 h to obtain a homogeneous solution. A nanospinning system (Nano-NC, Korea), equipped with a high voltage power supply unit, was used to provide high voltages in the range of 0–30 kV. Spinning solutions were carefully loaded in a 10-ml syringe with a stainless steel 21-gauge blunt needle, taking care to avoid the entrapment of air bubbles. The positive electrode with a high voltage power was connected to the needle tip. Aluminum foil wrapped around the collector drum acted as a negative electrode as well as fiber collector. The electrospinning process was carried out at ambient temperature with a relative humidity of 45 ± 5%. A fixed electrical potential of 15 kV was applied, and the distance between the collector and needle tip was kept at 15 cm. The solution feed rate was controlled at 0.5 ml/min using a single syringe piston pump. The collected nanofibers were dried overnight at 40 °C to remove residual solvent. The spun nanofibrous material was carefully peeled from the aluminum foil and cut in to specified dimension (1 cm × 1 cm) for further pharmaceutical evaluation. The polymer composition that produced fine and smooth nanofibers was selected for drug and AgNP's loading and for pharmacological evaluation.

Drug and AgNP's loading

The one-pot procedure was followed for the *in situ* drug and AgNP's (AgNP's) loading. Herein the sulfanilamide (50 w/w% to that of the total polymer) and silver nitrate (10 w/w% Ag to that of the total polymer) were dissolved in 8 ml 4:1 DD water:IPA mixture prior to PVA addition. The respective quantity of PVA to obtain a 12% w/v solution was added to the mixture, and heated at 90 °C to obtain a homogenous PVA drug solution. Subsequently, 2 ml 3% CS solution in solvent (B) was added, and the solutions mixed thoroughly until a homogeneous solution was obtained. The solution was drawn into the syringe and fibers were formed using the above-described electrospinning procedure (note: due to the formation of AgNP's, the color of the solution changes from colorless to dark brown to black while mixing solvent B mixture to PVA mixture).

Characterization

The surface morphology of the electrospun nanofibers was studied using a JEOL-JSM 5600 scanning electron microscope (JEOL Ltd., Japan). The samples were sputtered with gold plasma *via* a sputter-coater (Cressington, Sputter Coater-108 auto) before visualization under the SEM. The diameters of the electrospun nanofiber were measured using the image visualization software, Image-J (National Institutes of Health, USA). The average fiber diameters were determined by measuring 30 fibers at random from the SEM micrographs. The morphological characteristics and particle size of the AgNP's were observed using a Hitachi tunneling electron microscope (Hitachi-800 TEM) with the solution of nanoparticles in the solvent with the polymer. The solution was placed over a copper grid and allowed to dry at room temperature, and then placed in the sample holder of the TEM instrument and analyzed. Fibers loaded with AgNP's were also examined TEM. To determine the crystal structural changes of the polymer and AgNP and drug loaded polymer XRD was recorded using Rigaku Miniflex diffractometer using CuKα radiation (λ = 1.54 Å). The diffraction data were recorded in the 2θ range of 10–80° with a 0.1° step size and a 1-s step time. SCINCO DSC N 650 differential scanning calorimeter was used to record the DSC traces of the pure sulfanilamide, PVA–CS and PVA–CS-sulfanilamide nanofiber mats with a heating rate of 10 °C/min under helium atmosphere (40 ml/min) to get the information on drug and polymer crystalline changes after fabrication. Further to confirm the formation of the AgNPs, the polymeric solution was subjected to UV–vis spectral scanning using a Shimadzu UV mini-1240 UV–visible spectrophotometer with 1-cm quartz cells. A solution without reducing agents, formic acid, or CS was used as a blank. Fourier-transform infrared (FTIR) spectra were obtained on a Nicolet 6700 FT-IR spectrometer at room temperature following the formation of a