International Journal of Biological Macromolecules 51 (2012) 980–986

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Preparation of regenerated silk fibroin/silk sericin fibers by coaxial electrospinning

Yichun Hang, Yaopeng Zhang, Yuan Jin, Huili Shao*, Xuechao Hu

State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, College of Materials Science and Engineering, Donghua University, Shanghai 201620, PR China

ARTICLE INFO

Article history:
Received 13 April 2012
Received in revised form 25 June 2012
Accepted 13 August 2012
Available online 20 August 2012

Keywords:
Coaxial electrospinning
Silk fibroin
Silk sericin
Morphology
Structure

ABSTRACT

The coaxial electrospinning using the regenerated silk fibroin (SF) and silk sericin (SS) aqueous solutions as the core and shell spinning dopes, respectively, was carried out to prepare regenerated SF/SS composite fibers with components and core–shell structure similar to the natural silkworm silks. It was found from the scanning electron microscope (SEM) and transmission electron microscope (TEM) results that the core dope (SF aqueous solution) flow rate (Q_c) and the applied voltage (V) had some effects on the morphology of the composite fiber. With an increase in Q_c, the diameter nonuniformity and eccentricity of the core fiber became serious, while the increasing V played an inverse role. In this work, the suitable Q_c for the fiber formation with better electrospinnability was about 6 μL/min, and the corresponding optimum V was 40 kV. Moreover, the results from Raman spectra analysis, modulated differential scanning calorimetry (MDSC), thermogravimetry (TG) measurement and mechanical property test showed that, compared with the pure SF fiber, the coaxially electrospun SF/SS fiber had more β-sheet conformation, better thermostability and mechanical properties. This was probably because that SS played significant roles in dehydrating SF molecules and inducing the conformational transition of SF to β-sheet structure.

1. Introduction

The silkworm (*Bombyx mori*) silk has a core–shell structure and is constituted by a pair of silk fibroin (SF) core fibers covered with silk sericin (SS). The SF is the predominant component and constitutes about 75% of the total silk weight, while the SS is a kind of hydrophilic "glue-like" protein that serves as not only a cover of the SF monofilament but also an adhesive to bind two SF monofilaments together [1,2]. As a typical fibrous protein, silkworm silk shows remarkable mechanical properties and has been used in textile industry for thousands of years. More recently, regenerated SF-based biomaterials with desirable properties have attracted considerable attentions of the researchers in the field of polymer science. They have been successfully processed into different forms including films [3], 3D porous scaffolds [4], hydrogels [5], as well as fibers via wet spinning [6] or electrospinning [7].

In order to understand the formation mechanism of silkworm silk, many works have been performed to mimic the spinning process of silkworm and ultimately fabricate the silk fibers in a regenerated way. The regenerated SF fibers were first prepared through wet spinning process, usually by using polar or organic solvents and extruding the spinning dope into a coagulation bath [8–10]. However, most of these solvents either severely degrade the SF molecules or are toxic for potential industrial application. The wet spinning process has the recycling problem of the solvent used in dissolving and coagulation bath, and is rather different from the dry spinning process of silkworm using water as solvent. Recently, the electrospinning process has been developed to prepare SF-based biomaterials with a submicron diameter. In most of prior studies about the electrospinning of SF solution, the SF spinning dopes were also formed by using organic solvents [11–14], or further blending with poly (ethylene oxide), chitosan, collagen and other synthetic or nature polymers [15–17]. Only few researches were implemented using water as solvent and pure SF aqueous solution as spinning dope [18–20]. No composite spinning process using SF aqueous solution as core dope and SS aqueous solution as shell dope has been reported.

It is well accepted that SF is an ideal biomaterial for tissue engineering due to its impressive mechanical properties, biocompatibility and biodegradability [21]. On the other hand, scientific investigations have also revealed that SS is resistant to oxidation and UV radiation, anti-bacterial, biocompatible, and can absorb and release moisture easily. Moreover, it exhibits many biological activities, such as tyrosinase activity inhibition, anticoagulation function, anti-cancer activity, promoting digestion and so on [22–25]. However, with weak structural properties and high solubility, pure SS biomaterials are usually fragile and difficult to

* Corresponding author. Tel.: +86 21 67792953; fax: +86 21 67792955.
E-mail addresses: hangyc_0430@mail.dhu.edu.cn (Y. Hang), zyp@dhu.edu.cn (Y. Zhang), jinyuan@mail.dhu.edu.cn (Y. Jin), hlshao@dhu.edu.cn (H. Shao), xchu@dhu.edu.cn (X. Hu).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.08.010

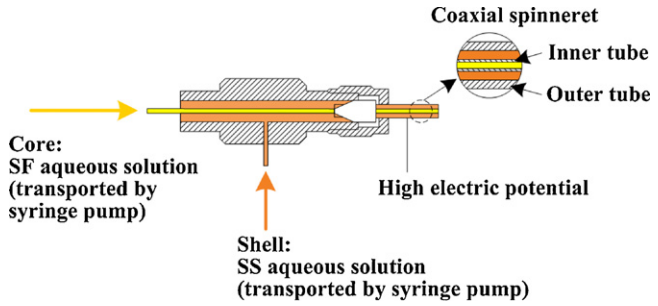

Fig. 1. Spinneret illustration for coaxial electrospinning.

fabricate [26]. In the present work, a coaxial electrospinning technique was utilized to fabricate the regenerated SF/SS fibers, which may not only mimic the compositions and core–shell structure of silkworm silk, but also achieve the mutual complementarity of SF and SS. During the electrospinning, the regenerated SF aqueous solution and SS aqueous solution were used as the core and shell spinning dope, respectively. The effects of the processing parameters, such as the core flow rate (Q_c) for SF aqueous solution and applied voltage (V), on the formation of core–shell fibers were investigated. Then, the secondary structure of the resultant electrospun SF/SS fibers were further characterized by Raman Spectroscopy and compared with the pure SF fibers. Moreover, their thermal and mechanical properties were also investigated. It is predictable that the regenerated SF/SS fibers will have great potential in the tissue engineering applications.

2. Experimental

2.1. Preparation of spinning dopes

B. mori cocoons were degummed twice in boiling Na_2CO_3 (0.5 wt%) aqueous solution for 30 min each at a bath ratio of 1:50 (w/v), then thoroughly rinsed to extract sericin and dried at room temperature to get degummed natural silk. The degummed silk was dissolved in 9.0 M LiBr aqueous solution at a ratio of 1:10 (w/v) at 40 °C for 2 h. After being diluted, centrifugalized and then filtered, the resultant regenerated SF solution was dialyzed in deionized water at 10 °C for about 3 days using the cellulose semi-permeable membrane (MWCO: 14,000 ± 2000). Finally, a 33 wt% regenerated SF aqueous solution was obtained after being subsequently condensed and used as the core spinning dope. Meanwhile, a 60 wt% regenerated SS aqueous solution was prepared by directly dissolving the SS powder (provided by Wuxi Smiss Technology Co., China) into the deionized water under gentle stirring and used as the shell spinning dope.

It is known that the molecular weights (MWs) of the heavy chain and the light chain of natural silk fibroin in *B. mori* cocoons are 350 kDa and 26 kDa, respectively [27]. In this work, during the preparation of the spinning dope, the SF molecules were inevitably degraded due to the scission of the chains. The MW of the resultant SF after dissolution was at a range from 40 kDa to a value over 97 kDa measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method as reported previously by us [28]. In addition, the MW of the water-soluble SS powder was detected to be around 2 kDa using a matrix-assisted laser desorption ionization mass spectrum (MALDI-MS, Applied Biosystems Co., USA) with a 4800 proteomics analyzer.

2.2. Electrospinning process

Fig. 1 shows the spinneret illustration for coaxial electrospinning. The core (SF) and shell (SS) spinning dope were extruded simultaneously through the coaxial spinneret by two separate syringe pumps. The inner tube of the spinneret has an inner diameter of 0.45 mm and an outer diameter of 0.80 mm, while the outer tube has an inner diameter of 2.00 mm and an outer diameter of 3.20 mm. Coaxial electrospinning was performed at a core flow rate (Q_c) ranging from 2 to 8 μL/min and at a constant shell flow rate (Q_s) of 2 μL/min. The applied voltage (V) was changed from 30 to 50 kV and the distance from the spinneret to collector was fixed to be 10 cm. The coaxially electrospun SF/SS (core/shell) fibers were collected on a grounded aluminum foil. Several copper meshes coated with carbon were previously placed on the aluminum foils to collect the fiber samples for transmission electron microscope (TEM) analysis. In this work, the electrospun pure SF fibers were also prepared as described previously for comparison [29].

2.3. Characterization

The morphology of coaxial regenerated SF/SS fibers was observed using a JSM-5600LV scanning electron microscope (SEM, JEOL Co., Japan) at 15 kV. The average value (AV) and standard deviation (STDV) for the diameter of the fibers were calculated by measuring 100 individual fibers shown in the SEM images.

The core–shell structures of the coaxial regenerated SF/SS fibers were investigated using an H-800 transmission electron microscope (TEM, Hitachi Co., Japan) with an accelerating voltage of 200 kV. The fiber samples deposited onto copper meshes were directly obtained by coaxial electrospinning of SF and SS dopes (see Section 2.2).

Raman spectra of the electrospun fibers were obtained using a LabRam-1B microscopy Raman spectrometer (Dilor Co., France). The 632.81 nm line of a He–Ne laser was used to generate an intensity of 6 mW on the samples and the spectra were recorded from 900 to 1800 cm^{-1}. The quantitative analysis of the amide I region was conducted under a deconvolution method reported by Zhou et al. [30]. The three main bands of secondary structures for SF molecules are commonly employed: 1670 ± 5 cm^{-1} is assigned to β-sheet conformation, 1655 ± 5 cm^{-1} to random coil/α-helix conformation, and 1680 ± 5 cm^{-1} to intermediate conformation (a conformation between random coil and β-sheet [30], or distorted β-sheet conformation [31]). In addition, the percentage of peak area of 1615 cm^{-1} band assigned to the phenyl group of tyrosine residues was used as an invariant internal standard to check the validity of each analysis. In our work, this value was controlled to be about 5% of the total peak area for the above four bands.

The thermal properties of the electrospun fibers were investigated using a MDSC 2910 modulated differential scanning calorimeter (TA Instruments Co., USA) under nitrogen atmosphere at a flow rate of 40 mL/min. The electrospun fiber mats were heated from room temperature to 350 °C at a heating rate of 5 °C/min with a modulation period of 60 s and temperature amplitude of ±1 °C. Meanwhile, the thermogravimetry (TG) measurements for the electrospun fibers were also performed from room temperature to 500 °C at a heating rate of 5 °C/min under nitrogen atmosphere (10 mL/min) using a TG209 F1 Iris thermogravimetric apparatus (Perkin Elmer Co., USA). The kinetic evaluation of the thermal degradation of the fibers was then conducted according to the method discussed by Jimenez and Li et al. [32–34].

The mechanical properties of the electrospun fiber mats (5 mm × 40 mm) with certain thickness were investigated using an Instron 5565 material testing instrument (Instron Co., USA) at 25 °C and 65 ± 5% RH. Tensile tests were performed at an extension rate of 1 mm/min, with a gauge length of 20 mm. The thicknesses of the samples were measured by using a CH-1-S thickness instrument (Shanghai Liuling Instruments Co., China) with a resolution of 0.001 mm.