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Electrospun regenerated silk fibroin mats with enhanced mechanical properties

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ABSTRACT

In this paper, a simple and effective method was applied to enhance regenerated silk fibroin (RSF) mats electrospun from aqueous solution. The mats were first mechanically drawn in 90 vol.% ethanol aqueous solution and then immersed in the same solution for 30 min. The morphology, structure, thermal and mechanical properties of the RSF mats with different draw rates and draw ratios were investigated by scanning electron microscope (SEM), Raman spectroscopy, wide angle X-ray diffraction (WAXD), differential scanning calorimetry (DSC) and tensile test. Results revealed that the content of β-sheet conformation, the crystallinity and the number of fibers aligned to the drawing direction increased evidently with the draw ratio. The breaking strength and breaking energy of the post-treated mats at 1.4× draw ratio and 0.1 mm/s draw rate were 8.6 MPa and 172.2 J/kg, respectively. However, those of the as-spun mats were only 1.8 MPa and 93.2 J/kg, respectively. The enhanced RSF mats prepared from entirely aqueous solutions may have extensive applications for tissue engineering.

1. Introduction

Natural silk from the silkworm, *Bombyx mori*, has been used as biomedical suture material for centuries [1]. In recent years, silk fibroin (SF) has fascinated scholars for excellent biocompatibility, biodegradability and outstanding mechanical properties [2–7]. Various forms of SF materials have been used in biomaterials including films, fibers and sponges. Among these forms, electrospun regenerated silk fibroin (RSF) fibers have various advantages, such as circular cross-section, appropriate porosity and nanoscale diameter [8–10]. This material can greatly mimic the extracellular matrix (ECM) and be suitable for cell attachment, multiplication and growth [11].

It is well known that the poor mechanical properties of electrospun RSF mats limit their specific applications, such as blood vessel, ligament, tendons, urethra and so on [12]. Researchers have tried to reinforce electrospun RSF mats through blending or post-treatment. Zhou [13] obtained electrospun RSF/collagen blending mats post-treated by immersion in methanol for 40 min, the breaking strength of the blending mats was only 1.93 MPa although breaking elongation reached 30.47%. Jin [14,15] and Meinel [16] prepared electrospun RSF/PEO mats which were post-treated through immersing in 90 vol.% methanol aqueous solution, but the complicate process needs removing PEO from the mats. Chen et al. [17] immersed pure RSF mats in 90 vol.% methanol aqueous solution, but the stress and strain of the mats were only 1.5 MPa and 1.6%, respectively.

In general, drawing can promote the orientation of molecular chains parallel to extension direction [18], which prevents the growth of cracks and produces high tenacity [19]. Thus, post-drawing has been widely applied to fabricate materials with good mechanical properties. Gandhi et al. [20] post-treated electrospun RSF mats by immersing in methanol aqueous solution firstly and then drawing manually in the air. The post-treated mats have higher breaking strength than those only immersed in methanol aqueous solution. Wei et al. [21] improved the post-treatment process and found that drawing with a subsequent immersion in ethanol aqueous solution was more effective than immersion with a following drawing in post-treatment agent. However, the post-treatment was not accurate and convenient due to the hand-drawing process. In this paper, we demonstrate a new stable approach to control the microstructure and the macrostructure of the electrospun RSF mats *via* a mechanical post-drawing device on our previous basis of dry-spinning and hand-drawing post-treatments. Draw rate and draw ratio vary during the post-treatment. The approach allows us to accurately control the mechanical properties, thermal stability and degradability.

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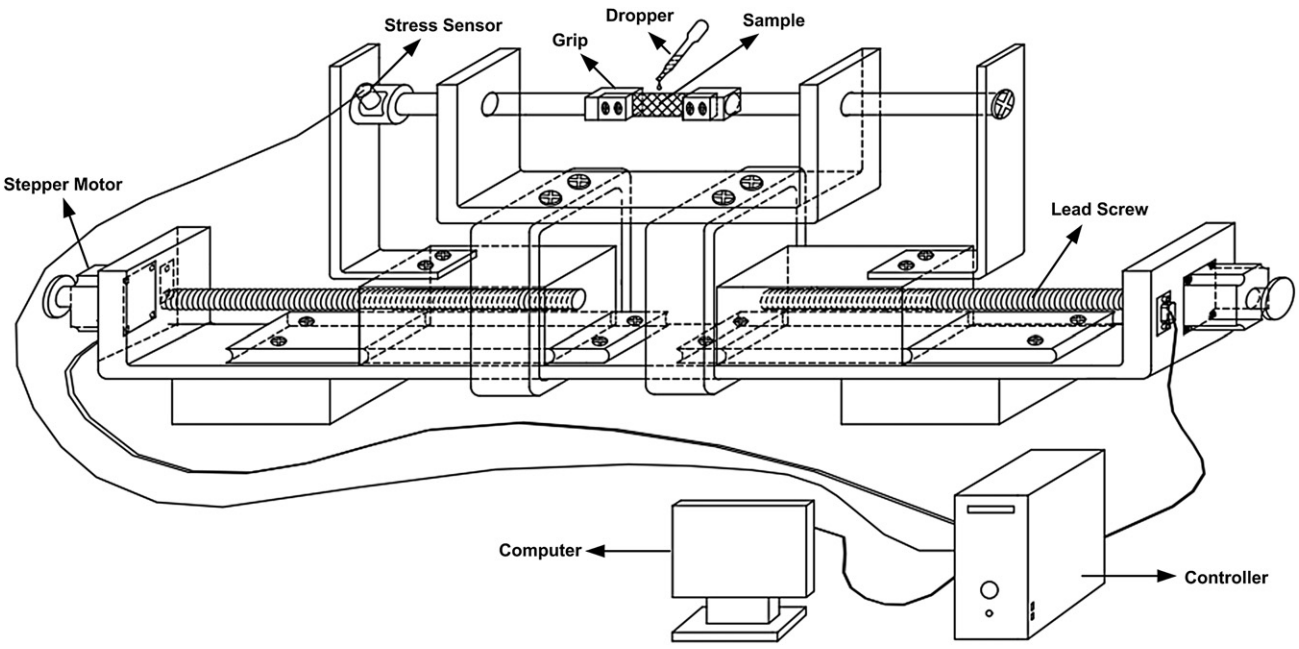

Fig. 1. Experimental setup of post-drawing device for RSF mats.

2. Experiments

2.1. Preparation of electrospun RSF mats

B. mori cocoons from Zhejiang province, China, were degummed twice in boiling 0.5 wt.% Na_2CO_3 solution for 30 min, then rinsed with distilled water to extract sericin thoroughly, and dried at room temperature to prepare degummed silk. Subsequently the degummed silk was dissolved in 9.0 M LiBr aqueous solution at 40 °C for 2 h yielding a 10% (w/v) solution. After being diluted, centrifugalized and filtered, the solution was dialyzed in deionized water at 10 °C for 3 days with a cellulose semipermeable membrane (molecular weight cut off (MWCO): 14,000 ± 2000) to remove the salt. Finally, a 33 wt.% regenerated silk fibroin (RSF) aqueous solution was obtained through condensing by forced airflow.

In electrospinning process, a high electric potential of 20 kV was applied to a syringe needle with an inner diameter of 0.6 mm, while a collection plate covered with an aluminum foil was grounded. The flow rate of the RSF solution was 1.2 mL/h by the pump and the electric field was 2 kV/cm. The electrospinning process was carried out at an ambient temperature for 6 h to obtain about 130 μm thick mats for relevant tests. After a complete drying in air for 15 days, the mats were peeled off from the aluminum foils.

2.2. Post-treatment of electrospun RSF mats

The post-treatment of the electrospun RSF mats was composed of drawing process and immersing process. The drawing process was applied by using a drawing device shown in Fig. 1. The sample was fixed by grips and kept wetting by 90 vol.% ethanol aqueous solution from a dropper during the drawing process. The draw rate and the draw ratio were controlled by a computer equipped with a controller. Meanwhile, the wetted as-spun mats (5.0 cm × 2.5 cm) were firstly drawn at certain times and then immersed in 90 vol.% ethanol aqueous solution for 30 min with a fixed length. The draw rate and the draw ratio were changed separately. The draw rate was initially changed from 0.1 to 0.9 mm/s, while the draw ratio was fixed at 1.17×. Then the draw ratio ranged from 1.1× to 1.4×, while the draw rate was kept constant. The post-treated mats were further dried at room temperature for at least 24 h to remove surface moisture.

2.3. Characterization

The morphologies of electrospun RSF mats were examined using a JSM-5600LV (JEOL Co., Tokyo, Japan) scanning electron microscope (SEM) at 10 kV.

Raman spectra of electrospun RSF mats were obtained using a LabRAM-1 B microscopy Raman spectrometer (Dilor, France). The 632.8 nm line of a He–Ne laser was used to generate an intensity of 6 mW on the mats. The wavenumber ranged from 900 to 1800 cm^{-1}.

To investigate the crystalline structure of the RSF mats, synchrotron radiation wide angle X-ray diffraction were measured at the BL15U1 beamline in Shanghai Synchrotron Radiation Facility (SSRF) at a wavelength of 0.7746 nm. The samples were glued to a sample holder and the exposure time was 30 s/pattern. The distance between detector (Rayonix SX-165) and sample was calibrated with lanthanum hexaboride to 187 mm. Data analysis was performed with FIT2D software (version 12.077, Andy Hammersley/ESRF, Grenoble, France). The crystallinity of the mats was estimated by separating the Bragg reflections from the broad short-range order background.

About 5 micrograms of each sample was encapsulated in an aluminum pan and heated in a MDSC 2910 differential scanning calorimeter (TA Instruments Co., New Castle, DE) with nitrogen gas flow (40 mL/min) and equipped with a refrigerated cooling system. The RSF mats were heated from 35 to 270 °C at a heating rate of 5 °C/min.

To evaluate the mechanical properties of the RSF mats, stress–strain curves were obtained using an Instron 5969 material testing instrument at 25 °C and 50 ± 5% RH. Tensile tests were performed using a 100 N load cell at an extension rate of 1 mm/min with 20 mm gauge length. The thickness of the samples was measured using a CH-1-S thickness measuremeter (Shanghai Liuling Instruments Co., Shanghai, China). The suture retention strength was also determined using the Instron 5969 material testing instrument. Sample (5 mm wide × 40 mm long) was fixed in the lower grip of the instrument and inserted with a suture (Polyglactin 910,