Morphology and Cell Compatibility of Regenerated Ornithoctonus Huwenna Spider Silk by Electrospinning

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Abstract: Ornithoctonus huwenna spiders can be bred in mass production, and potential applications of the spider silk in medical materials should be paid attentions. Regenerated nano-scale spider silk nonwovens were prepared by electrospinning from tanglesome Ornithoctonus huwenna spider silk. The morphologies of the electrospun spider silk fibers were investigated, and the cell compatibilities were explored. The results showed that the electric field strength was an important factor for the diameter, degree of crystal and molecular conformation of electrospun Ornithoctonus huwenna spider silk. With the increase of electric field strength, fibers became fine and even, and the crystallinity and β-sheet structure were improved. The electrospun nanofiber nonwoven had good compatibility with rat bone marrow stromal cells (rBMSCs). The cell survival rates were above 98% on the fiber surfaces.

Keywords: Ornithoctonus huwenna spider, spider silk, electrospinning, molecular conformation, crystallinity, cell compatibility

1. Introduction

Orb-web spiders secret unique silk fibers named as dragline silks from the major amplullate glands, which are used as radial threads that support a spider's web and dragline to make spiders rapid vertical descents [1]. Spider dragline silk is a flexible, lightweight fiber with extraordinary strength and toughness [2], so that researchers have being focused on the fiber since last decade, especially recent 10~15 years. The dragline silk, a protein fiber, has important applications in impact-proof textiles or other structural fabrics. Moreover the inherent biocompatibility makes it to be used in the fields of controlled release biomaterials and scaffolds for tissue engineering. Therefore spider silk has been studied extensively as a kind of protein-based biomaterial and achieved great fruits [3-8]. Biomimetic production of dragline silks has had great progress [9-14]. However, if we want to make commercial use of the biosynthetic fibers, firstly, we must develop a method of spidroin solution mass production, then, an appropriate spinning technology is necessary to make spidroin solution into fine and even fibers. Electrospinning is a simple spinning method widely used in fabrication of various nano-scale polymer fibers, such as PVA, PLA, and PAN etc [15-18]. Electrospun SF fibers have been obtained as well for the following: vascular grafts, wound dressings or tissue engineering scaffolds due to the very high surface area to volume ratio and its biocompatibility and biodegradability [19-21]. However, except that Zarkoob et al first reported the electrospinning of Nephila clavipes dragline silks, there are few of research on electrospinning spider silk [22]. The author Pan has developed an electrospinning method to obtain nano-scale spider silk fiber nonwoven from dragline and egg-case silk of *Araneus ventricosus* spiders [23].

Ornithoctonus huwenna, also named as cutworm, is one of the bigger spiders with the length and weight 6~9 cm, 14~16g respectively. Some special farms breed Ornithoctonus huwenna spiders for extracting toxin as new medicine materials [24-26]. At the same time, a lot of spider silk can be obtained. In our research, Tanglesome Ornithoctonus huwenna spider silk was regenerated, and nano-scale fiber nonwovens were prepared by electrospinning. SEM, FTIR and WAXD were used to investigate the morphologies of the regenerated spider silk fibers. Meanwhile, the cell compatibility of the nanofiber nonwoven was explored.

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2. Experimental

2.1 Materials

Ornithoctonus huwenna spider silk was kindly provided by North spider breeding institute, Nanning city, Guangxi province. 99% Hexaflorisopropanol (HFIP) was purchased from Dupont Chemical Solution Enterprise.

Ornithoctonus huwenna spider silk was dissolved in 99% HFIP for 10h at room temperature. Regenerated nano-scale spider silk nonwovens were prepared from the 0.9wt% spidroin solution by electrospinning.

2.2 Measurements

2.2.1 Longitudinal Shape and Diameter

The electrospun nano-scale fiber nonwoven was fixed on a sample holder by double side adhesive tape and coated with gold. Then, the longitudinal images of fibers were observed and recorded by Hitach S-4700 SEM. The fiber diameters were calculated with HJ2000 software. 100 fibers were processed for every sample and average diameter was obtained.

2.2.2 Molecular Conformation

About 2 mg powdered sample was mixed with 200mg spectrum degree KBr, and pressed in slice on standard procedure. Then the FT-IR spectrum was measured with NICOLE 5700 FT-IR instrument (USA). Every sample was scanned 32 times with wave numbers range 4000-500cm^{-1} and spectrum resolution 4cm^{-1}.

2.2.3 Crystalline Structure

X′Pert Pro MPD X-ray diffraction system (CuKα target, acceleration voltage 40 KV, electric current 40mA, λ= 0.154 nm) of Holand PAN alytical company was used to measure X-ray diffraction intensity curves of powdered samples, and diffraction angle 2θ ranged from 5° to 45°.

The resulting plots of X-ray diffraction intensity versus 2θ were analyzed using the profile fitting program Peak Fit 4.1(AISN Software Inc.). Each peak was modeled using a Gaussian-Lorentzian peak shape. Areas of the peaks obtained from the analysis were used to estimate the degree of crystallinity, i.e. the ratio of the areas of the crystalline reflections to that of total area of the scattering curve (amorphous+crystalline). In general, agreement between model predictions and the data were exceptional, yielding coefficients of determination, i.e.R^2 above 0.98.

2.2.4 Cell Compatibility

rBMSCs were inoculated on the surface of regenerated spider silk nonwoven electrospun at voltage 12KV and distance from capillary tip to collecting screen 8cm from 0.9wt% Ornithoctonus huwenna spider silk / HFIP solution . After being inoculated for 24 h, 48 h and 72 h, the sample was leached two times by L-DMEM without blood serum, then, incubated in 1 mM Calcein and 1 mM ethidium homodimer at 37°C for 30 min. Cell survival rate was obtained by using an Olympus inverted fluorescence microscope.

3. Results and Discussion

3.1 Effects of Electrospinning Parameters on the Fiber Microstructures

3.1.1 Longitudinal Shape and Diameter

Table 1 shows the SEM images, distribution of diameter and average sizes of electrospun regenerated Ornithoctonus huwenna spider silk (EROHS) fibers at different distance from capillary tip to collecting screen (C-SD) at Voltage 12kv. Ornithoctonus huwenna spidroin could be electrospun into smooth and continuous nano-scale fibers. Nanometer scale diameter of the protein fibers would be a useful feature in some biomaterial and tissue engineering applications to enhance surface area while maintaining high porosity.

Their diameters mainly distributed 50~150nm, and average diameters were 105.2±33.5nm, 110.8±40.5nm, 117.6±41.7nm, 123.8±32.9nm and 135.9±45.4nm respectively when C-SD were 8, 10, 12, 14 and 16cm.

With the increase of C-SD, the electric field strength decreases, thus, the electric force loaded on the spinning jet weakens and fiber diameters augment. On the other