Analysis of structure and properties of biodegradable regenerated silk fibroin fibers

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In this paper, the molecular weight change of native silk fibroin fibers when they are dissolved in neutral salt solution, and the relationships of structural change of the regenerated SF fibers with their mechanical properties and degradability have been studied. The results shows that the mechanical properties of regenerated SF fibers are lower than those of native SF fibers, but the biodegradability is raised. © 2006 Springer Science + Business Media, Inc.

1. Introduction
Native silk fibroin (SF) fibers are one kind of protein which consists of 18 amino acids, such as glycine, alanine and sericine. Recently, there are increasing reports about silk used as biomedical materials because of its good biocompatibility with human body. SF has no toxicity, no irritability and no irritation [1, 2]. Particularly, recent research indicated that similar to collagen, SF was ideal for attaching animal cells cultured in vitro, and was also important for maintaining cell function. For example, Wu et al. [3] randomly wound SF fibers to form net where animal chondrocytes were three-dimensionally cultured, and their results showed that SF could be used as good scaffolds for chondrocytes in three-dimensional culture.

The studies on SF fibers have histories of near 40 years. Masumoto [4] reported regenerated SF fibers were spun by self-dialysis in 1996, Oskar [5] reported the SF fibers were spun by dissolving SF with Hexafluoro-2-propanol (HFIP) in 1998, and Yao [6] reported the SF fibers were spun by dissolving SF with HFA in 1998. Particularly, Ishizaka et al. [7] dissolved SF with phosphorus acid in laboratory, then wet-spin the solution with ammonium sulfate or sodium sulfate as coagulant, and then regenerated SF fibers which had strength 2.2 g/d and elongation at break 10% were made after drawing in methanol.

In this paper, the regenerated SF fiber structure and its relationships with mechanical properties and degradability have been studied, and some proofs are provided for the regenerated SF fibers used as tissue engineering materials.

2. Experimental
2.1. Preparation for spinning solution
The domestic (Bombyx mori) silks were boiled three times (each time for 30 min) in 0.5% (w/w) Na2CO3 solution with a liquor ratio of silk to the solution 1:20 (w/w) in order to remove sericin.

The certain amount of the pure dried SF fibers were dissolved with triad solvent LiBr·H2O·CH3CH2OH for 4 h at 78 ± 1°C. The prepared solution was purified by dialyzing against tap water and deionized water for three days, and then the solution was spread on the polyethylene board and dried in air to form film, some of which was taken to dissolve in HFIP from Acros Organics to make SF protein solution. After filtering and deaerating silk fibroin solution with concentration 10% (w/w) was obtained.

2.2. Measurement of SF molecular weight
Molecular weight of SF after dissolved in lithium bromide was measured by use of SDS-polyacrylamide gel-electrophoresis made by Hoefer mini VE. The main composition used for gel-making was 30% mother-liquor(Acr:Bis = 29:1). Dyeing liquor is 40% methanol and 10% acetic acid aqueous solution containing 0.1% coomassie brilliant blue 250, and decolour liquor is 10% methanol and 10% acetic acid aqueous solution.

2.3. Wet-spinning for the SF solution
Regenerated as-spun fiber was formed using wet-spinning process with ethanol as coagulant through double-diffusion between solvent and coagulant, and then the as-spun fiber was drawn and heat-set.

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2.4. Measurement of crystallinity and degree of orientation

2.4.1. Crystallinity

I–2θ curve of SF fiber was obtained by use of silk fibroin fiber. According to the method described by the researchers [8, 9], the I–2θ diffraction curve of SF fiber powder was divided into crystalline area and non-crystalline area. The crystallinity was calculated as

\[ X_c = \frac{I_c}{I_c + I_a} \times 100\% \]

where \( X_c \) is crystallinity, \( I_c \) is diffraction intensity of crystalline area, \( I_a \) is diffraction intensity of non-crystalline area.

2.4.2. Degree of orientation of crystalline area

After carded and paralleled, SF fibers were measured using the highest diffraction intensity to obtain I–ψ curves, on which the half-width (H°) was calculated. The degree of orientation (Rc) of crystalline area was estimated according to

\[ R = \frac{(180 - H^\circ)}{180} \times 100\% \]

2.5. Measurement of enzyme degradation of SF fibers in vitro

Degradation experiment for the silk fibers was performed with actinomyces enzyme bought from Sigma Co. at pH 7.0 and temperature 37°C. The enzyme was dissolved in phosphonate buffer solutions of pH 7.0 to form solutions containing 5 unit actinomyces enzyme per ml. The regenerated and native SF fibers were respectively put in the enzyme solutions with a liquor ratio of SF fibers to the solution 1:20 (w/w). Then the solutions were put in the test tubes which were then sealed by film, the tubes were put in water bath at constant temperature 37°C, and one of the tubes containing regenerated SF fibers or native SF fibers was taken out every 5 days. Then the fiber taken from the tube was washed with water and heated to a constant weight in oven at 105°C. The solutions except the remained fibers in the other test tubes were changed with new enzyme solutions, respectively, and after the process described above was repeated for several times, the fibers after degradation for different times would be obtained. The degradability (D) was calculated according to

\[ D = \frac{(w_1 - w_2)}{w_1} \times 100\% \]

where \( w_1 \) is weight of dried regenerated SF fibers and \( w_2 \) is weight of dried regenerated SF fibers after degraded certain days.

3. Results and discussion

3.1. SF molecular weight after dissolved in lithium bromide

As show in Fig. 1, after dissolved in lithium bromide, molecular weight of the SF solution mainly distributes below 100000, while molecular weight of native SF fiber is about 300000, indicating when native SF fiber was dissolved using lithium bromide, SF molecules degrade to a great extent, and biodegradability of regenerated SF fibers made by this solution will possibly be increased.

3.2. Raman spectroscopy

The Raman effect arises when the incident light excites molecules in the sample which subsequently scatter the light. The Raman scatter is related to the ability with which the molecules are polarized. When vibration and rotation energy of the molecules change, Raman absorption spectra are caused. In material research, Raman spectroscopy can serve as a complement of IR spectroscopy. In Raman spectroscopy, the Raman scatter intensity (Y-axis) is plotted against the Raman shift (X-axis). The Laser Raman spectroscopy can show characteristic band of peptide bond, backbones of main chains and sideway chains in proteins. According to the characteristic peaks of peptide bond, conformation characteristics of proteins can be analyzed [11]. In the Laser Raman spectroscopy polypeptide and protein molecular chains have many amide bands. Particularly, each of the amide characteristic peaks lower than 1700 cm\(^{-1}\) has intimate relations with protein molecular conformation [12].

As shown in Laser Raman spectra of regenerated SF fiber coagulated by ethanol and native SF fiber (Figs 2 and 3), there are certain differences between the spectra parallel and perpendicular to polarization direction of the laser. This mainly ascribes to anisotropism of molecular chain arrangement in the fibers. Combining the wavenum-