Analysis of structure and properties of biodegradable regenerated silk fibroin fibers

B. ZUO*, L. DAI, Z. WU
School of Materials Engineering, Soochow University, Suzhou, Jiangsu 215021, People's Republic of China
E-mail: bqzuo@suda.edu.cn

Published online: 12 April 2006

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.

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-spun 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. Exprimental

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) Na_2CO_3 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 $LiBr{\cdot}H_2O{\cdot}CH_3CH_2OH$ 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.

*Author to whom all correspondence should be addressed.
0022-2461
DOI: 10.1007/s10853-005-5384-z

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

$$X_c = I_c/(I_c + I_a)100\%$$

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 (R_c) of crystalline area was estimated according to

$$R = (180 - H^\circ)/180 \times 100$$

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

$$D = (w_1 - w_2)/w_1 \times 100\%$$

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.

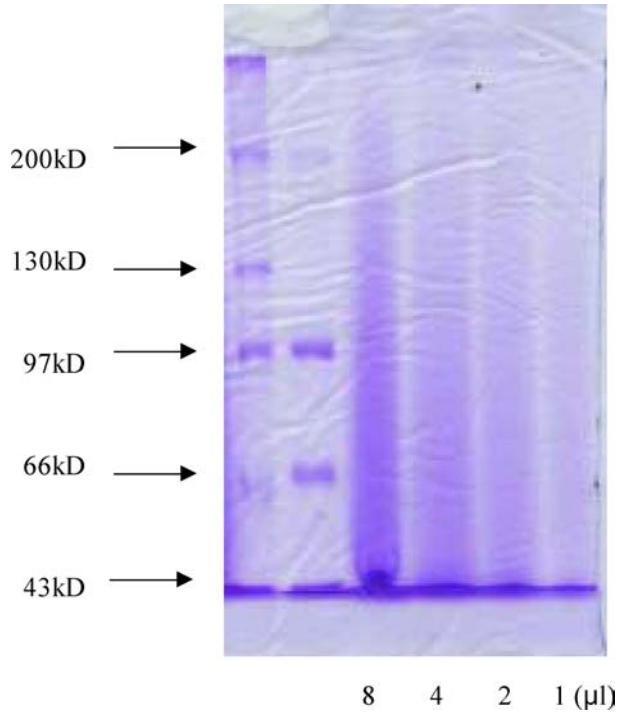

Figure 1 SF molecular weight after dissolved in lithium bromide.

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-

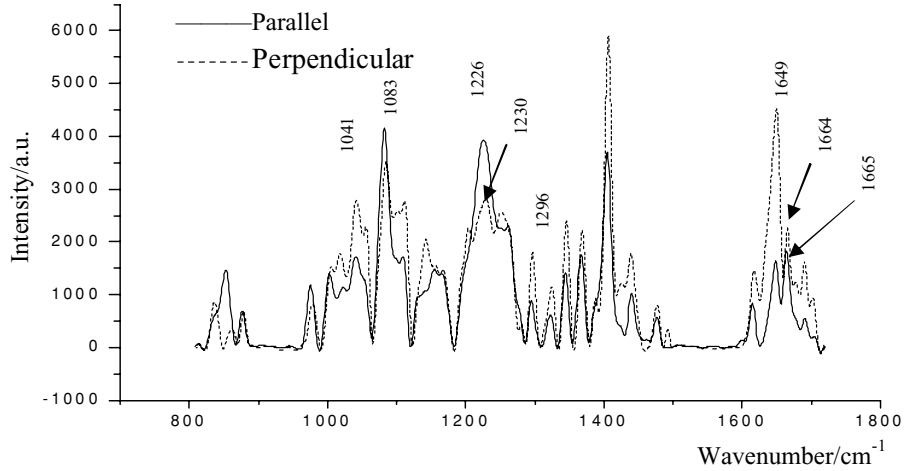

Figure 2 Laser Raman Spectra of regenerated SF drawn fiber.

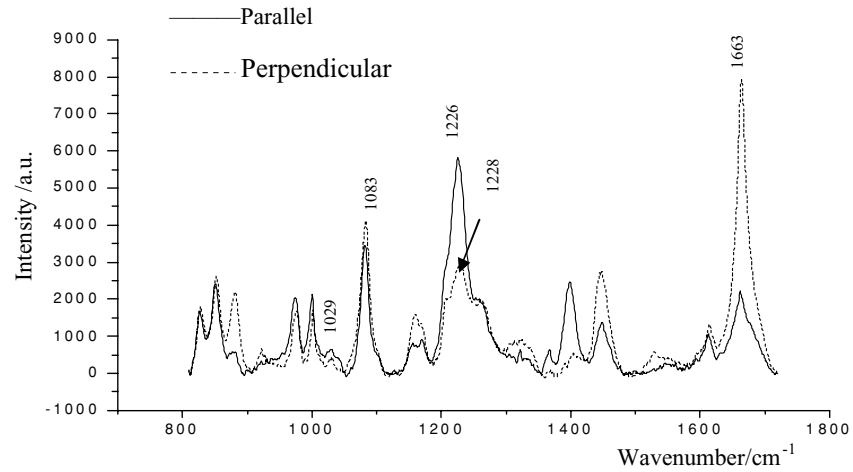

Figure 3 Laser Raman spectra of native SF fiber.

ber position of the characteristic peak with Raman spectroscope [12] of typical SF conformation, it is clear that 1665–1680 cm^{-1} corresponds to β-fold of amide I polytide segement, and also reflects distribution of β-turn conformation; 1660–1666 cm^{-1} corresponds to α-helix, and 1645–1658 cm^{-1} to random coil. Inside amide I, β-fold and α-helix coexist for native SF fiber, while α-helix and random coil coexist for regenerated SF fiber coagulated by ethanol. Near amide III absorption, there are higher intensities at 1226–1230 cm^{-1} for both of the fibers, which is related to β-fold and β-turn. Simultaneously, there is a peak in the vicinity of 1296 cm^{-1} for regenerated SF fiber, but not for native SF fiber, It is considered that the structure is compact and crystalization is good for native SF fiber, while it is incompact for regenerated SF fiber.

As shown in Figs 2 and 3, compared the wavenumber position of each characteristic peak with Raman spectra of typical conformation, and combined with the analytical results of X-ray diffraction, it is considered that β-fold coexistence with random coil and α-helix in regenerated SF molecules leads to incompact structure due to low molecular weight and insufficient drawing, while in native SF molecules, β-fold conformation and random coil coexist to form compact structure. It was reported that there was α-helix in silk proteins of silk gland [12, 13]. So it is possible that when draw ratio is not so high during SF fiber processing, there is some α-helix in regenerated SF fibers.

3.3. X-ray diffraction

X-ray diffraction curves of the regenerated as-spun SF fiber, regenerated drawn SF fiber and native SF fiber are shown in Fig. 4. Crystallinity and degree of orientation of regenerated as-spun SF fiber, regenerated drawn SF fiber and native SF fiber are shown in Table I.

The crystallinity and degree of orientation as shown in Table I and Fig. 4 indicate that the spinning solution begins to coagulate and crystallize after it is forced to coagulating bath. So the as-spun fiber has higher crystallinity. But crystallinity of the regenerated drawn SF fiber is less than that of native SF fiber. Unfortunately, because of the brittle property of the fiber the degree of orientation in crystalline area can not be measured.

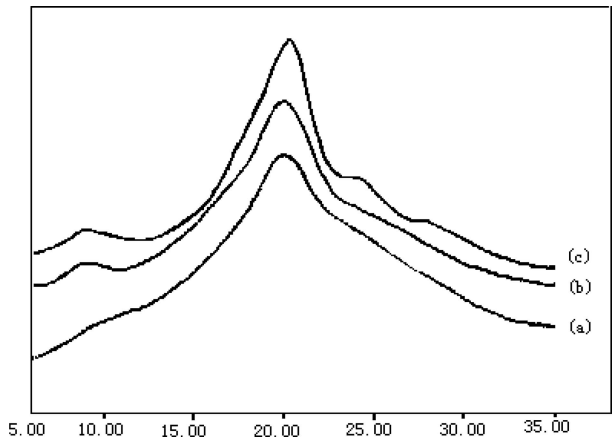

Figure 4 WAXD patterns of silk fibroin fibers, (a) Regenerated as-spun SF fiber, (b) Regenerated drawn SF fiber and (c) Native SF fiber.

3.4. Thermal gravity analysis

The TGA results of regenerated SF fibers and native SF fiber are shown in Table II.

Compared characteristic temperatures of regenerated drawn SF fiber with native SF fiber, based on the theory that the higher the degradation temperature, the higher the crystallinity, it is clear that the crystallinity of regenerated SF fibers lower than that of native fiber. However, the degradation temperature difference between the drawn fiber and the as-spun fiber is smaller, so the crystallinity difference is also smaller, which agreed with the result of Para. 3.3.

3.5. Mechanical properties

Mechanical properties of the regenerated fibers were measured using YG004A Single Fiber Electronic Strength Tester. The conditions of the measurement were as follows: The gauge length of the specimens was 10 cm, the close head speed 25 mm/min, the initiate tensile force 0.10 cN. each sample was measured 5 times and averaged.

TABLE I The crystallinity and degree of orientation

Samples	Crystallinity (%)	Orientation degree (%)
Regenerated as-spun SF fiber with ethanol as coagulant	47.2	—
Regenerated SF fiber with ethanol as coagulant	48.3	77.8
Native SF fiber	61.5	87.1

TABLE II Decomposition temperyure of SF fibers

Samples	Decomposition temperature (°C)
Regenerated as-spun SF fiber with ethanol as coagulant	301.8
Regenerated drawn SF fiber with ethanol as coagulant	307.9
Native SF fiber	331.1

TABLE III Mechanical properties of SF fiber

Samples	Properties		
	Coarseness (dtex)	Break strength (cN/dtex)	Elongation at break (%)
Regenerated drawn SF with ethanol as coagulant	49.08	0.82	25.04
Native SF fiber	17.11	2.56	9.30

Each of the data was an average obtained by measuring each sample 5 times.

As shown in Table III, compared the mechanical properties of the drawn SF fiber with native SF fiber, the break strength of the former are about 30% latter, while break elongation of the former is 2–3 times the latter, which is agree that the crystallinity and degree of orientation of the regenerated SF fiber are all lower than those of native fiber.

If spinning technology is improved, possibly the mechanical properties of regenerated SF fiber would be somewhat raised. But because it is inevitable that SF fiber degradation will be caused during dissolving, and at the same time, the crystallinity and orientation of regenerated SF fiber are also lower than the native fiber, the mechanical properties of the former will certainly lower than the native fiber. But it is anticipated that the degradation properties of regenerated fiber are higher than the native fiber, which is required using as biological medical materials and which is no other than the right significance of our research.

3.6. Enzyme degradation of SF fibers *in vitro*

Degradation experiment for the silk fibers *in vitro* was performed using actinomyces enzyme. The moisture regain of regenerated fibers used was 11.2% and that of native SF fiber used was 10.7%.

The degradation curves are shown in Fig. 5. According to the experiment, the degradability of regenerated fiber

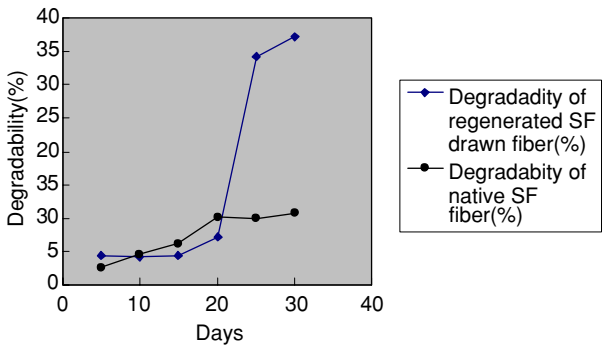

Figure 5 Actinomyces enzyme degradation test of SF fiber *in vitro*.

without actinomyces enzyme (blank test) after 30 days in phosphonate buffer solution is 1.97%.

As shown in Fig. 5, the degradability of the regenerated fiber using actinomyces enzyme after 20 days increases obviously, the degradability using the enzyme after 30 days reaches 37.2%, but the degradability of the native SF fiber using the enzyme is 10.7% and lower than the regenerated fiber.

4. Conclusions

1. In this paper, most of the molecular weights of regenerated SF fibers are below 100000, so their crystallinity, orientation index in crystalline area, heat degradation temperature and mechanical properties are all lower than native SF fiber. During coagulating and drawing for fiber formation by wet spinning, SF conformation is transformed from random coil into coexistence of β-fold, random coil and α-helix.

2. As X-ray and TGA indicated, the crystallinity and degree of orientation of the regenerated fibers are lower than that of native SF fiber, which can improve degradability of the regenerated fibers.

3. Actinomyces enzyme degradation for SF fibers prepared by HFIP process was performed *in vitro*. The degradability of the regenerated SF fibers coagulated by alcohol reaches 37.2% after 30 days' degradation, while the degradability of the native SF fiber after the same days' degradation is 10.7%, indicating the degradability of regenerated SF fiber is better than that of native SF fiber due to low molecular weight and incompact structure of regenerated SF fiber.

Acknowlegments

The present work is supported financially by the National Natural Science Foundation of China (No. 50373031).

References

1. L. NING, L. XUE and H. N. HUANG, *et al*., *China J. Mod. Med.* **9** (1999) 5.
2. ZHENGYU WU, *et al*., *J. Mater.* **14**(9) (2000) 63.
3. HAITAO WU, CUIPING CUI and YUNDI GU, *et al*. *Chinese J. Reparativ. Reconstr. Surg.* **14**(5) (2000) 301.
4. K. MATSUMOTO, *J. Appl. Poly. Sci*. **60** (1996) 503.
5. OSKAR LIIVAK *Macromolecules* **31** (1998) 2947.
6. JUMING YAO and HIROMI MASUDA, *et al*. *Macromolecules* **35** (2002) 6.
7. HIROKO ISHIZAKA, YOICHI WATANABE and KYOKO ISHIDA, *et al. J. Srric. Sci. Jpn.* **58**(2) (1989) 87.
8. A. WEIDINGER and P. H. HERMANS, *J. Appl. Phys.* **19** (1948) 491.
9. A. WEIDINGER, G. CHALLA and P. H. HERMANS, *Makromolekulare Chemie* **56** (1962) 169.
10. HUASHAN ZHAO, *et al*. "Polymer physics" (Textile Industry Press, Beiging, 1982) p. 235.
11. JIALAI PAN, "Application of laser Raman spectrum in organic chemistry" (Chemical Engineering Press, Beiging, 1986).
12. ZHENGZHONG SHAO, DONG WU and GUANGXIAN LI, *et al*. *Chinese J. Light Scatter.* **7**(1) (1995) 2.
13. MASUHIRO TSUKADA, MASANOBU NAGURA and HIROSHI ISHIKAWA, *SEN-I GAKKAISHI* **41**(6) (1985) 37.
14. GREGORY H. ALTMAN, *et al*. *Biomaterials* **23** (2002) 4131.

Received xx xx
and accepted 13 July 2005