

# Property of electrospinning silk fibroin nanofibers prepared by different dissolved methods

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**Abstract.** In the present study, silk was dissolved in CaCl<sub>2</sub>-formic acid and CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O to obtain silk fibroin solution. The dissolve process, morphology, structure and mechanical properties of nanofibers prepared by electrospinning were studied. It showed that nanofibrillar structures were exhibited in the silk fibroin solution in CaCl<sub>2</sub>-formic acid compared with traditional method dissolving in CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O solution, and the viscosity of the solution was much higher. The average diameter of nanofibers prepared by CaCl<sub>2</sub>-formic acid dissolution method was 375±26nm, and it showed well drawability, after stretching to 1 times, its breaking strength and elongation at break reaching 18.6±3.8MPa and 14.0±2.5%, respectively, was improved obviously relative to nanofibers prepared by CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O dissolution method. It demonstrated that the good mechanical property of fibers was contributed to the fibrillar structures in the solution.

## 1. Introduction

Natural silk from the silkworm, *Bombyx mori*, has been used as biomedical suture material for centuries[1]. Silk fibroin is a nature protein and is known to promote cell attachment and proliferation and tissue regeneration. It has been reported that the biological features of silk fibroin make it a very good choice for use as regeneration [2,3]. Silk fibroin has been widely explored in many biomedical applications due to its impressive biocompatibility, biodegradability, minimal inflammatory reactions [4,5]. Electrospinning is a unique method capable of producing nanoscale fibers from both synthetic as well as natural polymers for biomedical applications [6,7]. The cylinder collector rotating at high speed can obtain aligned fiber was a simple and common way relative to other methods to obtain aligned fiber[8]. However, if the speed of collector was too higher, the fibers were easily to fracture.

In the present study, silk was dissolved in CaCl<sub>2</sub>-formic acid and CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O to obtain silk fibroin solution. To obtain aligned and well mechanical properties electrospun silk fibroin nanofibers, we achieve an optimal combination of the revolve cylinder as equipment collector and physical stretching with the speed of 1000rpm. To compare the

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different properties of nanofibers prepared by different dissolved methods, the dissolve process, morphology, structure and mechanical properties of nanofibers prepared by electrospinning were studied.

## 2. Materials And Methods

### 2.1 Preparation of SF Regenerated Films

**CaCl<sub>2</sub>- formic acid dissolved method:** Raw silk fibers were degummed twice with 0.05% (w/w) Na<sub>2</sub>CO<sub>3</sub> solution at 100°C for 30 min and then rinsed thoroughly with deionized water for three times to remove glue-like sericin proteins. The degummed silk fibroin(SF) was dissolved in a mixture of solvents composed of CaCl<sub>2</sub>: formic acid=5:95(w/w) for 2h under room temperature. The SF membrane was prepared by spread the SF solution on the polyethylene plastic board and dried under the room temperature, was named SF1 films.

**CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O dissolved method:** Raw silk fibers were degummed twice with 0.05% (w/w) Na<sub>2</sub>CO<sub>3</sub> solution at 100°C for 30 min and then rinsed thoroughly with deionized water for three times to remove glue-like sericin proteins. The degummed SF was dissolved in a mixture of solvents composed of CaCl<sub>2</sub>:C<sub>2</sub>H<sub>5</sub>OH:H<sub>2</sub>O=44:45:11(w/w) for 6h at 80°C. The solution was then dialyzed in cellulose tubular membrane (molecular cutoff = 8000~14000, Sigma, USA) against distilled water for 3 days. The water was changed every 2 h. The SF solution was filtered and air-dried to obtain the regenerated SF films, was named SF2 films. Desalt treatment in deionized water for 3-4h and air-dried to obtain the regenerated SF2 films.

### 2.2 Electrospinning and Post-Treatment

The 8% SF electrospinning solution was prepared by dissolving regenerated SF1 and SF2 films in 98% formic acid for 1 and 3h, respectively. In the electrospinning process, the SF solutions were placed into the syringe with a stainless needle connected to the high voltage power supplier, a voltage of 12 kV was applied to the stainless needle and a distance of 10 cm between the syringe tip was employed. High voltage power supply (DW-P503-4AC, Dongwen High Voltage Power Tianjin Power Supply Plant, China), a syringe pump (WZ-50C66 T, Medical Instrument Corporation of Zhejiang University, Zhejiang, China). Cylinder collector(manmade), the rotation speed of the collector was studied at speed of 1000rpm.

All electrospinning experiments were performed with the same processing conditions. As the voltage increases, a drop of SF solution formed at the tip of the needle, and then a jet was ejected. With the solvent evaporating, the SF nanofibers formed on the collection screen. Then ethanol treatment of SF nanofibers were immersed in 75/25 (v/v) ethanol/water for 30 min to induce crystallization of SF, and then dried in vacuum at room temperature for 24 h. The nanofibers prepared by different dissolved method were named SF-CaCl<sub>2</sub>-formic acid and SF-CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O, respectively.

### 2.3 Characterizing Techniques

The morphology of SF nanofibers were observed using SEM (S-5700, Hitachi, Tokyo, Japan). Samples were mounted on copper plate and sputter-coated with gold layer 20-30nm thick prior to imaging. The diameters of the fibers were acquired from randomly collected

SEM images and measured by Image J. For each sample, the average fiber diameter and distribution were determined based on 100 random measurements.

Rheological studies were run on a rheometer (Rheometer, TA Instruments, New Castle, DE) with a 35 mm cone plate. The normal force applied on the sample during lowering of the top plate was limited to 0.1N. The shear rate was linearly increased from 0.1 to 5000 1/s at 25°C.

Mechanical properties of samples were determined by a Universal Testing Machine (model 3365, Instron corp., Canton, MA, USA) at 20°C, and 65% RH. Various parameters were set as follows: force accuracy, 1cN; and elongation accuracy, 0.01 mm. Strips measuring 50×10 mm were glued on a paper frame and then mounted on Instron tensile tester. The sample was broke by elongation rate 20 mm/min with a gauge length of 20 mm. Average tensile properties from five specimens were measured.

### 3. Results And Discussion

#### 3.1 Morphology and Viscosity of SF solution

In this work, a novel dissolve method for producing fibers from a  $\text{CaCl}_2$ - formic acid solution, hierarchical fibrillar structures were exhibited in the solution, as showed in Fig.1(A). The viscosity of solution was showed in Fig.1(B), the viscosity was higher compared with traditional method dissolving in  $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  solution, and was able to satisfy the electrospinning process.

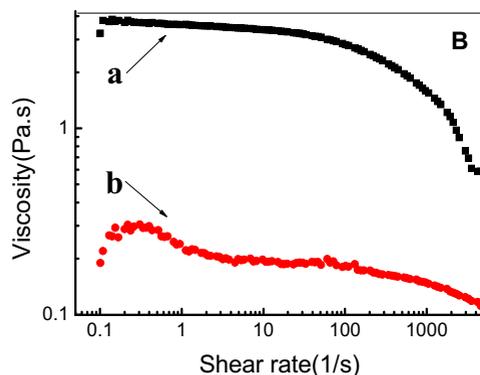
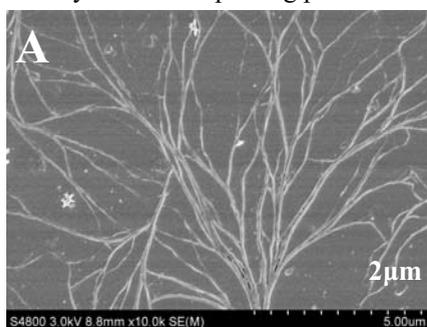


Fig.1 Morphology(A.  $\text{CaCl}_2$ - formic acid solution) , Viscosity(B) of SF solution: a.  $\text{CaCl}_2$ - formic acid solution; b.  $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  solution.

#### 3.2 Morphology of SF Nanofibers

The morphology of SF nanofibers was varied with different dissolved method in the same parameters, as showed in Fig.2. The average diameter of nanofibers prepared by  $\text{CaCl}_2$ -formic acid dissolution method was  $375\pm 26\text{nm}$ , which much higher than SF- $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  with diameter of  $132\pm 15\text{nm}$ . The SF- $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  was more uniform and smooth. After stretching, the majority fibers were oriented along the longitudinal axis to form a unique aligned structure, while, SF- $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  showed uneven thickness along the fibers, as showed in Fig.3.

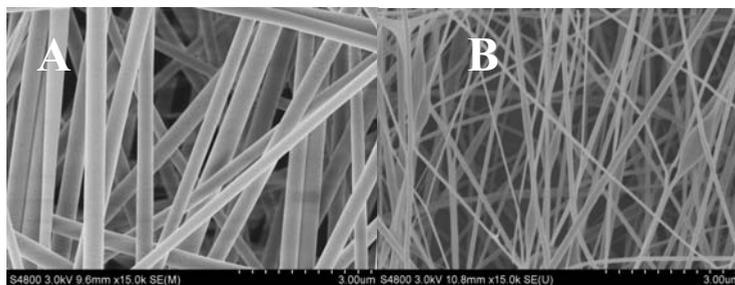


Fig.2 Morphologies of SF nanofibers: A. SF-CaCl<sub>2</sub>- formic acid, 375±26nm; B. SF-CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O, 132±15nm.

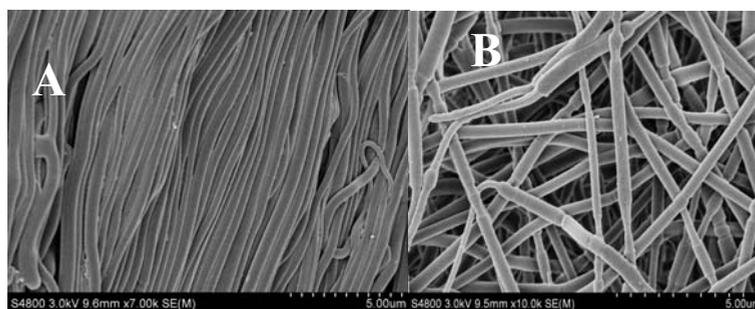


Fig.3 Morphologies of SF nanofibers after stretching: A. SF-CaCl<sub>2</sub>- formic acid; B. SF-CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O.

### 3.3 Mechanical Property

The mechanical properties of electrospun SF nanofibers were tested, since it was a key factor for applications in tissue engineering. According to the results of mechanical property test, as showed in Table 1, the mechanical properties of SF-CaCl<sub>2</sub>-formic acid were much higher than SF-CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O. The stress of SF-CaCl<sub>2</sub>- formic acid was nearly 4 times to nanofibers obtained by the traditional dissolved method[9]. Therefore, it is believed that the improvement of the mechanical properties of SF-CaCl<sub>2</sub>-C<sub>2</sub>H<sub>5</sub>OH-H<sub>2</sub>O, was likely due to the preserved nanofibrils in electrospinning solutions. Above all, to improve the mechanical property of fibers, post-drawing was applied on the fibers. After 1 times stretching, the breaking strength and elongation at break reaching 18.6±3.8MPa and 14.0±2.5%.

Table 1 Mechanical properties of silk fibroin nanofibers

Dissolved solution	Drawing times	Stress (MPa)	Strain at break (%)
CaCl <sub>2</sub> - formic acid	0	12.5±2.3	16.8±3.4
	0.5	14.6±1.4	15.2±4.2
	1	18.6±3.8	14.0±2.5
CaCl <sub>2</sub> -C <sub>2</sub> H <sub>5</sub> OH-H <sub>2</sub> O	0	3.6±1.5	4.8±2.3
	0.5	4.8±2.6	3.2±1.7

## 4. Conclusion

In this paper, a novel method for dissolving degummed silk in  $\text{CaCl}_2$ -formic acid system was applied, and hierarchical nanofibril structures were exhibited in the solution compared with traditional method dissolving in  $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  solution, and the viscosity of the solution was higher. Silk was dissolved in  $\text{CaCl}_2$ -formic acid and  $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  to obtain SF solutions. The dissolve process, morphology, structure and mechanical properties of nanofibers prepared by electrospinning were studied. It showed that nanofibril structures were exhibited in the SF solution in  $\text{CaCl}_2$ -formic acid compared with traditional method dissolving in  $\text{CaCl}_2$ - $\text{C}_2\text{H}_5\text{OH}$ - $\text{H}_2\text{O}$  solution, and the viscosity of the solution was much higher. The average diameter of nanofibers prepared by  $\text{CaCl}_2$ -formic acid dissolution method was  $375\pm 26\text{nm}$ , and it showed well drawability, after stretching to 1 times, its breaking strength and elongation at break reaching  $18.6\pm 3.8\text{MPa}$  and  $14.0\pm 2.5\%$ , respectively.

## 5. Acknowledgments

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