

## Effect of Polyalcohol on the Gelation Time and Gel Structure of Silk Fibroin

Si-Yong Xiong, Ying-Dong Cheng, Yu Liu, Shu-Qin Yan, Qiang Zhang, Ming-Zhong Li\*

*National Engineering Laboratory for Modern Silk, College of Textile and Clothing Engineering, Soochow University, No. 199 Ren'ai Road, Industrial Park, Suzhou 215123, China*

**Abstract:** The *Bombyx mori* silk fibroin gels were prepared by adding various types of polyalcohol (Ethylene glycol, Polyethylene glycol 600) into the regenerated silk fibroin aqueous solution. The gelation time was recorded respectively. Gel structure was analyzed by X-ray diffraction, Fourier transform infrared spectroscopy, laser Raman spectroscopy and scanning electron microscopy. It was found that adding polyalcohol into the regenerated silk fibroin aqueous solution can accelerate the gelation process, with increase in the percentage of the polyalcohol except at 100%. Silk fibroin gels with Ethylene glycol mainly consist of silk II crystal structure. Ethylene glycol almost has no effect on the molecular conformation or crystal structure of silk fibroin gels. However, adding PEG600 to the solution can promote the silk fibroin molecules to form clusters rapidly, while it can also obstruct the structural transformation of silk fibroin molecules from random coil or  $\alpha$ -helix to  $\beta$ -sheet. The crystallinity of silk gels was found to decline with an increase in the adding percentage of PEG600. While adding 100% polyalcohol of the mass of silk fibroin into the solution, whether it was Ethylene glycol or PEG600, the silk fibroin gels have more uniform morphology and pores distribution.

**Keywords:** Silk fibroin, gel, gelation time, ethylene glycol, PEG 600, polyalcohol, structure.

### 1. Introduction

Silk from *Bombyx mori* are natural fibrous polymers that have been increasingly studied as new biomaterials in recent years due to its biocompatibility, degradability and excellent mechanical properties. As the structural protein of *Bombyx mori* silk fiber, silk fibroin has also been prepared or designed into various formats such as nanospheres, films, porous materials, for controlled release-delivery of bioactive agents [1-3], cell culture and tissue engineering [4-8].

Among many *Bombyx mori* silk fibroin materials, silk fibroin gel with three a dimensional structure is an important form to be developed in tissue engineering materials. Besides the flexibility and plasticity, it also has permeability for some small molecular or polymer materials. Silk fibroin gel has been studied or applied in artificial skin, contact lenses, drug controlled-release carrier, enzyme immobilization carrier, cell culture matrix [9-11]. Therefore, it is necessary to explore the formation mechanism of silk fibroin gel and its influencing factors.

During the formation of silk fibroin gel, it mainly includes hydrophobic interactions, hydrogen bond formation and electrostatic interactions [12-16]. There are many influencing factors such as temperature, silk fibroin concentration, pH and ions, which significantly

affect the gelation process. Gelation will occur in the regenerated silk fibroin solution under certain conditions, mainly due to a structural transition from random coil or  $\alpha$ -helix to  $\beta$ -sheet [17]. Hirabayashi et al [18] reported the formation mechanism of silk fibroin gel and considered that the gel formation was due to hydrogen bond formation between silk fibroin molecular chains. Akira Matsumoto et al. [19] studied mechanisms of silk fibroin sol-gel transitions by monitoring the process under various physicochemical conditions with optical spectroscopy at 550 nm. This study indicated that there are two stages during the gelation process. In the early stage small precipitates ( $<10 \mu\text{m}$ ) first appeared then sizes of these precipitates gradually increased. In the later stage, larger aggregates ( $10\text{-}100 \mu\text{m}$ ) became immobile in the bulk or partially gelled state.

In vitro [20-22], the silk fibroin molecules in aqueous solution will turn into  $\alpha$ -helix and  $\beta$ -sheet conformation after gelation. This sol - gel transition process was related to the concentration of silk fibroin solution, temperature, pH, and ion concentration. M. Fini et al [23] induced to prepare silk fibroin hydrogel for healing the critical size defects of rabbit distal femurs by adjusting the pH of the silk fibroin solution. Xiaoqin Wang et al [24] studied sonication-induced gelation of silk fibroin for cell encapsulation and found

\*Corresponding author's email: mzli@suda.edu.cn  
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that ultrasonication could accelerate the formation of physical cross-links responsible for gel stabilization, because it initiated the formation of  $\beta$ -sheets by alteration in hydrophobic hydration, and then formed gels finally. Ung-Jin Kim et al [25] reported that strong hydrophilic substances such as PEO could facilitate the intermolecular or intramolecular interactions among the protein molecules and the subsequent formation of the  $\beta$ -sheet structure. Thus it promoted the sol-gel transitions, which could be induced by direct addition of PEO into the fibroin aqueous solutions or via separation from the aqueous solutions across a dialysis membrane (with PEG).

In the present study, we have studied in detail the strong hydrophilic substance such as polyalcohol (mainly Ethylene glycol or PEG 600) that influence silk fibroin sol-gel transitions, because it is one of the important factors which should not be overlooked. The Bombyx mori silk fibroin gels were prepared by adding various quality of polyalcohol (Ethylene glycol, PEG 600) into the regenerated silk fibroin aqueous solution. The gelation time of regenerated silk fibroin aqueous solutions with various qualities of polyalcohol was studied. The morphology and the structure of silk fibroin gels were also studied.

## 2. Materials and methods

### 2.1 Preparation of regenerated silk fibroin solution

According to the literature [26], the Bombyx mori silks were treated three times with 0.05 wt %  $\text{Na}_2\text{CO}_3$  solution at 98~100°C for 30 min respectively to remove sericin. Then they were rinsed and air dried. The pure silk fibroin fibers were dissolved with triad solvent  $\text{CaCl}_2 \cdot \text{CH}_3\text{CH}_2\text{OH} \cdot \text{H}_2\text{O}$  (mole ratio=1:2:8) at  $78 \pm 2^\circ\text{C}$  through stirring. The prepared solution was purified by dialyzed against water for three days, to obtain 2.7 wt % silk fibroin solution. The regenerated silk fibroin solution was prepared, and then it was stirred slowly at room temperature to make it to evaporate and concentrate up to 3.0 wt %.

### 2.2 Preparation of regenerated silk fibroin gels

The 25mL silk fibroin solutions at concentrations of 3.0 wt% were injected into two groups of culture dishes (diameter 8.5 cm, each group with six culture dishes). Added Ethylene glycol to the first group and PEG 600 to the second group, according to these percentages (0%, 100%, 300%, 500%, 700%, and

900%) of solute mass, respectively. Stirred while adding polyalcohol, and then were put aside at room temperature. Gelation time of each sample was recorded. After freeze-drying, silk fibroin gels were prepared.

### 2.3 X-ray diffraction

X-ray diffraction was performed by a Rigaku D/Max-3C diffractometer with Cu-K $\alpha$  radiation ( $\lambda = 0.15418$  nm). The X-ray source was operated at 40 kV and 40 mA. Diffraction intensity was measured in reflection mode at a scanning rate of  $2^\circ/\text{min}$  for  $2\theta = 5\text{--}40^\circ$ .

### 2.4 FTIR spectroscopy

Fourier transform infrared (FTIR) spectra were obtained by a Nicolet Avatar-IR360.

### 2.5 Laser Raman Spectroscopy

Raman spectra were recorded using a Dilor LabRam-1B spectrometer, operating at a resolution of 1  $\text{cm}^{-1}$ . The Spectra Physics Model 164 argon ion laser was operated at 632.8 nm with about 6mW power.

### 2.6 Scanning electron microscopy

The gel samples were cut horizontally at middle depth. The cross-sectional surface was examined with a Hitachi S-570 Scanning Electron Microscope (SEM).

## 3. Results and discussion

### 3.1 The effects of polyalcohol on the gelation time

Adding ethylene glycol or PEG600 into the regenerated silk fibroin aqueous solutions can accelerate the gelation process with an increase in percentage except at 100%. The gelation time of silk fibroin solution without polyalcohol (the control sample) was 116h (Figure 1). This is because the water-bonding capacity of the strong hydrophilic substances, such as Ethylene glycol, PEG 600, broke hydration layer around the fibroin molecules in the solution. It then competed for water molecules with silk fibroin molecules, which reduced water molecules among the silk fibroin chains and facilitated to collide, leading to the acceleration of silk fibroin molecular aggregation and coagulation. In addition, while silk fibroin molecules collided, the hydrophobic