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The Effect of Shearing Force that Influences Structural Transitions in Silk Fibroin

Yu Liu, Ying-Dong Cheng, Si-Yong Xiong, Peng-Ju Li, Yan-Qiong Wei, 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: Silk hydrogel has been utilized to deliver drug and growth factor and replace tissue. Therefore, exploring the structural changes of silk fibroin for variety of applications has attracted public concern. It has been reported that gel transition is influenced by temperature, pH, and shearing force. In this paper, we have studied the effect of shearing force that influences silk fibroin structural transitions. After stirring the silk fibroin solution at various shearing rates, the gelation time was observed. To investigate the effect of shearing force during the process, the molecular conformation of gel was investigated by CD, XRD, FT-IR and Raman spectroscopy. It was found that shearing force accelerated the gel rate, and with the increase of rate, gelation time decreased. The rate of 150r/min was more effective to promote gelation. β-sheet structure was formed by the shearing force, which was observed by X-ray diffraction. The crystallinity was increased effectively. After being lyophilized, the sample exhibited network and sponge-like structures. Remarkable changes in porosity were observed after using different shearing rates. It was observed that the higher the shearing rate, the lesser the pores formed.

Keywords: Shearing force, gel, structural transition, regenerate, silk fibroin.

1. Introduction

The Bombyx mori (B. mori) silk fibroin, consists of mainly glycine, alanine, and sericine, isa spun fibroin protein with strong intramolecular and intermolecular hydrogen bonding. Silks have been used as textile fibers for thousands of years because of their unique gloss, handle, and mechanical properties. Recently, studies based on silk fibroin for biomaterials have become more and more popular. Silk fibroin provides an important set of material options in the fields of controlled release, biomaterials, and scaffolds for tissue engineering because of combination with impressive mechanical properties, biocompatibility, biodegradability, and cell interaction [1-5]. For example, as a surgical suture application in clinical medicine has a history for many years [6-9]. The most applicable performance, such as water resistance, strength and elongation, softness, biodegradability is closely related to the molecular conformation and crystal structure of silk fibroin.

Hydrogel preformed by chemical or physical cross-linking are a special class of polymers that imbibe a considerable amount of water while maintaining their shape. Hydrogel are considered useful scaffolds for encapsulation and delivery of cells and bioactive molecules, such as in tissue engineering and cell therapeutic applications. Hydrogel used in these types of applications have mechanical and structural properties similar to some tissues and extra cellular matrices (ECM) [10, 11], therefore, they can be implanted or used for tissue restoration or local release of therapeutic actors. To encapsulate and deliver cells. Moreover must be formed without damaging cells. Furthermore must be biocompatible and have suitable mass transport capability, sufficient mechanical integrity & strength and controllable biodegradability [12-16].

Many researches on hydrogel with respect to drug delivery and biomedical devices have been extensive over the last few decades because of their biocompatible properties and easy control of solute transport [17]. Silk fibroin hydrogel are of interest for many biomedical applications. Fini et al. [18] used low-pH-induced silk fibroin hydrogel as a bone-filling biomaterial to heal critical-size chancellor's defects in rabbit distal femurs.Silk gels show better bone healing than the control material.

In vitro, silk fibroin aqueous solutions undergo self-assembly into β-sheet structures and form hydrogel. This sol-gel transition is influenced by temperature, pH, and ionic strength [19-23]. It is generally believed that shearing stress plays an important role in the transformation of molecular

*Corresponding author's email: mzli@suda.edu.cn
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conformation of silk fibroin. There are many researchers who studied the rheological behavior of silk fibroin on the dilute aqueous solution. The shearing rate has effect on silk fibroin gel and the morphology of silk fibroin gel [24]. Hence, we aimed to investigate in detail the factors of shearing rates that influence silk fibroin sol-gel transitions and structural changes in silk fibroin

2. Materials and methods

2.1 Preparation of silk fibroin solution

B. mori silk fibroin was degummed in 0.2% on the weight of fibroin aqueous solution containing 0.2% Na_2CO_3 at 90–100°C for 60 min and repeated 3 times. The degummed silk fibroin was then dissolved in a mixture of ethanol (2M), calcium chloride (1M), and water (8M) at 72°C for 1 h. The silk fibroin solution was obtained by dialyzing for 4 days at room temperature with a cellulose tube (molecular weight cut-off 3,500) to remove the excess salts. The concentration of the silk fibroin solution was adjusted to 3% (v/v).

2.2 Preparation of silk fibroin gels

The 25mL silk fibroin solutions at concentrations of 3.0 wt% was stirred at various shearing rates (150,240 and 380r/min) for different times (15, 30, 45, 60 and 90min) respectively, then injected into culture dishes (diameter 8.5cm). Then were put aside at room temperature and the gelation time of each sample was recorded. Gelation time was determined when the sample had developed an opaque white color and did not fall from an inverted dish within 30s. After freeze-drying, silk fibroin gels were prepared.

2.3 Circular Dichroism (CD) Analysis

Measurements were taken using a J-715 spectropolarimeter equipped with a slab (NESLAB RTE-111) and purged with N_2 gas at a flow rate of 35 mL/min. The aqueous silk protein solution was stored in 0.1cm path length cells for detection. Spectra were recorded from 190 to 250 nm wavelengths with a resolution of 0.2 nm and an accumulation of six scans. The scan speed was 100nm/min.The response time was 0.25 s.

2.4 X-ray diffraction

Wide-angle X-ray diffraction (XRD) curves was recorded with a Rigaku D/Max-3C diffractometer with Cu-Kα radiation (λ= 0.15418nm). The X-ray source was operated at 40kV and 40mA. Diffraction intensity was measured in reflection mode at a scanning rate of 2°/min for 2θ=5-40°.

2.5 FTIR spectroscopy

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

2.6 Laser Raman spectroscopy

Raman spectra were recorded using a Dilor LabRam-1B spectrometer. The Spectra Physics Model 164 argon ion laser was operated at 632.8 nm with about 6mW power.

2.7 Scanning electron microscopy (SEM).

Silk fibroin hydrogel were frozen at -40°C and then lyophilized. The samples were fractured in liquid nitrogen and examined using a LEO Gemini 982 field emission gun SEM. To check for artifactual morphological changes due to freeze-drying, an alternative preparation used Karnovsky's fixative at room temperature for 4h was done. Hydrogel with and without fixative treatment showslittle morphological change upon freeze-drying.

3. Results and discussion

3.1 Precipitation of silk fibroin

During the process of shearing silk fibroin solution, there is a growing milky white flocculent material and precipitation near the sub-shaft area. After washing the portion of this material by deionized water, the sample looked like non-woven fabric.It was soft and insoluble. It was speculated that shearing led to the precipitation of silk fibroin.

3.2 CD of silk fibroin solution effected by shearing

CD is usually used to test dilute aqueous solutions of different protein conformation [25]. Studies have shown that structure of silk fibroin (α-helix, β-sheet, β-angle, random coil) in the far ultraviolet zone (178-