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Stability of Silk Fibroin Blend Films with Silk I Structure ⋆

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Abstract

The crystalline structure of silk fibroin Silk I is generally considered to be metastable structure and will transition to the crystalline structure of Silk II, however, under what conditions this crystalline structure is stable and under what conditions the transition will occur is not definite. In this paper, silk fibroin protein solution was prepared from silkworm cocoon, the glycerol/silk fibroin protein blend film containing Silk I crystalline structure was prepared with a glycerol/silk fibroin mass ratio of 20: 100, and a pure silk fibroin solution was used as a raw material to prepare a silk fibroin protein film rich in random coil structure. Different concentrations of methanol and ethanol were used to soak the above two materials to investigate the influence of monohydric alcohol on the crystalline structure of silk fibroin materials. X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), Raman scattering spectroscopy (Raman) and Thermogravimetric analysis (TGA) were used to characterize the structure of silk fibroin before and after the treatments. The results showed that after methanol and ethanol treatment, the pure silk fibroin protein film with random curled structure was transformed into Silk II crystal structure, while the glycerin/silk fibroin protein film still showed the crystal structure of Silk I without obvious transformation. Two experimental methods of high temperature wetting and high temperature soaking were set, and four temperature gradients of 60 °C, 80 °C, 100 °C and 120 °C were set to explore the influence of high temperature on the structure of silk fibroin protein materials. In order to investigate the time stability of crystal structure of Silk I, glycerin/silk fibroin blend film and pure silk fibroin protein film were placed in an oven at 60 °C for 32 days. The structure of the material was examined by XRD, FTIR and Raman test techniques before and after placement. The results showed that the glycerin/silk fibroin film with crystal structure of Silk I had no obvious structural changes and had good humidity and heat stability and time stability. The random coil structure of pure Silk film gradually changed to the more regular crystal structure of Silk I and Silk II. Glycerin/silk fibroin protein blend film has considerable stability and can be used as biomaterials.

Keywords: Silk fibroin; Film; Silk I crystalline structure; Crystal structure; Stability; Glycerol

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1 Introduction

Silk fibroin is a kind of fibrin, which is not only rich in resources, but also has excellent biocompatibility and mechanical properties. Based on these properties, silk fibroin has moved from the traditional textile industry to the biomedical field. Silk protein can be processed into different forms and has a very broad application prospect [1,2]. At present, the silk fibroin materials that have been developed and applied mainly include 3D printing [3], tissue repair [4,5], drug controlled release [6,7], disease model [8], bone tissue scaffold and skin wound dressing [9].

Silk fibroin has a polypeptide chain, which is composed of hydrophobic heavy chain (H-chain, 390 kDa), hydrophilic light chain (L-chain, 25 kDa) and P25 glycoprotein chain (P25, 30 kDa) [10]. H-chain and L-chain are connected by disulfide bond. One P25 glycoprotein and six H-L chain aggregates are assembled by hydrophobic interaction. That is, the molar ratio of H:L:P25 = 6:6:1 [11]. Silk fibroin contains crystalline region and amorphous region. H-chain runs through the crystalline region and amorphous region, while L-chain only exists in the amorphous region. The amorphous region is composed of non-repetitive sequences. Amino acids such as tryptophan, phenylalanine and tyrosine with large side chain groups exist in amorphous state in the amorphous region. While the GAGAGS repetitive sequence in the crystal region forms an antiparallel β-sheet structure by intermolecular and intramolecular forces [12].

The exploration of the crystal structure of silk fibroin can be traced back to Shimizu Masanori, who obtained two crystal states of silk fibroin through experiments, which were named silk fibroin α and silk fibroin β [13]. Later, Kratky [14] and others found that silk fibroin has two types of crystals, which are called Silk I and Silk II. Subsequently, the researchers [15] found that Silk I crystal structure model is a repeat unit dipeptide, and the molecular chain is crankshaft type, which is neither a α-helix nor a β-sheet structure belongs to orthorhombic system. The Silk II crystal structure model is a layered structure formed by β-antiparallel folding, belonging to the monoclinic crystal system [16]. Regina [17] et al found a new crystal on the gas-liquid interface, which is called Silk III, its structure is similar to triple helix. The crystal structure of Silk III is similar to that of polyglycine II, which belongs to hexagonal system.

At present, silk fibroin materials of Silk II crystal structure have been studied and applied in many fields. However, studies on the crystalline structure of Silk I is slowly developing. At present, it is found that silk fibroin mainly transforms to Silk I when the film forming temperature is lower than 40 °C and the concentration is low; Lu [18] and others successfully induced silk fibroin to form Silk I crystal structure by adding polyols with specific hydrophile-lipophile value. Compared with Silk II crystalline materials, Silk I crystalline materials have better hygroscopicity, flexibility and higher degradation rate [15,19]. Generally speaking, the crystal structure of Silk I is a kind of metastable structure with poor time stability, which is easy to be induced to Silk II crystal structure. In order to explore the change of silk fibroin Silk I crystal structure, the stability of silk fibroin blend film with Silk I crystal structure was discussed. The stability after soaking in methanol and ethanol and time stability of glycerol/silk fibroin blend film were studied, so as to expand the application of Silk I as biomaterials.

2 Materials and methods

2.1 Experimental materials

Fresh mulberry silkworm cocoons of Bombyx mori (Suzhou siruibao Biological Technology Co., Ltd., China), sodium carbonate, sodium bicarbonate, lithium bromide (Tiancheng Chemical