

Condensed Structure of Regenerated *Antheraea pernyi* Silk Fibroin Porous Materials Prepared by Freeze-drying

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Abstract: Regenerated *Antheraea pernyi* (*A. pernyi*) silk fibroin porous material was prepared by freeze-drying. By means of X-ray diffraction, Fourier transform infrared spectrometry, ¹³C NMR and Raman spectroscopy, the molecular conformation of *A. pernyi* silk fibroin porous materials prepared under different freezing temperatures and different concentrations of silk fibroin solution were investigated and analyzed. The results indicated that the molecular conformation of silk fibroin inside regenerated *A. pernyi* silk fibroin porous materials prepared by freeze-drying was due to the coexistence of α -helix and random coil structure, which was different from that of *Antheraea pernyi* *A. pernyi* silk fibroin fibers. The content of α -helix structure of freeze-dried material prepared under higher temperature had a tendency to increase. Crosslinking by PEG-DE can promote the transformation of silk fibroin from random coil to α -helix structure.

KeyWords: *Antheraea pernyi*, silk fibroin, porous material, molecular conformation.

1. Introduction

Compared with *Bombyx mori* silk fibroin, *Antheraea pernyi* (*A. pernyi*) silk fibroin contains special Arg - Gly - Asp (RGD) tripeptide sequence, which favors cells to attach. It also contains certain amount of amino acid with positive charges. Thus *A. pernyi* silk fibroin is more beneficial for cells of human being and many kinds of mammals to adhere and proliferate than that of *Bombyx mori* silk fibroin [1-3]. So it is expected to prepare *A. pernyi* silk fibroin porous materials with controllable molecular conformation, biodegradation and excellent biocompatibility and apply it into biomedical field such as drug controlled-release carrier, tissue engineering and tissue inducing scaffold. The degradation rate of scaffolds is an important parameter for biomaterials and scaffolds for tissue engineering to match new tissue ingrowth. Silk fibroin material can be considered a degradable material in vivo, and the rate of degradation may be highly variable depending on the crystallization or the molecular conformation inside the material [4-6]. In this paper *A. pernyi* silk fibroin porous materials were prepared by freeze-drying and the influence of different technical conditions on the molecular conformation of silk fibroin inside porous material was studied. It was also tried to investigate the transformation behaviour of *A. pernyi* silk fibroin molecular conformation and provide a new kind

material with less β -sheet structure and excellent biodegradation for biomedical fields such as tissue engineering.

2. Experiment

2.1 Preparation of regenerated *Antheraea pernyi* silk fibroin solution

100g *A. pernyi* silk were boiled for 30-45 mins three times in 5000 ml aqueous solution of 2.5 g/L Na₂CO₃. After being rinsed and air dried at 60 °C, the degummed *A. pernyi* silk fibroin fibers would be obtained. 10 g of degummed *A. pernyi* silk fibroin fibers were placed in 100 ml 10 M aqueous lithium thiocyanate solution, stirred to dissolve at 40 °C for 60 min. The cooled solution was respectively dialyzed in cellulose tube against water for 4 days to obtain the regenerated *A. pernyi* silk fibroin solution.

2.2 Preparation of *Antheraea pernyi* silk fibroin porous materials

A. pernyi silk fibroin solution (or add polyethylene glycol diglycidyl ether (PEG-DE) which is 20% of silk fibroin weight) were poured into metal utensils and frozen for 6hrs in -70, -50, -30, -20 and -10 °C. Then they were dried in vacuum for about 36 hrs using a vacuum freeze drier. Spongy porous *A. pernyi* silk fibroin materials were prepared.

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2.3 Morphology observation

The surface and sectional morphology of porous silk fibroin materials was observed by a Hitachi.S-570 Scanning Electron Microscope (SEM).

2.4 X-ray diffraction

X-ray diffraction was performed by a Rigaku D/Max-3C diffractometer with Cu K α radiation from a source operated at 40 kV and 40 mA. Diffraction was measured in reflection mode at a scanning rate of 2°/min. The diffraction intensity curves with 2 θ from 5° to 40° would be gained.

2.5 FT-IR

Fourier transform infrared (FT-IR) spectra were obtained with a Nicolet Avatar-IR360. The samples were prepared in KBr pellets.

2.6 Raman spectroscopy

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

2.7 ¹³C NMR spectra

¹³C NMR spectra was obtained by high resolution NMR spectrometer (FT-NMR(600 MHz) AVANCE 600, Bruker, Germany) to determine the molecular conformation of silk fibroin in materials.

3 Results and Discussion

3.1 Morphology

Figure 1 shows the surface and section SEM photographs of regenerated *A. pernyi* silk fibroin porous materials. It indicates that the top-surface of *A. pernyi* silk fibroin porous material is a compact structure and the undersurface is a porous structure with small porosity. Its interior is porous structure and the pore shape is anomalous. The pores are run-through from top-surface to undersurface. The porosity and the average pore diameter of *A. pernyi* silk fibroin porous materials are bigger and the pore density is smaller when the material is prepared in higher freezing temperature, which is consistent with our earlier study results [7].

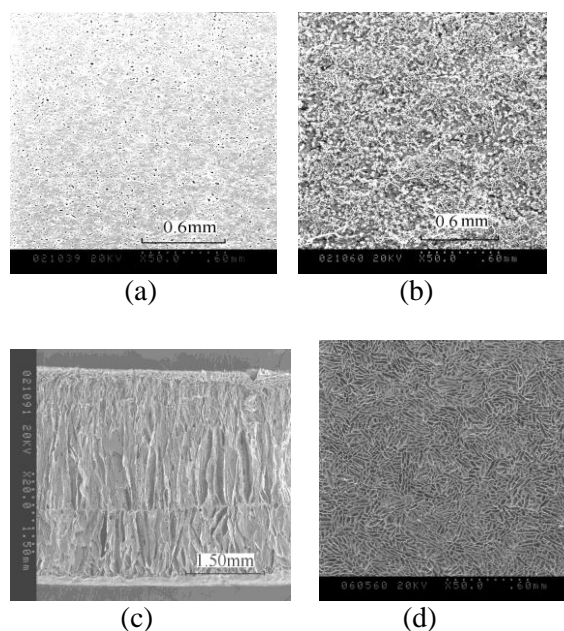


Figure 1 SEM photographs of regenerated *A. pernyi* silk fibroin porous materials. (a) top-surface, (b) undersurface, (c) longitudinal section and (d) Cross section. Freeze-drying temperature: -70 °C.

3.2 X-ray diffraction

According to the studies on *A. pernyi* silk fibroin molecular conformation [8-11], with CuK α radiation, the X-ray diffraction patterns have been determined as follows: 11.8° and 22.0° for α -helix structure, and 16.5°, 20.2°, 24.9°, 30.90°, 34.59°, 40.97° and 44.12° for β -sheet structure. There appears intense peaks around 17° and 20.5° and a medium strength peak around 23.5° in XRD curve of *A. pernyi* silk fibroin fiber (Figure 2, curve (a)) which indicate much β -sheet structure and high crystallinity of *A. pernyi* silk fibroin fiber. Compared with that of *A. pernyi* silk fibroin fibers, the XRD curves of porous silk fibroin materials change obviously. Non-crosslinked *A. pernyi* silk fibroin porous materials (curves (b) to (f)) show major peaks around 22° and 12°, which are attributed to the coexistence of random coil structure and α -helix structure. Crosslinked *A. pernyi* silk fibroin porous materials by PEG-DE (curves (g) and (k)) also show strong peaks around 22° and 12° and additionally appearing new diffraction peak around 11°, which indicates that silk fibroin molecular conformation of crosslinked materials by PEG-DE is still mainly random coil structure and α -helix structure. Whether the new diffraction peak around 11° was caused by the new crystallization structure or the minor crystal of α -helix structure, will be further analyzed as