Study on the Properties of Nano-TiO_2 Particles Modified Silk Fibroin Porous Films

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ABSTRACT: Using the freeze-drying method, Nano-TiO_2/silk fibroin porous films were synthesized with different ratios of TiO_2 to silk fibroin solution. Through scanning electron microscopy (SEM), X-ray diffraction (XRD), thermogravimetric analysis (TGA), tensile strain, and water-solubility tests, the structures and properties of these porous films were characterized. The SEM results indicated that the pores of the nano-TiO_2/silk fibroin porous films were uniformly distributed by the freeze-drying method. The XRD analysis indicated that the formation of nano-TiO_2 particles might induce a conformational transition of silk fibroin from the typical Silk I to the typical Silk II structure partly with an increase in the crystallinity of the porous films. Compared with the pure silk fibroin porous films, the mechanical properties of nano-TiO_2/silk fibroin porous films were improved, and its heat transition temperature was also enhanced; however, the water-solubility of this material was diminished. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 116: 468–472, 2010

Key words: freeze-drying method; nano-TiO2; silk fibroin; porous film

INTRODUCTION

Silk is a well described natural fiber produced by the silkworm, *B.mori*, which has been used traditionally in the form of thread in textiles for thousands of years.[1] Due to its excellent biological compatibility and mechanical properties, silk fibroins, have been explored for many biomedical applications including osteoblast, hepatocyte, and fibroblast cell support matrixes.[2,3] As an attractive tone tissue engineering biomaterials, silk is also widely studied because of its biocompatibility,[4,5] mechanical properties, and slow biodegradation.[6]

Usually, silk will be degummed before as biomaterial to remove the sericin, then the degummed silk is regenerated to make the desirable biomaterials, such as porous scaffolds. However, it has long been a problem that the regenerated SF films in the dry state show poor mechanical properties and brittleness compared with their original form.[7] These problems may be solved by blending with other natural or synthetic polymers. Blends with poly(vinyl alcohol),[8] sodium alginate,[9] cellulose,[10] chitosan,[11] and so on, have been extensively studied to improve the mechanical properties or membrane properties of silk films, and some blend films showed the improved properties.

In this article, we tried to use low content nano-TiO_2 particles to control the crystal structure of the silk fibroin, and then prepared nano-TiO_2/silk fibroin porous films using freeze-drying method. It was found that the pores of the films distributed uniformly, and nano-TiO_2/silk fibroin porous films showed lower solubility in the water, higher swelling ratio, and better mechanical properties.

EXPERIMENTS

Materials and reagents

Calcium chloride (AR, from Shanghai the Second Chemical Reagent Factory, China); Titanium(IV) Butoxide (AR, from Shanghai Chemical reagent Co., China); Methyl alcohol and Ethanol (AR, from Hangzhou Changzhen Chemical Reagent Factory, China); Cocoons of *B.mori* silkworm silk (kindly supplied by Silk Museum, China) were employed in this investigation.

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Preparation of Regenerated *B. mori* silk fibroin solution

To remove the sericin, the cocoon was degummed using fatty acid neutral soap [ω(weight percentage) = 0.02] and sodium carbonate (ω = 0.001) in hot water (98°C) two times, each for about 1 h. Degummed silk fibroin was washed thoroughly with deionized water for 30 min to remove any remaining sericin and surfactants and then gently dried in air. To dissolve the fibroin, the degummed silk fibroin was dissolved by 9.5*M* LiBr solution.

Three gram degummed silk fibroin and 40 mL 9.5*M* LiBr solution was mixed together in a round-bottom flask, and mechanical stirring was applied to provide adequate physical agitation to the system. A heater was used to keep the temperature of the system at 60°C. It took about 4 h to dissolve the silk fibroin in the above system. The fibroin-salt solution was dialyzed against deionized water for 3 days until the conductivity of the aqueous solution was less than 0.8 μs/cm. The dialyzed fibroin solution was kept in a desiccator for subsequent use, the concentration of silk fibroin solution was about 8 %[w/w(weight:weight)].

Preparation of nano TiO_2/silk fibroin porous films

1.0 mL Ti $(OC_4H_9)_4$ was added dropwise into 10 mL ethanol in a fume hood under vigorous stirring. The proper amount Ti $(OC_4H_9)_4$/ethanol mixed solution was added dropwise into the regenerated silk fibroin solution according to the different ratios of TiO_2: silk (0, 0.002, 0.004, 0.006, 0.008), and then the mixed solution was concussed by the ultrasonification for 30 min. Finally, the mixtures were put into the glass disks and then frozen in the low-temperature freezer at −80°C for 2 h, then freeze-drying in the Freeze Dryer (ALPHAI-2 LD) for 24 h, then the drying porous nano-TiO_2/silk fibroin composite matrices were obtained.

Characterization

Tensile strength and elongation at break were measured with an Instron 5540 at 20°C, 65% RH, with head speed of 0.2 mm/min. The samples were 10 mm wide, 40 mm long between clamps.

The X-ray diffraction (XRD) data were obtained in an ARL-X'TRA diffractometer using CuKα radiation, in 2Θ range of 2 Θ = 5–50° with a step size of 2°/min and a counting time of 10 s. The room temperature measurements were performed with samples spread on a conventional glass sample holder.

The thermal stabilities of porous films were characterized using a Pyris Diamond TGA thermogravimetric analyzer. All the sample weights were about 1 mg, and all measurements were carried out under a nitrogen atmosphere. The specimens were heated up from 50 to 650°C at the same rate of 10°C/min. The thermographs of the whole process were used for analysis.

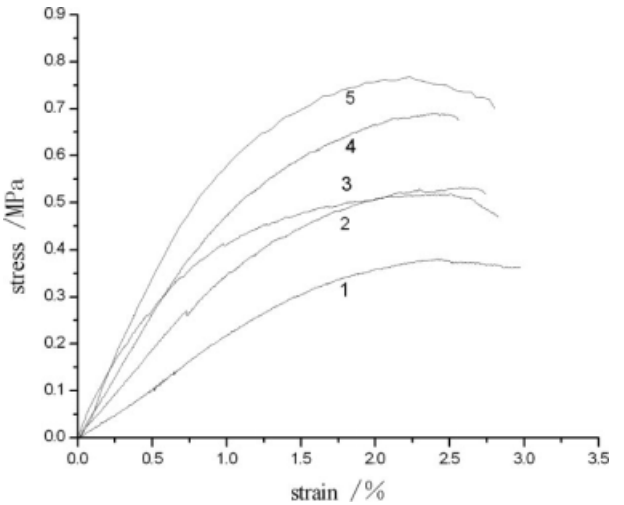

Figure 1 The stress–strain curve of Nano TiO_2/silk fibroin porous films 1-5 showing the quality ratios of the Nano TiO_2/silk fibroin is 0/100, 0.2/100, 0.4/100, 0.6/100, 0.8/100, respectively.

Scanning electron microscopy (SEM) was used to observe the microstructural cross-sectional morphology of the samples. The instrument was Japan JSM-5610LV JEOL SEM with an acceleration voltage of 20 kV. Before the scanning process, all samples were coated with gold in a vacuum sputter coater. The SEM provided detailed imaging information about the morphology of individual samples.

Samples (W_s) were dried at 100°C for 24 h in the oven and the dry weight of the porous films was marked as W_d. Samples were immersed into the distilled water at 37°C for 3 h then dried at 100°C for 24 h in the oven and the weight was marked as W_{ds}. Samples were immersed into the distilled water at 30°C for 30 min, then excess water was removed, the wet weight of the films was marked as W_w.

$$\text{Moisture ratio}(\%) = (W_s - W_d)/W_s * 100$$

$$\text{Water solubility}(\%) = [W_S * (1 - \text{moisture ratio}) - W_{ds}]/[W_s * (1 - \text{moisture ratio})] * 100$$

$$\text{Swelling ratio} = (W_s - W_W)/W_W * 100$$

RESULTS AND DISCUSSION

Mechanical properties

Mechanical properties including strength and Young's modulus of silk fibroin porous films are shown in Figure 1 and Table I. From Figure 1 and

TABLE I
Mechanical Properties of Nano TiO_2/Silk Fibroin Porous Films

Sample	Length (mm)	Thickness (mm)	Width (mm)	Young's modulus (MPa)	Strength (MPa)
1	40.0 ± 0.1	0.800	10.0 ± 0.1	20.614	0.380
2	40.0 ± 0.1	0.940	10.0 ± 0.1	39.341	0.529
3	40.0 ± 0.1	1.012	10.0 ± 0.1	75.997	0.517
4	40.0 ± 0.1	0.920	10.0 ± 0.1	48.217	0.689
5	40.0 ± 0.1	0.850	10.0 ± 0.1	69.156	0.767

Table I, the strength and Young's modulus of pure silk fibroin porous films were very low (only 0.38MPa and 20.614MPa, respectively), and the strength and Young's modulus of porous films increased with the increase of added nano-TiO_2 particles. When the content of nano-TiO_2 particles was increased to 0.8%, the strength and Young's modulus reached to 0.767 MPa and 69.156 MPa, respectively. Compared with the pure silk fibroin porous films, the mechanical properties of nano-TiO_2/ silk fibroin composite porous films were enhanced remarkably, indicating that the formation of nano-TiO_2 particles in the silk fibroin films could improve the mechanical properties, and further impart the flexibility.

X-ray diffraction

X-ray diffraction was used to characterize the structure of the silk fibroin. Generally, there have been two types of crystalline structures proposed for silk, Silk I and Silk II. Figure 2 showed that the main diffraction peaks of the pure silk fibroin porous films was at 20.7° and there were two weak peaks at 12.2° and 17.2°, the peak at 20.7° was attributed to silk II and the peaks at 12.2° and 17.2° were attributed to silk I, which indicated that Silk I and Silk II crystalline structure could coexist in pure porous silk film. With the adding of nano-TiO_2 particles, the intensity of the peaks at 20.7° became big gradually, and the new weak peaks at 18.9° and 24.3° were showed gradually. However, two weak peaks at 12.2 and 17.2° were disappeared. It could be explained that the crystalline structure of the fibroin molecules could be induced to transform from Silk I to Silk II by the formation of nano-TiO_2 particles inside the silk fibroin, and the ratio of the silk II was increased gradually in the composite porous silk films.

Generally, inorganic particles, such as SiO_2 or TiO_2, could hydrate with water molecules to produce an amount of hydroxyls on their surface. It could be speculated chemically and molecularly that the carbonyls or hydroxyls of alkaline amino acids (Arg, Lys, and His) and acidic amino acids (Asp and Glu) on fibroin chain could interact with hydroxyls produced by TiO_2 particles, to form ester or the intermolecular hydrogen bonds.[12] Thus, the interaction between hydroxyl groups of TiO_2 surface and SF molecule could increase the crystallinity, and induce the partial transformation to Silk II conformation.

Thermogravimrtric analysis

The thermal behavior of the pure silk fibroin and nano-TiO_2/silk fibroin porous films was investigated by thermogravimetric analysis. The degradation temperature, known as a criterion of thermal degradation, was calculated based on differential TGA curves. Additionally, the location of peaks observed in the detrivative thermogravimetric (DTG) curve also provided information on the component and the effect of the composite components on the temperature scale.[13] Figure 3 shows the TGA and DTG curves of the pure silk fibroin and nano-TiO_2/silk fibroin porous film samples.

As shown in Figure 3, there was only one distinct heat conversion temperature about 300°C of the pure silk fibroin porous films and the nano-TiO_2/ silk fibroin porous films. The initial decomposition temperature of the pure silk fibroin porous films

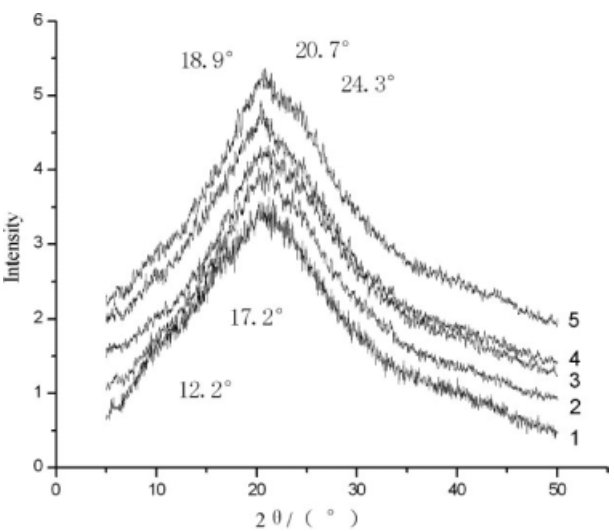

Figure 2 X-ray diffraction of Nano TiO_2/silk fibroin porous films 1–5 showing the quality ratios of the Nano TiO_2/silk fibroin is /100, 0.2/100, 0.4/100, 0.6/100, 0.8/100, respectively.

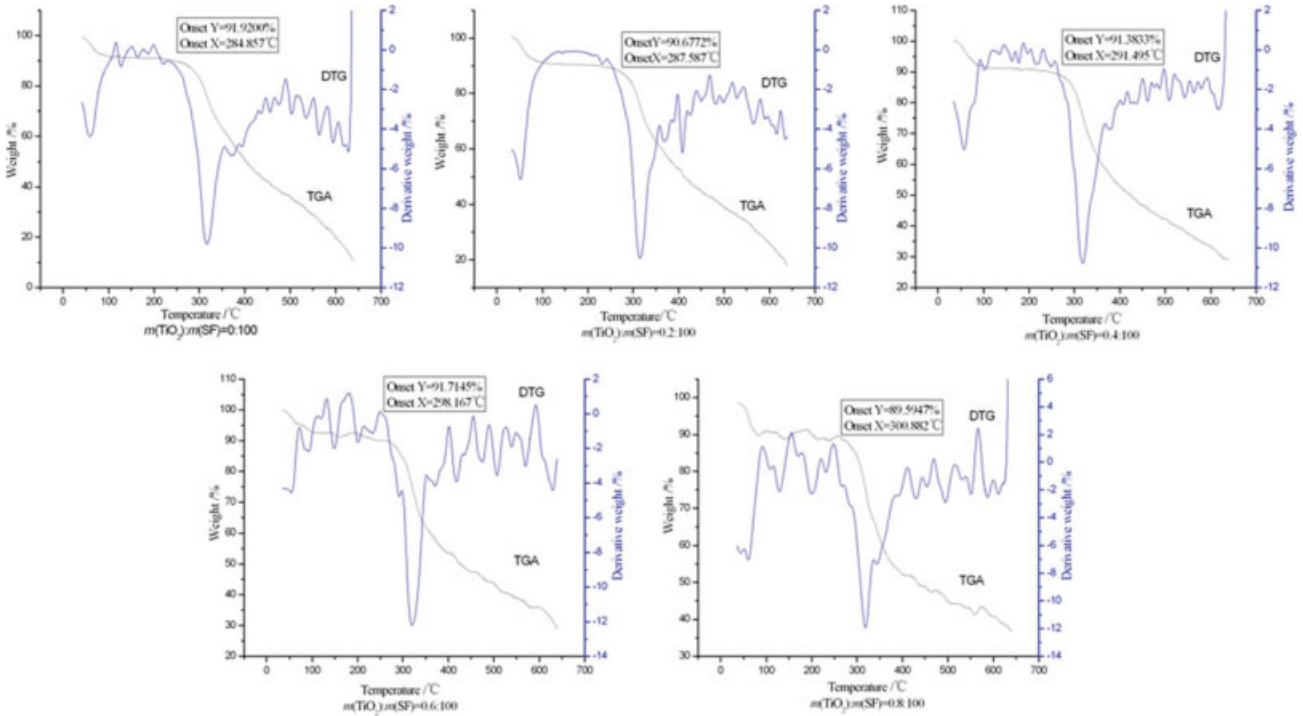

Figure 3 TGA and DTG of Nano TiO_2/silk fibroin porous films. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

was (ca.) 284°C, however, the initial decomposition temperature of the nano-TiO_2/silk fibroin porous films became high gradually with the increase of the content of nano-TiO_2 particles from (ca.) 287°C to 300°C. This indicated that the nano-TiO_2/silk fibroin porous films have better thermal stability than the pure silk fibroin porous films. It was likely that the increase of the initial decomposition temperature was associated with the crystalline structure transition from Silk I to Silk II, which was consistent with the X-ray diffraction' results.

Moisture ratio, water-solubility and swelling ratio

Figure 4 showed the moisture, water-solubility, and swelling properties of nano-TiO_2/silk fibroin porous films. The moisture ratio of the nano-TiO_2/silk fibroin porous films was very low, almost under 10% after freeze-drying handle, and the water solubility of the pure silk fibroin porous films was very high (about 57%). However, with the adding of nano-TiO_2 particles, the water solubility of porous films decreased markedly, when the content of nano-TiO_2 was up to 0.8%, the water-solubility dropped only to 9.47%. It could be explained that the nano-TiO_2 particles induced the structures of silk fibroin to transition and obtained more ß-crystalline structure in the nano-TiO_2/silk fibroin porous films, and resulted in the reduction of the water solubility. The swelling ratio of porous pure silk fibroin was 2.02%, with the increase of the nano-TiO_2 particles the swelling ratio was up to 7.95%.

Morphology of porous films

The porous structure of the pure silk fibroin porous film and nano-TiO_2/silk fibroin porous film were displayed in Figure 5. As shown in Figure 5, the

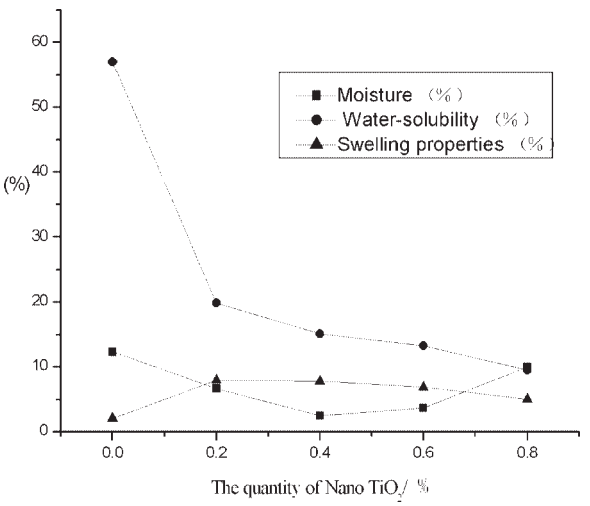

Figure 4 The moisture, water-solubility, and swelling properties of Nano TiO_2/silk fibroin porous films.

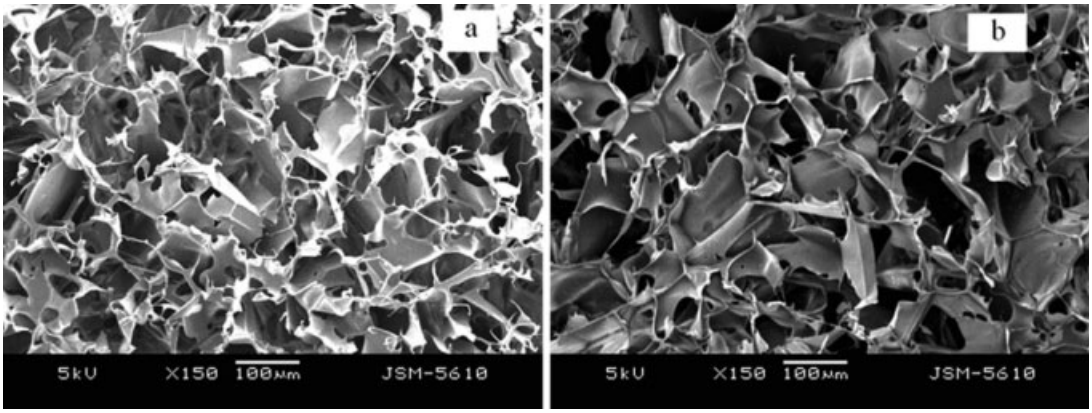

Figure 5 SEM images of Nano TiO_2/silk fibroin porous films [(a) m(Nano TiO_2):m(SF) = 0/100; (b) m(Nano TiO_2):m(SF) = 0.2/100].

shape of these pores were random polygonal, pores were linked to each other, and distributed uniformly. Through the comparison of the images between pure silk fibroin porous film [Fig.5(a)] and nano-TiO_2/silk fibroin porous film [Fig.5(b)], it could be determined that the pore diameter of pure silk fibroin porous film is larger than that of the nano-TiO_2/silk fibroin porous film.

CONCLUSIONS

Nano-TiO_2/silk fibroin porous films were successfully prepared using freeze-drying method. These films were presented with the porous structure, and pores distributed uniformly. Compared with pure silk fibroin porous films, the mechanical and thermal properties of nano-TiO_2/silk fibroin porous films were improved, and the solubility in water was decreased because of the formation of structural transition from Silk I to Silk II during the preparation of nano-TiO_2/silk fibroin porous films.

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