

Note

PROPERTIES OF SILK FIBROIN/POLY(ETHYLENE GLYCOL)400 BLEND FILMS*

Song Wang**, Qing-hua Wang, Xin-lin Yang, Li-yong Wang and He-sun Zhu
Research Center of Materials, Beijing Institute of Technology, Beijing 100081, China

Abstract The blend film of silk fibroin (SF) and poly(ethylene glycol)400 (PEG400) with a blend ratio of 2/1 (wt/wt) was prepared simply by dropping a little PEG400 into the SF solution and then casting the mixed aqueous solution at 50°C. The resulting film exhibited much better mechanical properties in the dry and wet state than SF itself, owing to the conformational change of SF in the blends from the random coil to the β -sheet structure and intermolecular hydrogen bond formation between SF and PEG400. Thermogravimetric analysis showed that the initial thermal decomposition temperature of the blend film was 170°C, which was 80°C lower than that of SF (250°C) and 20°C higher than that of PEG400 (150°C), and indicated a strong interaction between two components of the blend. No crystalline peaks were observed in the X-ray diffraction curve of the blend film. Cell culture test showed that SF/PEG400 was a suitable substrate for the growth of human umbilical vein endothelial cells (HUVEC).

Keywords Silk fibroin, Poly(ethylene glycol)400, Blend film, Cell culture

INTRODUCTION

Silk fibroin (SF) has proven to be an invaluable biomaterial due to its inherent biocompatibility, good oxygen and water vapor permeability and biodegradability. In recent years, the application of SF films has progressed to yield, *e.g.* the immobilization matrices for of enzymes, drug delivery systems, burn wound dressings^[1–3]. However, pure SF films are very brittle. To improve the mechanical properties, blend SF films have been prepared by mixing SF with other synthetic or natural polymers, for example, poly(vinyl alcohol) (PVA), sodium alginate, chitosan, and cellulose^[4–7].

Poly(ethylene glycol) (PEG) is widely used as a biomaterial. The chemical modification of SF with 2,4-bis[*o*-methoxypoly(ethyleneglycol)]-6-chloro-s-triazine (actPEG2, $M_w = 104$)^[8] and PEG diacrylate macromer ($M_{w, PEG} = 8000$)^[9] was reported. It was found that the tensile strength and elongation of SF/PEG were higher than those of SF itself. But the cell (L929) attachment and growth decreased significantly after PEG was introduced into SF. In addition, the modifiers and initiators used in chemical reactions are usually toxic, and it is not easy to remove them thoroughly from the products. In this study, a very low molecular weight PEG400 was chosen as a modifier of SF. Since it is a liquid, we can obtain the blend films simply by dropping PEG400 into the aqueous solution of SF and then casting the mixed solution. The resulting film of SF/PEG400 (2/1) shows a good mechanical properties in the dry and wet state, and it is also a suitable substrate for the growth of human umbilical vein endothelial cells (HUVEC).

* This work was supported by the National Basic Science Research and Development Grants (973) of China (No. G1999064705).

** Corresponding author: Song Wang (王松), E-mail: songwl@95777.com

Received April 6, 2002; Revised May 16, 2002; Accepted May 20, 2002

EXPERIMENTAL

Materials

Degummed silk was dissolved in a mixed solvent of CaCl_2 , H_2O , and ethanol in a molar ratio of 1:8:2. The solution was then dialyzed in a cellulose tube against running water. The fibroin concentration of regenerated silk was normally about 15 mg/mL. Analytical grade PEG400 from Beijing Yili Chemicals Ltd. was used without further purification.

Preparation of Films

The aqueous SF solution of 1% (g/mL) and PEG400 were carefully mixed in the ratio of 1000:5 (V/V). The films of SF and SF/PEG were obtained by casting the solutions onto tissue culture polystyrene (TCPS) dishes and drying at 50°C.

Measurements

The tensile strength and elongation at break were measured by using a TOM 500J model tensile testing machine at ambient relative humidity at room temperature. In the case of measurements in the wet state the samples were first equilibrated with distilled water for a few minutes. The experimental conditions were 5 kg initial load cell, 20 mm/min tensile speed, $60 \times 8 \times 0.03$ mm sample dimensions.

To measure the solubility in water, seven preweighed dry samples (190–220 mg) were immersed in distilled water (10 mL) at various time intervals (3, 6, 9, 12, 15, 18, and 21 days), respectively. The water was changed once every three days. Finally the removed samples were dried and weighed again. Dissolved fibroin was determined by spectrophotometry. The weight loss and the fibroin loss of the film were calculated from the following equations:

$$\text{Weight loss} = (W_0 - W_1)/W_0$$

$$\text{Fibroin loss} = (\Sigma 10C_i)/W_0$$

where W_0 and W_1 are the weights of the films before and after the immersion, 10 is the volume of the water in which the samples were immersed, and C_i is the concentration of fibroin in the water after the immersion for 3 days.

FTIR spectra were obtained using a Perkin-Elmer 2000 spectrometer in the spectral region of 2000–400 cm^{-1} . X-ray diffraction patterns were obtained with a D/max-2400 Diffractometer, and $\text{CuK}\alpha$ radiation with a wavelength of 0.154 nm. Thermogravimetric analysis (TGA) was run under a flow of nitrogen gas at a scanning speed of 10 K/min using a DuPont 2000 thermogravimeter.

Cell Culture Test Procedure

The films of SF and SF/PEG for the cell test were made by pouring each aqueous solution (0.5 mL) onto four-well tissue culture plates and drying at 50°C. The pure SF films were immersed in alcohol for 30 min to make them water-insoluble. All sample films were sterilized by UV irradiation for 3 h.

Human umbilical vein endothelial cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium), supplemented with 20% fetal bovine serum, 100 U/mL penicillin and 100 $\mu\text{g/mL}$ streptomycin. Aliquot (0.5 mL) suspensions of HUVECs at 1×10^5 cells/mL were plated, and maintained at 37°C in water saturated atmosphere (5% CO_2 – 95% air) for 72 h. Non-attached cells were removed by discarding the culture medium and then the films were carefully washed twice with phosphate buffered saline solution. The number of viable cells was measured by MTT (3(4,5-dimethylthiazol-yl)2,5-diphenyl tetrazolium bromide)-based colorimetric assay^[10] and the absorbance at 550 nm ($\text{OD}_{500\text{nm}}$) that was proportional to the number of cells on the sample films was recorded by a MB-III enzyme immune analyzer. The same procedure was repeated with the cells without films as a control. All data were analyzed using the means of duplicated wells of experiments. The data were subjected to analysis by Student's *t* test. Differences were considered significant when the *p* value was < 0.05.

RESULTS AND DISCUSSION

Mechanical Properties

There were significant differences in the mechanical behavior between SF and SF/PEG400 films. Pure silk films were extremely brittle in the dry state and soluble in water, so they were unsuitable for measurements. However, films became soft and water-insoluble after PEG400 was introduced into SF. In particular, the breaking tensile strength of SF/PEG with a blend ratio of 2/1 (wt/wt) in the dry and wet state was 16.5 and 2.9 MPa, the elongation at break was 10% and 50%, respectively. These strong and soft blend films were used in subsequent measurements.

It was found that the effect of the blend ratio on the mechanical behavior of the blend was remarkable; it will be discussed in a following paper.

Solubility

Table 1 shows the weight loss and fibroin loss of polymer blends composed of SF and PEG400 after immersing them in distilled water at 37°C for 3, 6, 9, 12, 15, 18 and 21 days, respectively. It was found that the blend films contain 33% and 23% PEG400 before and after the immersion and no more PEG diffused into water after 3 days. The total fibroin loss of the blend was 1.8% after 21 days' immersion, where the 3-day fibroin loss decreased as the immersion time increased. The first 3-day loss was the most significant at 0.55%, but it was still less than one tenth of that of pure silk film under the same immersion conditions (7%). This means that SF became nearly water-insoluble after PEG400 was introduced.

Table 1. Weight loss and fibroin loss of SF/PEG400 blend films after immersion in water

Immersion/d	3	6	9	12	15	18	21
Weight loss (%)	10.34	10.39	10.51	10.45	11.72	–	10.70
Total fibroin loss (%)	0.55	0.90	1.19	1.45	1.59	1.73	1.80
Last 3-day fibroin loss (%)	0.55	0.35	0.29	0.26	0.14	0.14	0.065

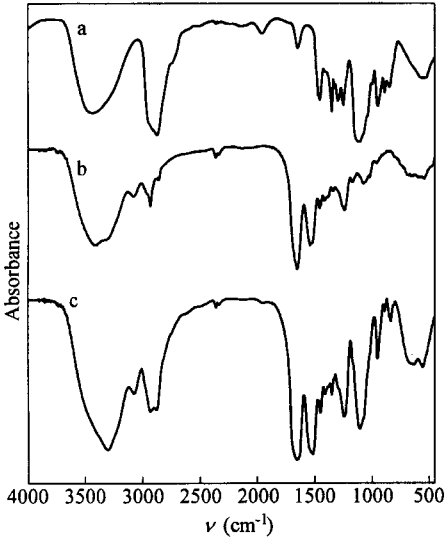


Fig. 1 FTIR spectra of PEG400 (a), SF(b), SF/PEG400 blend (c).

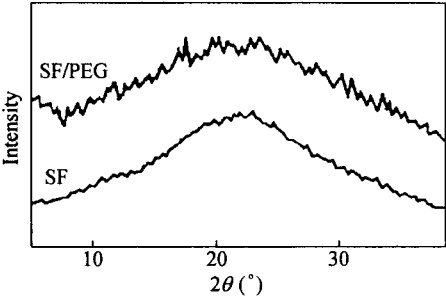


Fig. 2 X-ray diffraction curves of SF and SF/PEG400 blend

FTIR and WAXD Analysis

The FTIR spectra of PEG400 (a), SF (b) and SF/PEG400 (c) are shown in Fig. 1. SF exhibited absorption bands at 1652 (amide I), 1541 (amide II) and 1237 cm⁻¹ (amide III), attributed to the random coil conformation of SF.

PEG400 exhibited absorption bands at 3437 and 1108 cm^{-1} , attributed to the O–H and C–O–C stretching, respectively. On the other hand, the spectrum of the blend film revealed that the amide II band of SF at 1541 cm^{-1} shifted to 1518 cm^{-1} with increasing intensity, while the amide III band of SF at 1237 cm^{-1} became sharp and shifted to 1246 cm^{-1} . In the case of absorption bands of PEG400 in the blend, the O–H and C–O–C stretching bands shifted from 3437 and 1108 cm^{-1} to 3300 and 1105 cm^{-1} , respectively. These results suggested that the conformation change of SF occurred from a random coil to a β -sheet structure due to the formation of intermolecular hydrogen bonds between SF and PEG in the blend films.

Figure 2 shows the X-ray diffraction curves of SF and SF/PEG400. Both samples showed amorphous diffraction patterns.

Thermal Properties

Figure 3 shows the thermogravimetric curves of SF, SF/PEG400 and PEG400. The initial weight loss at approximately 100°C was due to the evaporation of water from SF. Then, the temperature increased and a sharp drop of the TGA curves appeared at 250, 170 and 150°C for SF, SF/PEG400 and PEG400, which indicated the beginning of the thermal decomposition accompanied by a greater weight loss. The temperatures at which SF, SF/PEG400 and PEG400 reached a 20% weight loss were 307, 275 and 275°C, respectively. These values indicated that the thermal stability of the blend film was intermediate between two pure components.

Figure 4 shows the differential thermogravimetric (DTG) curves of SF, SF/PEG400 and PEG400. The maximum decomposition temperatures of SF, SF/PEG400 and PEG400 were 305, 307 and 345°C, respectively. The blend film composed of SF and PEG400 showed one maximum, indicating that the molecular interactions between the two components were strong in the blend.

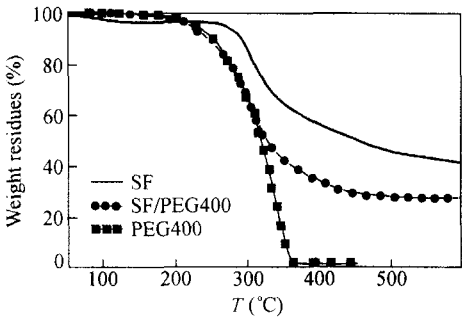


Fig. 3 Thermogravimetric curves of SF, SF/PEG400 blend and PEG400

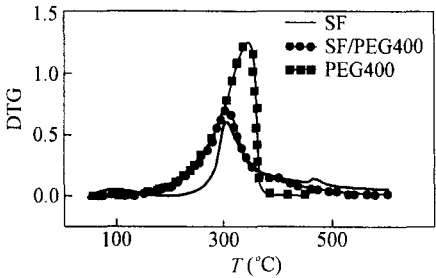


Fig. 4 DTG curves of SF, SF/PEG400 blend and PEG400

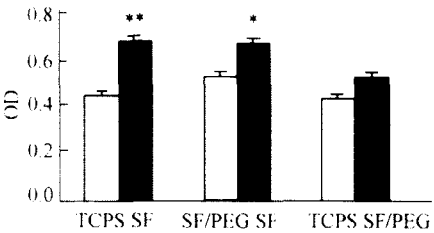


Fig. 5 Proliferation of HUVECs on SF, SF/PEG400 blend and TCPS. Values of OD are the average of eight independent measurements. Statistically significant differences between SF and SF/PEG400, TCPS: * $p < 0.05$ and ** $p < 0.01$

Interaction Between Cultured Cells and SF/PEG Blend Film

Figure 5 shows the proliferation of HUVECs on SF, SF/PEG and TCPS, where the values of $\text{OD}_{550\text{nm}}$ were proportional to the number of the viable cells growth on the films after 72 h of incubation. SF gave the highest

proliferation of cells, whereas SF/PEG gave the same proliferation of cells as the TCPS. The result suggested that SF/PEG400 is just as good a substrate for cell incubation as TCPS.

Minoura *et al.*^[8] have reported that SF-PEG ($M_w = 10000$) conjugates exhibited very low cell (L-929) attachment and growth, where the content of the PEG component in SF-PEG was about 67% by weight. The result has been explained in terms of the high excluded volume effect of PEG. In this paper, the content of the PEG400 in the blend was about 33% by weight and its molecular weight was one twentieth of that of the PEG10000 used in Ref. [8]. Therefore, the excluded volume effect of PEG on the cell incubation was small.

REFERENCES

- 1 Demura, M., Asakura, T. and Kuroo, T., *Biosensors*, 1989, 4: 361
- 2 Hanawa, T., Watanabe, A., Tsuchiya, T., Ikoma, R., Hidaka, M. and Sugihara, M., *Chem. Pharm. Bull.*, 1995, 43: 872
- 3 Minoura, N., Tsukada, M. and Nagura, M., *Biomaterials*, 1990, 11: 430
- 4 Yamaura, K., Kuranuki, N., Suzuki, M., Tanigami, T. and Matsuzawa, S., *J. Appl. Polym. Sci.*, 1990, 41: 2409
- 5 Liang, C.X. and Hirabayashi, K., *J. Appl. Polym. Sci.*, 1992, 45: 1937
- 6 Park, S.J., Lee, K.Y., Ha, W.S. and Park, S.Y., *J. Appl. Polym. Sci.*, 1999, 74: 2571
- 7 Freddi, G., Romarino, M., Massafra, M. R. and Tsukada, M., *J. Appl. Polym. Sci.*, 1995, 56: 1537
- 8 Yohko, G., Masuhiro, T., Norihiko, M. and Imai, Y., *Biomaterials*, 1997, 18: 267
- 9 Kweon, H.Y., Park, S.H., Yeo, J.H. Lee, Y.W. and Cho, C.S., *J. Appl. Polym. Sci.*, 2001, 80: 1848
- 10 Mosmann, T., *J. Immunol. Methods*, 1983, 65: 55