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SURFACE MODIFICATION OF BLEND FILMS COMPOSED OF SILK FIBROIN AND POLY(ETHYLENE GLYCOL) MACROMER AND THEIR *IN VITRO* ANTITHROMBOGENICITY*

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Abstract In order to improve the blood compatibility of silk fibroin (SF), poly(ethylene glycol) macromer (PEGM) in different amounts was added to the SF film to incorporate C=C group into the surface of blend films which were then modified by SO₂ gas plasma treatment. ATR-FITR and XPS were used to analyze the chemical change which had occurred on the film's surface. When the content of sulfur on the surface of blend films surpasses 1.59%, the antithrombogenicity of plasma treated films increases remarkably due to surface sulfonation. This result implies that SF with blend of PEGM after SO₂ plasma treatment have potential use for making blood-contacting biomaterials.

Keywords Silk fibroin, Poly(ethylene glycol) macromer, Plasma, Antithrombogenicity

INTRODUCTION

Silk fibroin (SF), as a natural biopolymer with outstanding physicomachanical properties, has been applied in biological and biomedical fields such as enzyme immobilization, oxygen permeable membranes, drug permeable films and so on^[1-3]. However, there also exist some disadvantages such as poor mechanical properties of SF films in the application of biomaterials. Poly(ethylene glycol) (PEG), as a widely used biomaterial, has been chosen to change SF properties by many researchers. Wang *et al.* blended SF and PEG to prepare films to enhance mechanical properties in the dry and wet state^[4]. Kawano *et al.* revealed that the PEG grafted on the surface of silk fibroin could decrease platelet adhesion, fibrinogen adsorption and complement consumption of silk fibroin^[5]. Many biomaterials must possess desirable blood compatibility, especially when they are considered for use as blood-contacting materials. The low-temperature plasma glow technique is an effective method for surface modification. A previous report has shown that sulfur-containing groups can be incorporated onto the substrate of some polymer materials by SO₂ plasma through surface sulfonation and thus reduce platelet deposition and thereby improve antithrombogenicity^[6, 7]. In the present work, poly(ethylene glycol) macromer (PEGM), having an acrylated-terminated group, was chosen as the additive of SF. SO₂ gas plasma was used to produce sulfonic groups covalently attached to the surface of SF blend film. Then the sulfonated film was tested for its antithrombogenicity by *in vitro* coagulation time tests, including the test of activated partial thromboplastin time (APTT). The results show that antithrombogenicity of SF/PEGM blend films could be significantly improved by surface sulfonation.

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EXPERIMENTAL

Materials

Raw silk was degummed twice with 0.5% weight-of-fiber (w.o.f) sodium carbonate solution at 100°C for 30 min and then washed with distilled water. Degummed silk was dissolved in $\text{CaCl}_2\cdot\text{H}_2\text{O}:\text{ethanol} = 1:8:2$ mixed solvent. The solution was then dialyzed in a cellulose tube against running water. The fibroin concentration of regenerated silk was normally about 20 mg/mL. PEG400 was purchased from Beijing Yili Chemicals Ltd. Acryloyl chloride was obtained from Acros Organics. Kits for *in vitro* coagulation time (APTT) test were obtained from Huashan Hospital of Shanghai Medical University. Human blood plasma was obtained from the China Red Cross Association. All other chemicals were extra pure reagent grade and the minimal water was removed with CaH_2 .

Synthesis of PEG Macromer

14 mL (36 mmol/L) PEG400 and 15 mL (107 mmol/L) triethylamine were dissolved in 70 mL dichloromethane in a 250 mL round-bottomed flask. 9 mL (110 mmol/L) acryloyl chloride dissolved in 100 mL of dichloromethane was added to the flask through a dropping funnel. The reaction mixture was stirred for 24 h at 0°C, and then kept for 48 h at room temperature (*RT*). The reaction mixture was filtered to remove triethylamine hydrochloride and then the filtrate was poured into an excess amount of petroleum ether to obtain the macromer. Finally, the macromer was dried at *RT* under reduced pressure for 24 h and then stored at a lower temperature.

Preparation of Blend Films

The 2% SF solution and PEGM were mixed in the ratio of 1:1, 2:1, 3:1, 4:1, 6:1 (*W/W*). The mixture was cast onto a polystyrene plate and incubated at 50°C for 24 h.

Surface Sulfonation of SF Blend Films by Using SO_2 Plasma

The plasma reactor system was designed by ourselves. The chemical grafting of sulfonyl group onto SF blend films was performed as described by Gu *et al.*^[8]. The processing parameters of SO_2 plasma treatment were set as follows: base gas pressure, less than 3 Pa; gas flow rate, 20 mL/min; working pressure, 20 Pa; net plasma power, 30 W; time, 10 min; temperature, *RT*.

Measurements

A FTIR spectrometer (Bruker) was used to confirm the structure of PEG macromer. ATR spectra were obtained using a Tensor spectrometer (Bruker) to identify the existence of $\text{C}=\text{C}$ group and sulfonated structure on the surface of the blend films after plasma treatment.

XPS analysis of SF films was performed by a PHI 5300 X-ray spectrometer (Perkin-Elmer) equipped with an $\text{MgK}\alpha$ non-monochromatic X-ray source. The surface elemental compositions of C_{1s} , O_{1s} , N_{1s} and S_{2p} were measured and a detailed scan of S_{2p} was made in the present experiments.

In vitro Coagulation Time Tests for SF Films

The antithrombogenicity of materials can be reflected by an *in vitro* coagulation time test (APTT). The main procedure was to put the testing film into a transparent plastic tube with healthy blood plasma and then the reagents were added for each coagulation time test immediately. The clotting time was measured by using a photo-optical clot detection instrument Coag-A-Mate[®]-XM (Organon Teknika, USA).

RESULTS AND DISCUSSION

ATR-FTIR Spectra

Figure 1 shows the FTIR spectra of (a) PEG and (b) the PEGM. The PEG macromer prepared by the reaction between PEG and acryloyl chloride shows new peaks at 1722 cm^{-1} and 1409 cm^{-1} , which are attributed to the stretching vibration band of carbonyl and deformation vibration band of the $\text{C}=\text{C}$ group, respectively, indicating the formation of PEG diacrylate^[9]. The ATR-FTIR spectra of (a) SF film, (b) SF/PEGM (1:1) blend film and (c) SO_2 plasma-treated SF/PEGM (1:1) blend film are shown in Fig. 2. SF exhibits absorption bands at

1637 (amide I), 1514 (amide II) and 1232 cm^{-1} (amide III), which are attributed to the β -sheet conformation on the SF film surface. On the other hand, the spectrum of the polymer blend composed of SF and PEGM is no more than an overlapping spectrum of SF and PEGM. In spectra b and c there is a peak at 1409 cm^{-1} ; however, its peak intensity in spectra c is weaker than that in spectra b (Fig. 2). That means the C=C groups existed on the surface of SF/PEGM blend film and the concentration of C=C group was lowered after SO_2 plasma-treatment. This means the C=C group takes part in the chemical reaction when SO_2 plasma-treated. The stretching vibration of S=O of sulfonate group should appear at 1120–1180 cm^{-1} , but in spectra c there is only one strong and broad peak at 990–1180 cm^{-1} , attributed to the C–O–C group. So the stretching vibration absorption of S=O group may be shielded by the absorption of the C–O–C group.

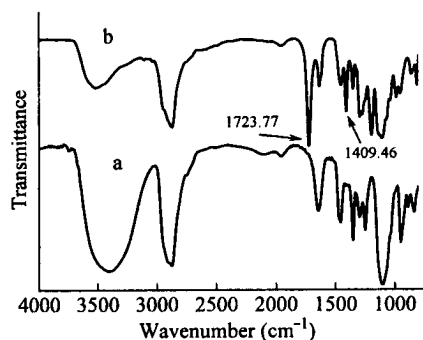


Fig. 1 FTIR spectra of (a) PEG and (b) PEGM ($M_{w, \text{PEG}} = 400$)

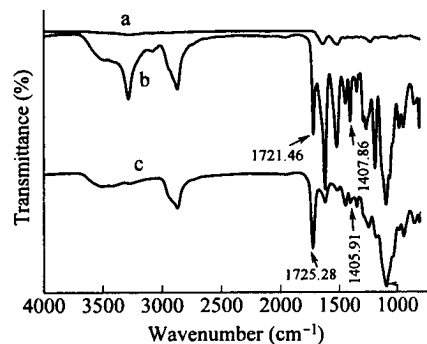


Fig. 2 ATR-FTIR spectra of (a) SF, (b) SF/PEGM (1:1) blend, (c) SO_2 plasma-treated SF/PEGM (1:1) blend films

XPS Analysis of Films

XPS analysis reveals the existence of carbon (C), nitrogen (N), oxygen (O), and sulfur (S) atoms on the surface of untreated and plasma-treated SF blend films. The surface atomic relative percentages in different SF films are listed in Table 1. The surface percentages of C, O, N, and S of SF/PEGM blend film are 72.80%, 24.27%, 2.82% and 0.10%, respectively. The O/C atomic ratios of the SO_2 plasma-treated SF blend films are all higher than that of untreated blend film, which indicates the strong oxidizing capability of SO_2 plasma^[7]. Compared with untreated sample, SF blend films treated by SO_2 plasma have more abundant surface sulfur as shown by the introduction of some sulfur-containing groups onto their surfaces. The percentages of S on the surface of films range from 1.59% to 3.9% (Table 1). First with the increase of PEGM content, the percentage of S increases and when the ratio of SF and PEGM increases to 3:1, the percentage of S reaches its maximum. It is presumed that when the PEGM content is low, the surface C=C groups may react mainly with SO_2 . In contrast, when the ratio of SF and PEGM is above 3:1, the percentage of S decreases gradually. It is believed that the surface C=C groups may mainly be involved in crosslinking reactions because they have strong chemical activities^[9]. In Fig. 3, spectrum C shows the XPS analysis results of the surface S_{2p} spectra of SF blend films treated by SO_2 plasma. There are two distinctive peaks near 169.1 eV and 164.2 eV in the S_{2p} spectrum of SO_2 plasma modified SF.

Table 1. Surface atomic relative ratios of C_{1s} , O_{1s} , N_{1s} and S_{2p} measured by XPS analysis in different modified SF films

Sample	Ratio (SF/PEGM)	C_{1s} (%)	O_{1s} (%)	N_{1s} (%)	S_{2p} (%)	O/C (Atomic ratio)	S/C
Silk fibroin	—	78.30	13.41	7.82	0.47	0.171	0.006
SF/PEGM blend film	1:1	72.80	24.27	2.82	0.10	0.333	0.001
SO_2 plasma-treated film	1:1	72.55	25.49	0.38	1.59	0.351	0.022
SO_2 plasma-treated film	2:1	69.18	25.10	3.39	2.33	0.363	0.034
SO_2 plasma-treated film	3:1	62.05	29.68	4.37	3.90	0.478	0.063
SO_2 plasma-treated film	4:1	67.22	24.55	5.90	2.34	0.365	0.035
SO_2 plasma-treated film	6:1	65.27	24.01	8.50	2.23	0.369	0.034

The higher energy peak may represent the sulfur atom at a higher oxidation state, assigned to sulfur atoms bonded to two or three oxygen atoms such as sulfone, sulfonate or sulfonic acid. In contrast, the lower energy peak may be attributed to sulfur atoms linked to carbon or to one oxygen atom^[7]. Interestingly, with the increasing PEGM content on the surface of SO₂ plasma-treated blend films, the relative contents of sulfur with around 170 eV binding energy gradually decrease while those of sulfur with around 165 eV binding energy increase gradually (Fig. 3).

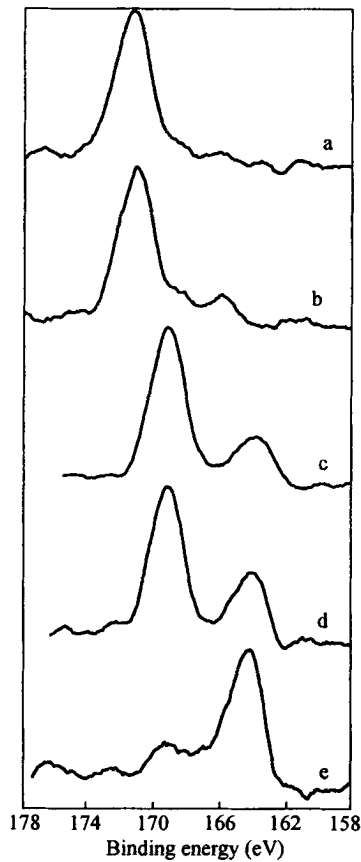


Fig. 3 XPS analysis of the S relative contents of the plasma treated films (SF/PEGM)
a) 6:1; b) 4:1; c) 3:1; d) 2:1; e) 1:1

Table 2. In vitro coagulation times (APTT) of human blood plasma incubated with SF and SO₂ plasma-treated SF blend films*

	Blood plasma	Untreated SF	SO ₂ plasma-treated film (1:1)	SO ₂ plasma-treated film (2:1)	SO ₂ plasma-treated film (3:1)	SO ₂ plasma-treated film (4:1)
APTT (s)	32.6	31.3	> 150	> 150	> 150	> 150

* The data are the mean value of three repeated determinations.

In vitro Coagulation Time Tests of SF Films

In vitro coagulation time tests (APTT) have widely been used for the clinical detection of abnormality of blood plasma, the primary screening of anticoagulative chemicals^[10] and the evaluation of antithrombogenicity of biomaterials. As the mean value of APTT time for a blood plasma is 32.6 s (Table 2), the normal range of APTT time is taken as 32.6 ± 10 s^[8]. It can be seen that when the blood plasma was incubated with untreated SF film,

the APTT is 31.3 s, indicating that SF shows neither antithrombogenicity nor thrombogenicity towards the blood plasma. However, when the blood plasma was incubated with SO₂ plasma-treated SF blend films with different ratios, the APTT time of all samples increase to above 150 s. Therefore, it is believed that the content of sulfur groups on the surface of blend films might have more influence on inhibiting the activities of some clotting factors of blood plasma involved in APTT tests but sulfur groups with different oxidation state on the surface of blend films might have little influence.

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