Blood compatibility of polyurethane immobilized with acrylic acid and plasma grafting sulfonic acid

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Sulfonic and carboxyl groups can effectively improve the blood compatibility of polyurethane. But it is difficult to obtain an optimum ratio of the two groups. In this article, polyurethane (PU) was dissolved with acrylic acid in a tetrahydrofuran solution and then spread on the glass plate to produce a film. At the same time, acrylic acid partly polymerized and immobilized with the PU films. The films (PU-AA) were exposed to sulfur dioxide plasma to graft sulfonic acid group on its surfaces. Through adjusting the quantity of acrylic acid and the plasma reaction condition, the antithrombin of polyurethane can be improved. The surface-modified PUs were characterized by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscope (XPS) and a contact angle goniometer. The blood compatibility of the films was examined by using thrombin time (TT), activated partial thromboplastin time (APTT) and prothrombin time (PT). The TT and APTT were significantly prolonged for the surface-modified films of PU-AA by sulfur dioxide plasma and only APTT was elongated for PU-AA. The results suggest that sulfonic acid and acrylic acid have the different effect on the blood compatibility of surface-modified PUs.

1. Introduction

The interactions between the biological environment and artificial materials are likely to be dominated by the materials' surface properties [1]. When a foreign material is exposed to blood, plasma proteins are absorbed onto the material surfaces, followed by the activation of clotting factors or the adhesion and activation of platelets, and finally the formation of a fibrin network [2]. Hence many efforts have been focused on understanding the relationship between polymer surface and thrombogenicity [3–5]. Many researches indicate that polyurethanes have excellent mechanical properties and relatively good blood tolerance and have been used in many blood-contacting devices [6–10]. But the antithrombogenicity of polyurethanes needs to be improved for the use in the small diameter blood vessel. To fulfill this requirement, heparin, a known anticoagulant, has been used frequently as an immobilizing biomolecule [11–15]. However the aim is not easy to fulfill because heparin often damaged its antithrombogenicity if not properly immobilized with polyurethanes.

Previous studies have reported that the blood compatibility of heparin was likely to be dominated by sulfonate and carboxyl groups [2]. And many sulfonated polymers have improved their blood compatibility [16]. But there are few documents to acclaim that both sulfonate and carboxyl groups are immobilized in the same surface and the clot time often prolong only TT or APTT when films were modified with different methods. In this study, polyurethane (PU) was blended with acrylic acid and then treated with sulfur dioxide plasma to incorporate acidic sulfur-containing functional groups and carboxylic acid. Because plasma discharge treatments are so active that various kinds of groups may react on each other partly, both carboxylic acid groups and sulfonic groups can be successfully immobilized on polyurethane surface. Through adjusting the content of acrylic acid and selecting optimal conditions of plasma discharge, the optimum ratio of content of carboxylic and sulfonic groups was achieved and the blood compatibility of films was improved. So the heparin-like surface of polyurethane was obtained and the *in vitro* blood compatibility of the treated polyurethanes was investigated.

2. Materials and methods

2.1. Materials

Polyurethanes used in this study were synthesized in the polymer laboratory of our university using a conventional prepolymer preparation procedure. Poly(ethylene oxide-tetramethylene oxide) (PET, $M_n = 4250$) and isophorone diisocyanate (IPDI) were added into a reactor

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to make an isocyanate terminated prepolymer at 60 °C for 5 h under nitrogen atmosphere. Then, 1,4-butanediol (BD) was added to extend the prepolymer and 0.05 wt % of dibutyltin diaurate (T-12) was used as a catalyst in tetrahydrofuran solvent. The chain extension reaction proceeded for 4 h at 50 °C, and was kept overnight at room temperature. The number average molecule weight of PU determined by gel permeation chromatograpy was about 64 000. The mechanical properties were measured in the Instron-6022 Test machine in which the stretching speed was 100 mm min^{-1} and the temperature was 298 K. The tensile strength of PU was about 8.34 MPa and ultimate elongation was 636%, which was similar to the typical biomedical polyurethane PYUA103 that the tensile strength was 10 MPa and the ultimate elongation was 1000%.

2.2. Preparation of polyurethane film

A 10 wt % tetrahydrofuran solution of PU blended with different ratio of acrylic acid (CH_2=CHCOOH, Aldrich Co.) was used for the preparation of the film. The solution was spread on a glass plate (10 cm diameter) and solvent evaporated in the air. The sample immersed in deionized water to separate the film from the plate and then dried in a drying oven at 80 °C for 24 h and acrylic acid polymerized in this time.

2.3. Treatment of plasma glow discharge

A plasma reactor consisted of a stainless-steel chamber with a pair of stainless-steel electrodes. The upper electrode (diameter 12 cm) was connected to a glow discharge generator and the lower electrode (diameter 12 cm) was grounded. The sulfur dioxide flow rate was controlled with a mass flow controller. The sulfur dioxide plasma treatment of PU films was carried out at a pressure of 300 m Torr and a power of 30 W with a velocity of 20 ml min^{-1} sulfur dioxide flow rate for a plasma treatment time of 10 min. After treatment, the gas flow was allowed to continue for 5 min in order to quench reactive sites.

2.4. Surface characterization

Surface compositions were analyzed by XPS (XSAM800, KRAOS Co, England). Carbon and sulfur superfine spectra were taken for analyzing the element bond environment. ATR-FTIR (System 2000, PE Co, American) was also used to determine the film structure.

The water contact angles of the modified PU surfaces were measured by putting a droplet of deionized water on the surface of polymer films using JY-82 contact angle goniometer. With each specimen, the measurement was repeated at three different points, and average values were obtained for contact angle.

2.5. The measurement of TT, PT and APTT

The nephelometry measurements, including PT, APTT and TT, were performed with the coagulation instrument Coag-A-Mate XM (Organon Teknika, USA) that measured the change of luminosity when light traversed plasma sample. The Coag-A-Mate XM instrument was operated in an automatic mode, with the clotting time defined as the time when the scattered light intensity differs 3% from the offset signal in that particular plastic tube, expressed as a percentage of the reference signal. The PT was measured as follow procedure. The tested films were incubated with 0.1 ml of healthy human blood plasma and mixed with 0.2 ml of calcium-prothrombin in a transparent plastic tube at 37 °C, then nephelometric measurements were performed to test PT. Similarly, the TT was also tested, which used 0.2 ml of healthy human blood plasma and mixed 0.2 ml of thrombin. The measurement of APTT has some difference with that of TT and PT. The films were incubated with 0.1 ml of healthy human blood plasma and mixed with 0.1 ml activated partial thromboplastin. After the mixture was activated for 3 min, 0.1 ml of 30 m mol l^{-1} $CaCl_2$ was added and then nephelometric measurements were performed. The measurement values were the average values of three time tests.

3. Results

3.1. Film surface characterization

Fig. 1 gave the ATR-FITR of the PU (a), PU-AA (b) and PU-AA treated with SO_2 plasma (c) samples. It was found that the spectra of the PU-AA film before and after plasma treatment were almost same. It was because the absorption peaks, corresponding to sulfonic acid groups (1050 cm^{-1}, 1230 cm^{-1}) overlapped with that of the ester groups (1105 cm^{-1}, 1237 cm^{-1}) in PU itself. However, the grafting of sulfonic acid group was confirmed by XPS and a contact angle goniometer. Comparing with the spectra of PU, a small peak (1409 cm^{-1}) was found in spectra of PU-AA and PU-AA treated by SO_2 plasma. It belonged to the plane deformation vibration of acrylic acid. There was also a peak at 811 cm^{-1} in b, c spectra that came from the wide band of polyacrylic acid, so the acrylic acid has partly polymerized on the surface of the films.

Fig. 2 showed the XPS survey scan spectra of PU (a), PU containing acrylic acid (PU-AA) (b) and PU-AA treated by plasma (c) as typical examples. PU (a) and PU-AA (b) showed three peaks corresponding to C-1s

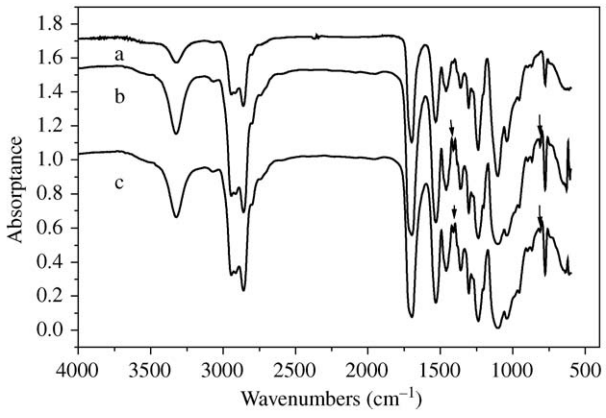

Figure 1 ATR-FITR spectra of (a) polyurethane (PU), (b) polyurethane immobilized with acrylic acid (PU-AA) and (c) polyurethane treated by plasma (PU-AA treated).

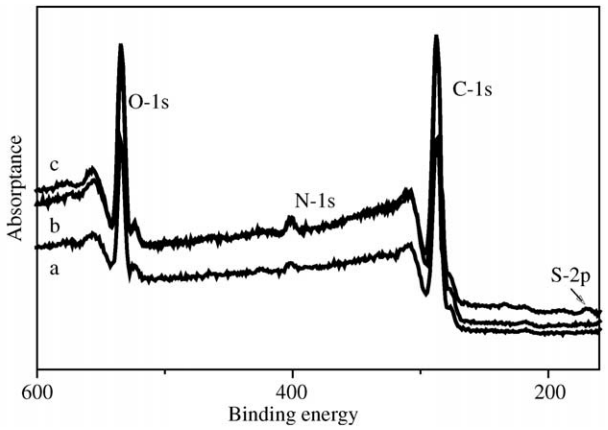

Figure 2 The XPS survey scan spectra of (a) PU, (b) PU-AA, (c) PU-AA (treated).

(284.6 eV), N-1s (400 eV) and O-1s (532.3 eV), PU treated with SO_2 plasma showed additional small peaks corresponding to S-2p (169 eV and 164 eV). The corresponding chemical compositions of the surfaces of the different films, calculated from the XPS spectra, were shown in Table I.

Comparing with PU, in sample PU-AA, the oxygen content (17.21%) was first slightly increased (18.13%) due to the adding of acrylic acid and then remained almost the same (18.14%) after sulfur dioxide plasma treatment. In addition, a sulfur component (1.94%) was observed on the surface of plasma modification film. It indicated that groups containing sulfur grafted on the PU surface.

Fig. 3 showed the C-1s superfine scan spectra of the above three samples as typical examples. The C-1s spectra could be deconvoluted into four peaks. The peak of 284.6 eV came from C–C bond, 286.1 eV peak corresponded to C–O bond, 288.1 eV one due to the carbon of C=O bonds and the O=C–O$^-$ group contributed to 289.1 eV peak. The relative areas of four peaks were shown in Table II.

When blended with acrylic acid, the peaks at 284.6 eV (C–C) and 289.1 eV (O=C–O$^-$) increased whereas the peaks at 286.1 eV (C–O) and 288.1 eV (C=O) both decreased significantly. The result suggested that the acrylic acid successfully existed on the surface and the surface composition changed to reduce the surface free energy. After sulfur dioxide plasma treatment, the peak at 289.1 eV (O=C–O$^-$) decreased because the COOH was relatively active and may partly react on other active groups because of the high energy of plasma treatment. Although the C–S peak was difficult to be seen in the C-1s superfine scan spectra because it was easily overlapped by C–C peak, the S spectrum could be seen in the S-2p spectrum of PU-AA modified through plasma.

TABLE I Chemical composition of surfaces of different samples

Substrate	Atomic percent (%)			
	C	O	N	S
PU	80.77	17.21	2.02	—
PU-AA	79.30	18.13	2.57	—
PU-AA (treated)	77.58	18.14	2.34	1.94

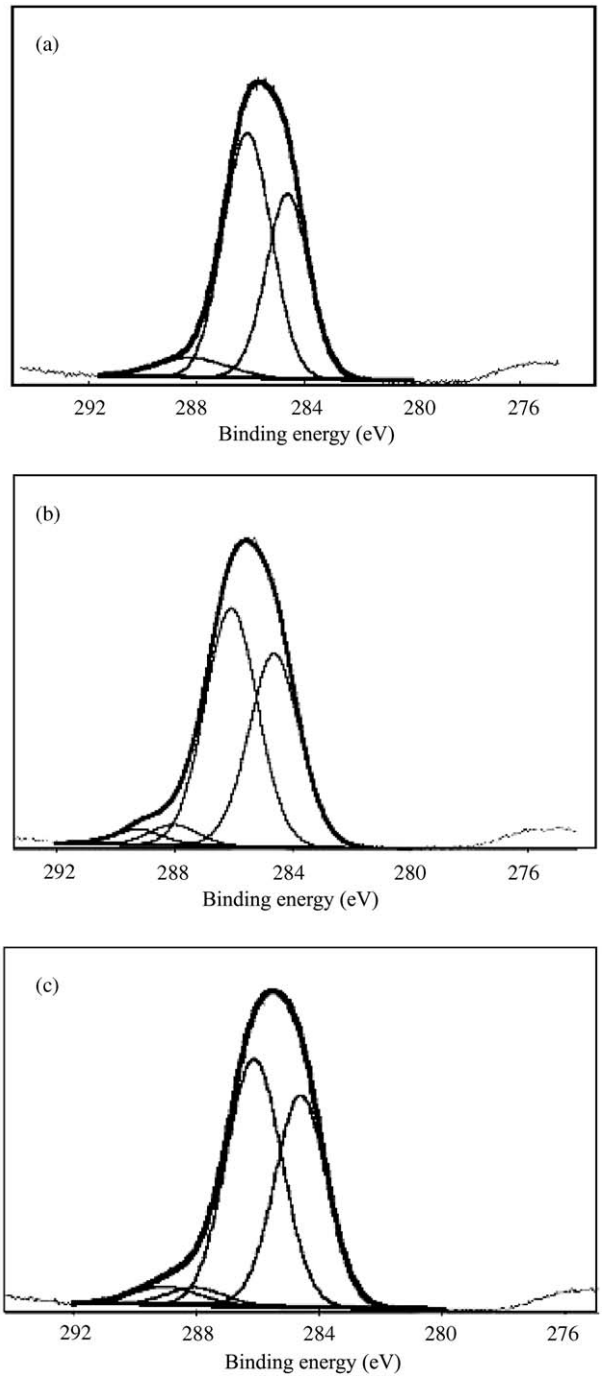

Figure 3 XPS carbon 1s core level scan spectra of (a) PU, (b) PU immobilized with acrylic acid (PU-AA) and (c) PU-AA treated by sulfur dioxide plasma.

Fig. 4 was the S-2p spectrum of PU-AA modified through plasma. There were two peaks in the spectrum. The peak at 168.9 eV belonged to the sulfonic acid group and the peak at 164 eV for sulfur group based on the C–S bond. So the sulfur groups were bonded on the surface of

TABLE II Percentage contribution of XPS C1s components for surface-modified PUs

Substrate	Contribution of C1s			
	C–C (284.6 eV)	C–O (286.1 eV)	C=O (288.1 eV)	COO (289.1 eV)
PU	37.80	55.63	6.57	—
PU-AA	41.04	50.85	3.72	4.39
PU-AA (treated)	41.02	51.64	4.12	3.22

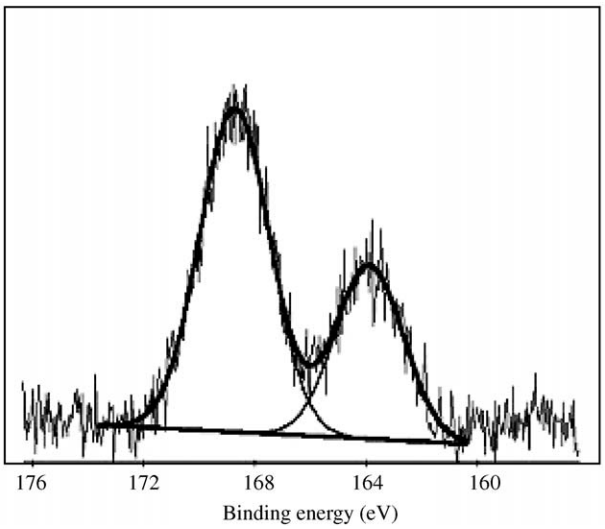

Figure 4 XPS S-2p spectra for plasma-modified surfaces of PU-AA.

the film and the active groups of sulfonic acid and carboxyl groups have both been grafted on the surface of the films.

3.2. Water contact angles and blood compatibility

Table III showed the contact angles of the different films. The water contact angle of PU (65°) increased with the introduction of acrylic acid (74°) because the hydrophobic group increased (C–C) and the hydrophilic groups (C–O and C=O) decreased. Because acrylic acid was added in tetrahydrofuran solution and then polyurethane films formed in the air, hydrophobic groups enriched on the film surface while hydrophilic groups such as acrylic acid mustered in the inside of the films in order to keep the least surface free energy. It was also why the contact angle increased when acrylic acid was introduced to the films. When the PU-AA was modified with the sulfur dioxide plasma, the water contact angle decreased significantly from 74° to 43°. It indicated that the film surface of PU was grafted with sulfonic acid group and became more wettable after surface modification.

Table IV showed the *in vitro* blood compatibility of samples. The PT had not the remarkable change in all samples (shown in Table IV). It indicated that both sulfonic acid and acrylic acid could not restrain the prothrombin activity separately. The APTT of the PU-AA was prolonged very much (> 200 s, significance level: $P = 0.0001$), which was different with other's study of the grafting of acrylic acid [9]. After it was treated with the sulfur dioxide plasma, the good blood compatibility could be maintained if the content of AA (1.3 g PU, 1 ml AA) was suitable and the TT would surpass the instrument maximal range (> 150 s, $P = 0.0001$). While, when the content of acrylic acid was low (1.3 g PU, 0.4 ml AA), the APTT of the film treated with the sulfur dioxide plasma lost this good property but the TT still remarkably elongated (> 150 s, $P = 0.0001$). Through the sulfur dioxide plasma, the sulfonic acid and acrylic acid was immobilized on the surface of PU-AA, so the heparin-like structure formed and the blood compatibility was very good (APTT > 200 s, TT > 150 s) except PT. In 20 days later, the measurement can be repeated (APTT > 200 s, TT > 150 s, 20 days). The result suggested that the modified film was rather stable.

TABLE III Water contact angles of surface-modified PU films

Substrate	Contact angle (°)[a]
PU	65 ± 2
PU-AA	74 ± 3
PU-AA (treated)	43 ± 3

[a]Measured by sessile droplet method.

4. Discussions

4.1. Surface characterization

Plasma glow discharge was used to chemically modify polyurethane film without causing any change to the bulk properties of the film. The sulfur dioxide plasma glow discharge treatment can create free radicals on the polymer surfaces and these radicals can then react with double bound or other groups. Through adjusting the content of acrylic acid and selecting optimal conditions of plasma discharge, the right ratio of the content of grafted carboxylic and sulfonic groups was achieved and the excellent blood compatibility then was obtained.

From the experimental results shown in Table I, it was concluded that the oxygen content hardly increased after the modification of sulfur dioxide plasma. It was believed that the carboxylic acid groups blended in the polyurethane partly participated in the reaction with active groups produced in plasma. And it was also found that the carboxylic acid groups really decreased after plasma modification.

4.2. The wettability and *in vitro* blood compatibility of polyurethane

When the polyurethane films blended with acrylic acid were produced, the contact angle of polyurethane surface increased rather than decreased. It was because the group composition had changed in the formation of films to keep the least surface free energy. So, as shown in Table II, the peaks at 284.6 eV (C–C) and the peaks at 286.1 eV (C–O) and 288.1 eV (C=O) both decreased significantly after incorporation of carboxylic groups. After the plasma modification, the contact angle decreased remarkably because of the grafted sulfonic groups.

As we know, the sulfonic groups have been frequently used as a useful group to improve the blood compatibility of polymeric materials [17–19]. And in this study, the sulfonic groups really improved the antithrombin and especially prolonged the TT time. In different circumstances and after treated by SO_2 plasma, whether blended with acrylic acid or not, the TT of polyurethanes was remarkably elongated (> 150 s). The results suggested that sulfonic acid could suppress the thrombin activity. In our study, the TT of PUs could maintain excellent (> 150 s) if the content of the sulfur groups remained in

TABLE IV Activated partial thromboplastin time (APTT), thrombin time (TT) and prothrombin time (PT) of PU and surface-modified PU films

Substrate	Condition	APTT(s)	TT(s)	PT(s)
PU		46.8 ± 3	15.1 ± 2.4	25.0 ± 1
PU (treated)	20 sccm, 15 Pa, 10 w, 5 min	45.9 ± 5	> 150	17 ± 3
PU-AA[a]	1.3 g PU, 0.4 ml AA	> 200	22.1 ± 2	—
PU-AA[b]	1.3 g PU, 1 ml AA	> 200	23.5 ± 3.5	18.5 ± 2
PU-AA[a] (treated)	20 sccm, 30 Pa, 30 w, 10 min	51.2 ± 4	> 150	11.9 ± 2.2
PU-AA[b] (treated)	20 sccm, 30 Pa, 30 w, 10 min	> 200	> 150	14.6 ± 2.7

Note: all the samples test three times and are considered effective only when the time surpasses the instrument maximal limit.

the scope 1–4.5%. On the other hand, when blended with different content acrylic, the APTT expressed the different results. It was because the content of acrylic acid decreased after the films were modified by SO_2 plasma (shown in Table II). The result suggested that in heparin the sulfonic and carboxyl groups affected different thrombin factors and although we do not know the precise content of the two groups, the right content would help to improve the blood compatibility of films. But the PT was hardly prolonged in all this research. It indicated that both sulfonic acid and acrylic acid could not affect the prothrombin activity and in heparin there must be other groups to improve the antithrombin. We have studied the other groups' effect such as methoxyl group, it was found that the PT could prolong very much when methoxyl group was introduced in PU. The relative result will be reported in another paper.

5. Summary

In this study, PU and acrylic acid dissolved in the tetrahydrofuran solvent and then made the films with spreading method. In the process of forming films, the acrylic acid polymerized and changed film structure. This method was simple and the content of acrylic acid was easy controlled. After sulfur dioxide plasma treatment, the sulfonic acid groups were immobilized on the surface of PUs. It was found that sulfonic acid could inhabit the activity of thrombin while acrylic acid could depress the activated partial thromboplastin in the suitable range content. Both groups had no effect on PT. Finally, this method makes it possible that the different molecular with the active groups can be synthesized first and then immobilized on the surface of the PUs.

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