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Surface modification of polyetherurethaneureas and their antithrombogenicity

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Polyetherurethaneurea (PU) films were treated by oxygen plasma discharge followed by acrylic acid (AA) grafting. The carboxyl groups of the AA-grafted PU (PU–AA) surface were coupled with bovine serum albumin and heparin via water soluble carbodiimide. Surface characterization of the modified PUs was carried out by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and electron spectroscopy for chemical analysis (ESCA). The amount of immobilized albumin and heparin on the PU surface was 1.8 and 1.5 $\mu g/cm^2$, respectively, as determined by the dye interaction method. Interactions between the surface-modified PUs and blood components such as plasma proteins and platelets were investigated to evaluate the blood compatibility of the samples. Plasma recalcification time (PRT) and activated partial thromboplastin time (APTT) of the albumin-immobilized PU (PU–Al) were almost the same as those of PU, while platelets were less adhered on the PU–Al than on PU. On the other hand, PRT and APTT of the PU–He were significantly longer than those of the PU, PU–AA, and PU–Al. Moreover, adhesion of platelets was effectively suppressed on the PU–He, leading to good *in vitro* blood compatibility.

1. Introduction

Radiation [1], plasma discharge [2, 3] and chemical treatment [4] have been widely used as means of surface modification to improve the blood compatibility of polymeric materials. The blood compatibility of common polymeric materials such as polyethylene and poly(vinyl chloride) can be improved to some extent by the radio frequency glow discharge (RFGD) technique [5]. It has been reported by Pitt *et al.* [3] that controlled hydrophobicity gradient surfaces could be prepared by the treatment of polymers with an RFGD technique under a movable shutter.

Barbucci *et al.* [6] developed a grafting method of poly(amido-amine) onto polyurethanes, using hexamethylene diisocyanate as a spacer. They proposed that heparin could be bound to the quaternized poly(amido-amine) parts of the grafted polyurethanes. Polyethylene oxide (PEO) has been frequently used for the surface modification of polymeric materials. Kim *et al.* [7] studied the surface modifications of commercial polyurethanes (Pellethane) by PEO grafting and/or heparin-immobilization for long-term biomedical applications. Although blood compatibility of polyurethane has been, to a certain extent, improved by heparin or PEO grafting, one limitation still remains, that is the restricted coupling for heparin-immobilization. To overcome this problem, Kim *et al.* [8] proposed a novel method to increase the concentration of immobilized heparin by introducing polyethylene imine on the surface of PU.

In this study, the surface of polyetherurethaneurea (PU) was chemically treated by oxygen plasma glow discharge, followed by direct reaction with acrylic acid (AA) to produce AA-grafted PU (PU–AA). Albumin and heparin were then coupled with carboxyl groups of the PU–AA. The PUs immobilized with albumin (PU–Al) and heparin (PU–He) were characterized by attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy and electron spectroscopy for chemical analysis (ESCA). The *in vitro* blood compatibilities of the PU–Al and PU–He were investigated.

2. Materials and methods

2.1. Preparation of films

PU was synthesized from 4,4′-diphenylmethane diisocyanate (MDI), polytetramethylene glycol (PTMG, MW 1000) and ethylene diamine as a chain extender in a molar ratio of 1.3:1:1. The number average molecular weight of PU determined by gel permeation chromatography was about 80 000. PU film was prepared by the solvent casting method [9].

2.2. Oxygen plasma treatment and acrylic acid grafting

The plasma reactor consists of a stainless steel chamber (≈8 l) with a pair of stainless steel discharge electrodes as shown in Fig. 1. The upper electrode

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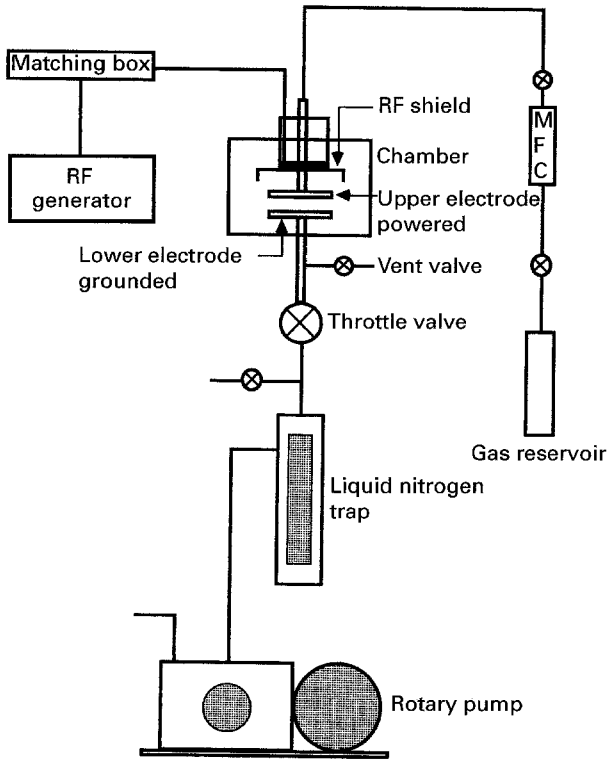

Figure 1 Schematic diagram of oxygen plasma discharge apparatus.

(diameter 12 cm) was connected to the 13.56 MHz radio frequency generator via an impedance matching circuit, and the lower electrode (diameter 12 cm) was grounded. The system pressure before discharge was monitored by a Hoyt thermocouple vacuum gauge (model PT 2500) connected to the downstream of the reactor. The flow rate of oxygen was measured by mass flow controller (Stec Inc., Kyoto, Japan, Model EC-400MK3). Oxygen plasma treatment of PU films was carried out according to the method described in a previous report [9]. The peroxide formed on the PU surface was determined using a 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method [10]. The oxygen plasma-treated PU was directly immersed in 10 wt % AA aqueous solution and incubated at 60 °C for 90 min. After the graft reaction, the film was washed with distilled water and then a 1% solution of Triton X-100 for 20 min using an ultrasonic cleaner. The film was finally washed with distilled water and dried under reduced pressure for 48 h at room temperature. The grafting of AA was confirmed by ATR-FTIR and the amount of carboxyl groups of PU–AA was determined by the modified dye-interaction method [11].

2.3. Immobilization of albumin and heparin

The PU–AA film (6.5 × 6.5 cm) was placed in 0.075 M sodium citrate solution (80 ml), containing 1-ethyl-3-dimethyl amidopropyl carbodiimide (WSC, 4.18×10^{-4} M) and kept at 2 °C for 30 min to activate the carboxyl groups for the immobilization. Bovine serum albumin (50 mg) and heparin (50 mg) are separately dissolved in 0.075 M citric acid (pH 4.7, 50 ml). Each solution was put into a glass vial containing the PU–AA film, previously activated with WSC, and kept at 2 °C for 24 h. The film was washed with citric acid and 0.1% Triton X-100 aqueous solution for 10 min and subsequently rinsed with distilled water in an ultrasonic cleaner for 10 min. The amount of albumin immobilized on the PU–AA was monitored by a method previously described [9]. The amount of heparin immobilized on the PU–AA was determined using the method previously reported by Ito *et al.* [4]. *In vitro* bioactivity of the heparin immobilized on PU was measured by chromogenic method [12].

2.4. Surface characterization

The ATR-FTIR spectra of the surface-modified PU films were obtained using a MIDAC spectrophotometer equipped with a ZnSe reflection element. The surface-modified PU films were also analysed using ESCA (ESCALAB MKII, V.G. Scientific Co., East Grinstead, UK) equipped with AlK_α at 1487 eV and 300 W power at the anode. Survey scan and carbon-1s core level scan spectra were taken and corrected for scattering cross-sections [13]. For the evaluation of surface wettability, the water contact angles of the surface-modified PU were measured at room temperature using a contact angle goniometer (Model 100-0, Rame-Hart, NJ, USA) [14].

2.5. *In vitro* blood compatibility

2.5.1. Thrombus formation

Human whole blood (30 ml) from healthy volunteers was collected and mixed with an aqueous solution containing D-glucose, sodium citrate and citric acid (ACD, 3 ml). The surface-modified PU film (3 × 3 cm) was attached to a watch glass (diameter 7.5 cm) and thrombus formation test was carried out according to the method previously reported [15].

2.5.2. Plasma recalcification time (PRT)

Platelet poor plasma (PPP, 300 µl) was incubated on the sample films. 0.025 M $CaCl_2$ aqueous solution was then added to the PPP and the plasma solution was monitored for clotting by manually dipping a stainless steel wire hook coated with silicone into the solution for detection of fibrin threads. Clotting times were recorded at the first signs of fibrin formation on the hook.

2.5.3. Activated partial thromboplastin time (APTT)

The sample film (3 × 3 cm) was attached to a watch glass (4 cm diameter and 1.5 cm height), pre-swelled with distilled water. Plasma solution (100 µl) and activated partial thromboplastin solution (100 µl) were added to the sample film, followed by addition of 0.025 M $CaCl_2$ solution (100 µl) after about 5 min. The clotting time of the plasma solution was observed as described in PRT experiment.

2.5.4. Platelet adhesion

Samples of platelet-rich plasma (PRP, 100 µl) were brought to contact with the surface-modified PU at

37 °C for 30 and 60 min. Phosphate-buffered saline (PBS, 6 ml) was then added to the PRP to stop further adhesion and kept for 1 min. The number of platelets adhered to the substrates was determined by measuring lactate dehydrogenase (LDH) activity of cells lysed with Triton X-100 [16]. The morphological observation of adhered platelets was done by the method previously reported [13].

3. Results and discussion

3.1. Oxygen plasma treatment and acrylic acid grafting

The plasma is known to create free radicals on polymer surfaces and these radicals can be converted into peroxide when they are exposed to air [17]. The concentration of radicals generated by plasma treatment depends on power level, exposure time, and reactor pressure [18]. Table I shows the effect of plasma exposure time on the concentration of the grafted AA. The peroxide concentration seems to increase up to 30 s exposure and to decrease thereafter. Ikada *et al.* [10] also observed such a maximum concentration of peroxide on polyethylene film when they had varied exposure time. The concentration of grafted AA increased with increases in peroxide concentrations. This indicates that the peroxides are effectively converted into radicals as initiators for the grafting of AA when they are immersed in AA solution at 60 °C.

When different pressures were applied for the oxygen plasma treatments, the maximum concentrations of peroxide (2.4 nmol/cm^2) and AA (1.7 μmol/cm^2) were found at 26 Pa, as shown in Table II. These results are consistent with those obtained with poly(methyl methacrylate) substrates [9].

The ATR-FTIR spectra of PU and PU–AA treated with sodium hydroxide aqueous solution are shown in Fig. 2. The absorbances at 1600 and 1410 cm^{-1}, which are based on symmetric and asymmetric stretching vibrations of carbonyls (–C=O), respectively, were largely increased. These results suggest that AA was successfully grafted on the PU surface.

TABLE I Effect of plasma exposure time on the concentration of peroxide and acrylic acid formed on the PU surfaces[a]

Exposure time (s)	Peroxide concentration[b] (nmol/cm^2)	Acrylic acid concentration[c] (μmol/cm^2)
10	2.1 ± 0.05	1.2 ± 0.05
30	2.4 ± 0.05	1.7 ± 0.05
60	0.6 ± 0.04	1.1 ± 0.05
120	0.7 ± 0.04	1.1 ± 0.05

[a] Pressure 26 Pa, power 120 W
[b] Measured by 1,1-diphenyl-2-picrylhydrazyl method
[c] Measured by dye-interaction method

TABLE II Effect of pressure on the concentration of peroxide and acrylic acid formed on the PU surfaces[a]

Pressure (Pa)	Peroxide concentration[b] (nmol/cm^2)	Acrylic acid concentration[c] (μmol/cm^2)
13.3	0.9 ± 0.04	1.3 ± 0.05
26.6	2.4 ± 0.05	1.7 ± 0.05
66.7	0.2 ± 0.05	0.3 ± 0.01
133.3	0.1 ± 0.01	0.3 ± 0.01

[a] Exposure time 30 s, power 120 W
[b] Measured by 1,1-diphenyl-2-picrylhydrazyl method
[c] Measured by dye-interaction method

3.2. Immobilization of albumin and heparin

It has been reported that albumin could be coupled onto crosslinked poly(vinyl alcohol) hydrogel membranes and ethylene-vinyl alcohol copolymer films grafted previously with oxidized starches having many pendent aldehyde groups [19]. Lyman [20] reported that platelets are less adhered on the surfaces coated with albumin. Heparin has also been widely used as the immobilizing biomolecules for the improvement of antithrombogenicity of material surfaces [21]. In this study, we chose albumin and heparin as the immobilizing biomolecules for the surface modification of PU film.

The ATR-FTIR spectra of the modified PUs are shown in Fig. 3. The spectrum of PU–He (b) was almost the same as that of PU itself (a). However, in the spectrum of PU–Al (c), amide I absorption newly appeared at 1650 cm^{-1}, indicating the presence of α-helical structure in immobilized albumin [14].

The ESCA C1s spectra of surface-modified PUs (Fig. 4) were resolved into four characteristic peaks. The peaks at 289.3, 288.2, 286.5 and 285 eV correspond

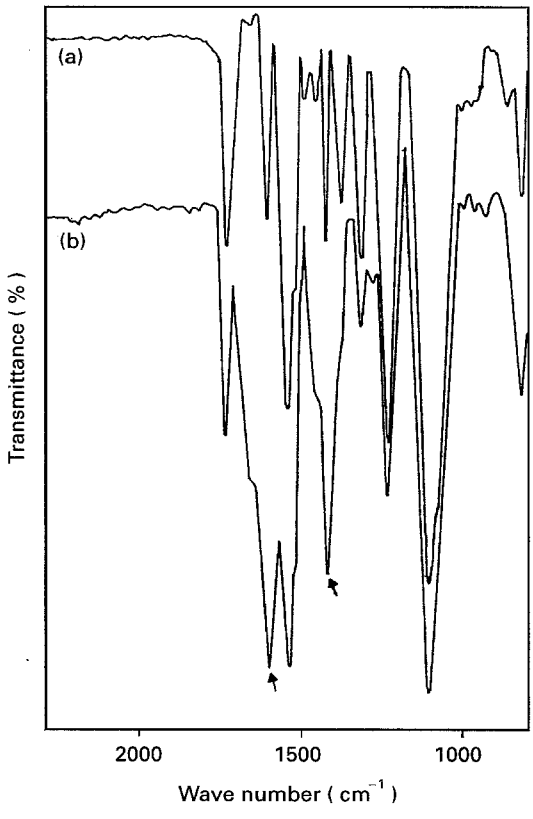

Figure 2 ATR-FTIR spectra of (a) PU and (b) PU–AA treated with sodium hydroxide aqueous solution.

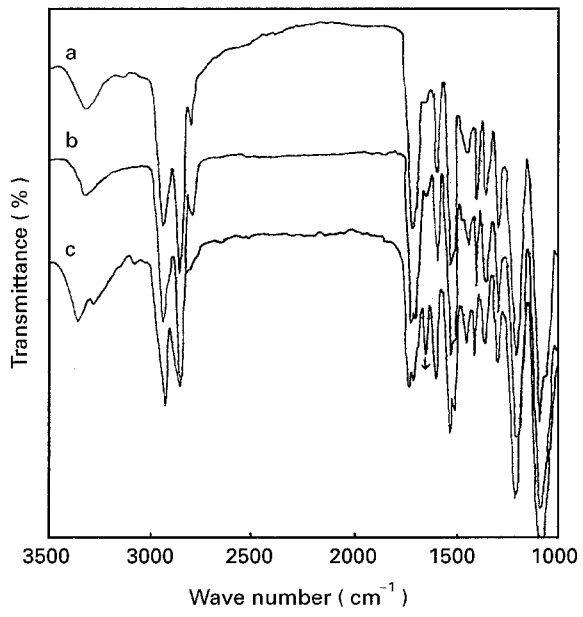

Figure 3 ATR-FTIR spectra of (a) PU, (b) PU–He and (c) PU–Al.

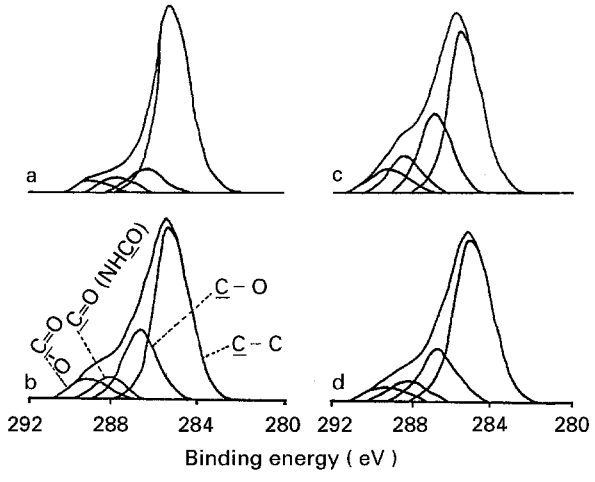

Figure 4 ESCA carbon 1s core level scan spectra of (a) PU, (b) PU–AA, (c) PU–Al and (d) PU–He.

TABLE III Peak area of ESCA C1s core level spectra of PU and surface-modified PU[a]

Substrate	Peak area of ESCA C1s components (%)			
	C–C	C–O	C=O (NHCO)	COO
PU	79	10	6	5
PU–AA	63	24	7	6
PU–Al	55	27	11	7
PU–He	71	23	4	2

[a] RFGD conditions: 120 W, 30 s, 26.6 Pa

to C1s core level of COO, C=O(–CONH–), C–O and C–C, respectively, on the surfaces of PU films [22]. The percentage contributions of C1s components of PUs are calculated from the data of Fig. 4 and the results are shown in Table III. The intensities of peaks at 286.5 and 289.3 eV were increased after AA grafting, suggesting the presence of carboxyl groups. The peak at 288.2 eV of PU–AA was also intensified after albumin immobilization, indicating that albumin was successfully immobilized on the surfaces of PU films. On the other hand, the peak at 289.3 eV of PU–AA was decreased after heparin immobilization.

TABLE IV Amount of heparin and albumin immobilized on the surface of the PU–AA film[a]

Substrate	Amount of immobilized biomolecules (μg/cm^2)	
	Rinsed by water	Rinsed by Triton X-100
PU–Al	5.6 ± 0.02	1.8 ± 0.02
PU–He	2.3 ± 0.03	1.5 ± 0.02

[a] RFGD conditions: 26.6 Pa, 30 s, 120 W

TABLE V Water contact angles of PU and surface-modified PU films

Substrate	Contact angle[a]
PU	68 ± 2
PU–AA	20 ± 3
PU–Al	26 ± 3
PU–He	25 ± 2

[a] Sample number, $n = 3$

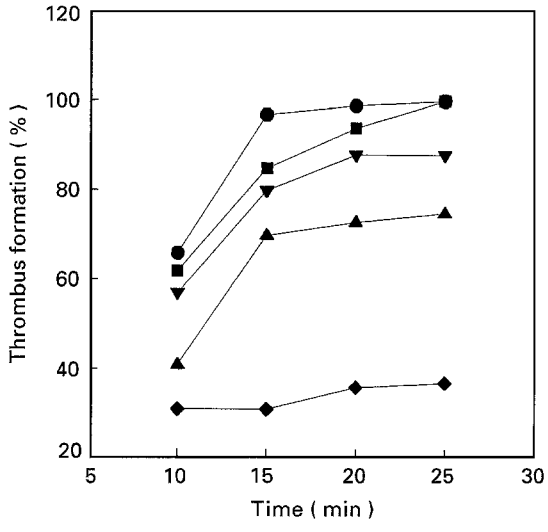

Figure 5 Thrombus formation on the surface-modified PUs as a function of incubation time: ● glass; ▼ PU; ■ PU–AA; ▲ PU–Al; ◆ PU–He ($n = 3$).

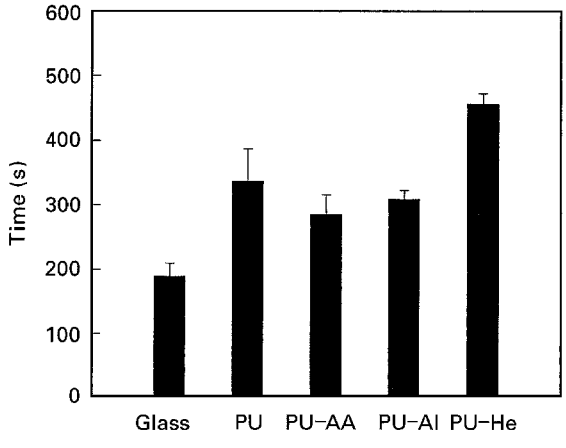

Figure 6 Plasma recalcification time (PRT) of surface-modified PUs ($n = 3$).

The amounts of albumin and heparin immobilized on the surface of PU films were 1.8 and 1.5 μg/cm^2, respectively, as given in Table IV. The amount of albumin immobilized on the PU surface was smaller than previously reported results on PMMA surface (6.25 μg/cm^2) [9]. This is probably due to the difference between the amounts of grafted carboxyl groups on the PU (1.7 μg/cm^2) and PMMA (9.48 μg/cm^2) [9]. It has been reported that the amount of heparin immobilized on the PU surface was 1.45–1.84 μg/cm^2 when the PU surfaces were treated with diisocyanate and subsequent reaction with heparin [7]. The amount of immobilized heparin (1.5 μg/cm^2) in this study was almost the same as that reported by Kim *et al.* [7]. From the experiment of heparin bioactivity, it was found that the concentration of active heparin, which is used to neutralize thrombin, was about 29%.

The water contact angles of the PUs are tabulated in Table V. PU showed a relatively hydrophobic surface with the contact angle of 68°. However, the contact angle was sharply decreased by AA grafting, heparin immobilization and albumin immobilization.

TABLE VI Activated partial thromboplastin time (APTT) on the PU and surface-modified PUs

Substrate	APTT[a]
Glass	22.6 ± 2
PU	26.3 ± 2
PU–AA	23.3 ± 2
PU–Al	24.0 ± 2
PU–He	32.6 ± 2

[a] Sample numbers, $n = 3$

3.3. *In vitro* blood compatibility

The relative amounts of thrombus formed on the surface of PUs are plotted as a function of incubation time, as shown in Fig. 5. The percentages of thrombus formed are calculated as weight fractions relative to that formed on a glass plate after 25 min incubation. Significant decrease of thrombus formation is apparent on the PU–He, while slight suppression is observed on the PU–Al.

As illustrated in Fig. 6, PRT of the PU–AA (280 s) and PU–Al (300 s) was relatively shorter than that of

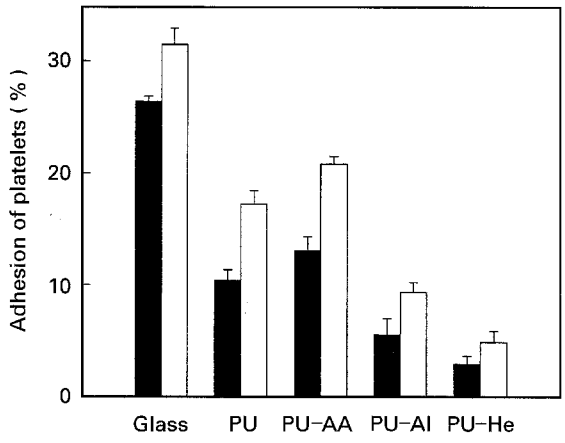

Figure 7 Adhesion of platelets on the surface-modified PUs: ■ 30 min; □ 60 min incubation ($n = 3$).

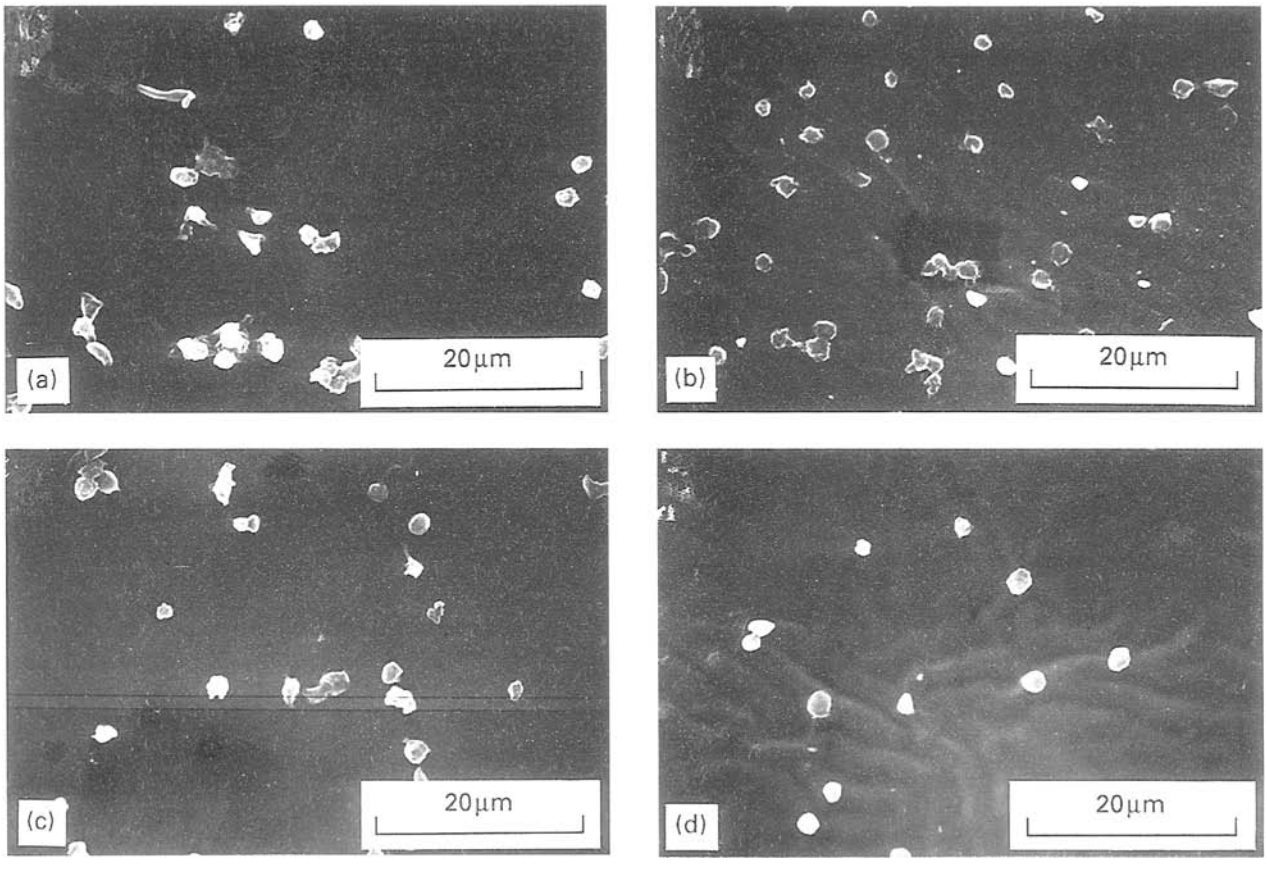

Figure 8 SEM photographs of platelets adhered to the surface-modified PUs after 1 h incubation: (a) PU; (b) PU–AA; (c) PU–Al; (d) PU–He.

PU (340 s). This indicates that blood-clotting factors are easily activated on the PU–AA and PU–Al compared to those on PU itself. On the other hand, PRT of the PU–He was significantly longer than that of PU, suggesting a compatible surface for blood-clotting factors.

To observe *in vitro* bioactivity of the immobilized heparin, the APTT, which exhibits the bioactivity of intrinsic blood coagulation factors, was examined, and the results are given in Table VI. The APTTs of the PU–AA and PU–Al were almost the same as that of PU within standard deviations. However, the APTT of the PU–He was significantly prolonged compared to that of PU. It is well known that heparinized surfaces show a longer APTT than non-heparinized surfaces [23].

Fig. 7 represents the percentage of platelets adhered to the surface of PUs. Adhesion of platelets was significantly suppressed on the PU–He, and moderately suppressed on the PU–Al. In contrast, PU–AA showed a higher level of platelet adhesion than PU. These results suggest that adhesion of platelets on PU was not suppressed by AA grafting but suppressed by albumin immobilization and heparin immobilization.

Fig. 8 shows scanning electron micrographs of platelets adhered to the surface-modified PU after 1 h incubation. Morphological change of platelets was not observed on PU–He, while slight change was observed on PU–Al, PU–AA, and PU.

4. Conclusions

AA was successfully grafted on oxygen plasma-treated PU surfaces, allowing the coupling of albumin and heparin to the surface of PU–AA. Plasma proteins were more activated on PU–Al than on PU, while platelets were slightly suppressed on PU–Al. On the other hand, the activation of plasma proteins and platelets were effectively suppressed on PU–He, leading to good *in vitro* blood compatibility.

Acknowledgements

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