

GRAFTING OF SULFOBETAINE ONTO A POLYURETHANE SURFACE TO IMPROVE BLOOD COMPATIBILITY*

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Abstract On the molecular level, it is believed that polymers containing zwitterionic structures should be compatible with blood. In this work polyurethane films were grafted with sulfobetaine by a three-step procedure. In the first step, the films' surfaces were treated with hexamethylene diisocyanate (HDI) in toluene at 50°C in the presence of di-*n*-butyl tin dilaurate (DBTDL) as a catalyst. The extent of the reaction was monitored by ATR-IR spectra; a maximum number of free NCO groups was obtained after a reaction time of 90 min. In the second step, the hydroxyl groups of *N,N*-dimethylethylethanolamine (DMEA) were allowed to react in toluene with NCO groups bound on the surface. In the third step, sulfobetaines were formed on the surface through the ring-opening reaction between tertiary amine of DMEA and 1,3-propanesultone (PS). The surfaces of the films were characterized by ATR-IR and XPS showing that the grafted surfaces were composed of sulfobetaine. The results of the contact angle measurement show that the surface was strongly hydrophilic. The platelet adhesion test demonstrated that the films grafted with sulfobetaine have excellent blood compatibility.

Keywords Polyurethane, Sulfobetaine, Surface modification, Platelet adhesion, Nonthrombogenic biomaterials

INTRODUCTION

Segmented polyurethanes (PU) have been widely used as blood-contacting biomaterials because they were found to exhibit excellent mechanical properties and favorable biocompatibility^[1]. In blood contacting applications, the surface properties of biomaterials are especially important, and it is therefore of interest to modify the surface of PUs in order to enhance their blood compatibility, and retain their excellent mechanical properties at the same time. Many studies have been performed aiming at producing blood-compatible surfaces by grafting of polymer surfaces with poly(ethylene glycol)^[2–4], heparin^[5–8], phospholipid polymer^[9–12], hirudin^[13]. Recently, sulfobetaines with $[-N^+(CH_2)_nSO_3^-]$, $n = 3, 4$ side groups have received increased attention as one of the most representative structures in the well-identified class of polymeric zwitterionic materials^[14–17]. Because of the strong dipolar structure of the zwitterionic lateral groups, these linear polymers display specific properties such as high chain rigidity and a unique antipolyelectrolyte behavior. The synthetic approach to obtain sulfobetaine is based on the reaction of tertiary amine groups with 1,3-propanesultone (PS) or 1,4-butanedisultone. It is believed that polymers containing an inner salt structure should be more compatible with blood as the zwitterionic groups could substantially maintain normal conformation^[18].

In this study, sulfobetaine was grafted onto PU used hexamethylene diisocyanate (HDI) as spacer arm. The reaction scheme is shown in Fig. 1. The scheme involves the coupling of HDI to the surfaces of PU through an allophanate reaction; the isocyanate groups attached to PU were then coupled through a condensation reaction

* Project of 973 State key Foundation Research and Development (No. 1999064705) sponsored and supported by Ministry of Science and Technology of China.

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Received July 11, 2002; Revised November 3, 2002; Accepted November 7, 2002

with hydroxyl groups of DMEA terminated with tertiary amine groups. Finally, sulfobetaine was grafted onto the PU surface through ring-opening reaction between tertiary amine and PS. Surface properties of PU were characterized by attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) analysis and contact angle measurements. The blood compatibility of PU was evaluated by platelet rich plasma (PRP) contacting experiments and the results were observed by scanning electron microscopy (SEM).

MATERIALS AND METHODS

Materials

The polyurethane employed was Pellethane 2363-80AE (Dow Chemical Co.). Hexamethylene diisocyanate (HDI, Acros) was used without further purification. *N,N*-dimethylethanolamine (DMEA, Aldrich) and 1,3-propanesultone (PS, Fluka) were used as received. Dehydrated toluene and *N,N*-dimethylformamide (DMF) were treated as usual. Di-*n*-butyltin dilaurate (DBTDL) was used as catalyst to promote the reaction between the isocyanate groups and urethane NH and used without further purification. 25% Glutaraldehyde water solution was provided by Shanghai Chemical Reagent Co.

Methods

Preparation of polyurethane films

A poly(ether urethane) membrane with a thickness of 0.3 mm was obtained by solvent casting. The pellets of poly(ether urethane) were dissolved in DMF to form a clear solution (10% *W/V*). The dilute solution was cast onto a polytetrafluoroethylene plate followed by drying in an oven filled with nitrogen at 40°C for at least 48 h to remove most of the solvent and then heated in a vacuum oven at 50°C for another 48 h to remove residual solvent. The films were extracted with methanol for 24 h, followed by vacuum drying at 50°C overnight.

Grafting of sulfobetaine onto PU surfaces

Sulfobetaine was grafted onto PU surfaces in three steps. The first reaction was conducted under N₂ atmosphere. Toluene (20 mL), HDI (2.0 g) and DBTDL (0.010 g) were introduced into the reaction flask. The PU film was totally immersed into the stirred reactive solution for 90 min at 50°C. The films were then rinsed with dry toluene before immersion in a toluene solution containing 10% (*W/V*) DMEA for 24 h at 50°C. After this reaction, the film was washed with dry toluene and immersed in a toluene solution containing 10% (*W/V*) PS for 24 h at 50°C. After each step, the film was washed alternately with toluene and dry acetone and was prepared for ATR-IR spectral characterization. The films were characterized on the air-facing side.

Surface analysis

XPS spectra were obtained on an ESCA spectrometer (Lab MK II V.G. Scientific Co. Ltd. UK) using AlK α radiation. Contact angle measurements were performed at 25°C by the sessile drop method using an instrument (Model 100-0, Rame-Hart Inc. USA). The drying films were weighed and immersed in purified water. After 24 h, the films were taken out of the water, wiped dry with tissue paper, and weighed again immediately. The water contents were calculated using the following formula:

$$\text{Water content (\%)} = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \times 100$$

where W_{wet} and W_{dry} are weights of the wet and dry samples, respectively.

Weight gain (add-on) was calculated from the increase in the weight of the dried original PU films after the chemical modification as follows:

$$\text{Add-on (wt\%)} = \frac{W_2 - W_1}{W_1} \times 100$$

where W_1 and W_2 represent the weight of PU films before and after chemical modification.

Platelet adhesion^[19]

The result of blood platelet adhesion *in vitro* was observed through a scanning electronic microscope (JSM Model 6300, JEOL, Japan). The treated films were rinsed with phosphate buffer saline (PBS) first and contacted at 37°C for 1 h and 3 h with freshly prepared PRP of human blood supplied by the Blood Center of Nanjing Red Cross. Samples were rinsed with PBS and then treated with 2.5% glutaraldehyde for 30 min at room temperature. The samples were washed with PBS again and dehydrated by immersion successively in a series of ethanol-water solutions (50, 60, 70, 80, 90, 95, 100% (V/V)) for 30 min each and allowed to evaporate at room temperature. The platelet-attached surfaces were coated with gold before being observed by SEM. The blank PU film was used as a reference.

RESULTS AND DISCUSSION

The aim of this research is to prepare a new biomaterial grafted with sulfobetaine. Two approaches of synthesis were explored as shown in Fig. 1 and Fig. 2. We have attempted to synthesize sulfobetaine monomer first and couple it with HDI (1:1 molar ratio) and then graft it onto PU surface through allophanate reaction. Sulfobetaine monomer is a highly polar compound and can not be dissolved in most solvents, but slightly in dimethyl sulphoxide at 120°C. Therefore, we chose the first reaction scheme (cf. Fig. 1), *i.e.*, HDI was first grafted onto PU film surfaces (PU-NCO) through allophanate reaction between urethane proton and isocyanate group of HDI in the presence of catalyst. The free isocyanate group remaining on the PU surfaces was then coupled through a condensation reaction to hydroxyl group on DMEA. The tertiary amine of DMEA grafted on PU was reacted with PS and finally sulfobetaine was grafted onto the surface of PU.

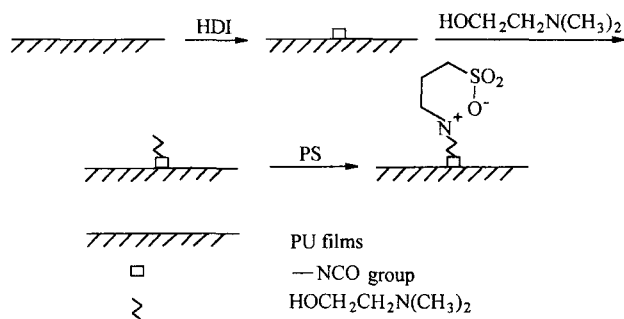


Fig. 1 Reaction scheme for grafting sulfobetaine onto PU surface

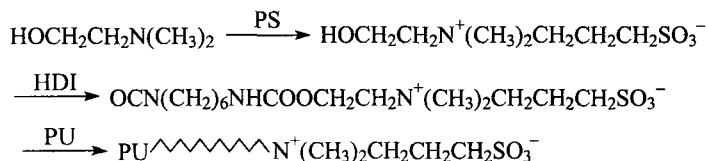


Fig. 2 The other reaction scheme for grafting sulfobetaine onto PU surface

The extent of the reaction was monitored by using ATR-IR. In Fig. 3(a) and 3(b), the ATR-IR spectra of untreated PU and PU grafted with HDI are shown. After reaction with HDI a sharp band at 2270 cm⁻¹, characteristic of NCO groups, appeared in the PU spectrum. The content of the newly formed free NCO groups which can be estimated from the intensity of 2270 cm⁻¹ band changes with the HDI reaction time. The ratio of band intensity of 2270 cm⁻¹ ν_{NCO} against the band intensity of 1600 cm⁻¹ ($\nu_{\text{C=C}}$) in spectrum was calculated, and illustrated in Fig. 4 as a function of the reaction time with HDI. As shown in Fig. 4, a maximum content of free NCO groups appeared after a reaction time of 90 min. The observed decrease in the content of free NCO groups at longer reaction times may be a result of ring formation through dimerization of NCO groups and/or allophanate reaction. After this step, the transparency of films decreased.

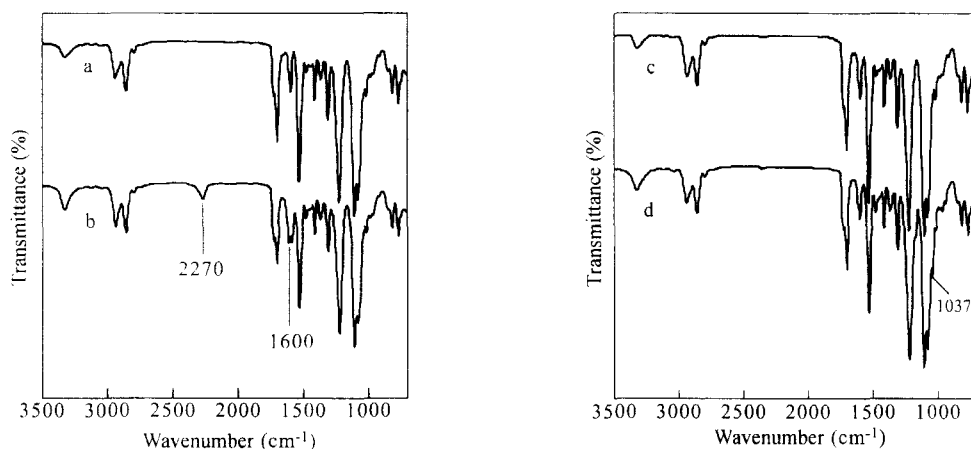


Fig. 3 ATR-IR spectra of (a) pure polyurethane, (b) coupled HDI, (c) coupled DMEA and (d) grafted sulfobetaine

It is worth mentioning that the $\nu_{\text{C}-\text{C}}$ band at 1600 cm^{-1} often splits into two peaks appearing at 1598 cm^{-1} and 1616 cm^{-1} , respectively. Therefore, we can also calculate the ratio of band intensities between 2270 cm^{-1} (NCO) and 1598 cm^{-1} or 1616 cm^{-1} in the spectrum. An attempt has been made to determine reaction time by using these ratios, and the final result was consistent.

In the second reaction step, the hydroxyl group of DMEA was allowed to react with the free NCO groups at the surface. To avoid the occurrence of side reactions, this reaction step was allowed to proceed for 24 h without DBTDL as catalyst. In ATR-IR, the peak at 2270 cm^{-1} disappeared, because to the NCO groups reacted completely with OH groups of DMEA. The C—N bond formed in the grafted DMEA had no characteristic peak, so no spectral evidence was shown in ATR-IR spectra. After this step, the transparency of the films decreased further.

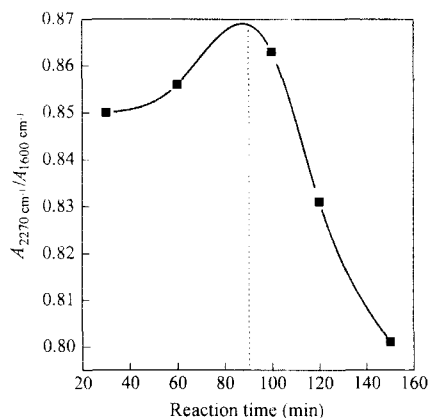


Fig. 4 Extent of reaction of HDI on PU surfaces, measured from ATR-IR spectra as the ratio of the NCO absorption at 2270 cm^{-1} and the phenyl group absorption at 1600 cm^{-1}

In the third step, we aim to construct sulfobetaine on the PU surface. The ring-opening reaction between tertiary amine and PS is the main approach to obtain sulfobetaine. This reaction step was carried out at 50°C for 24 h in order to reach high yield. The sulfobetaine was confirmed by the presence of SO_3^- vibration at 1037 cm^{-1} in the ATR-IR spectrum (Fig. 3d). The films after this step became non-transparent.

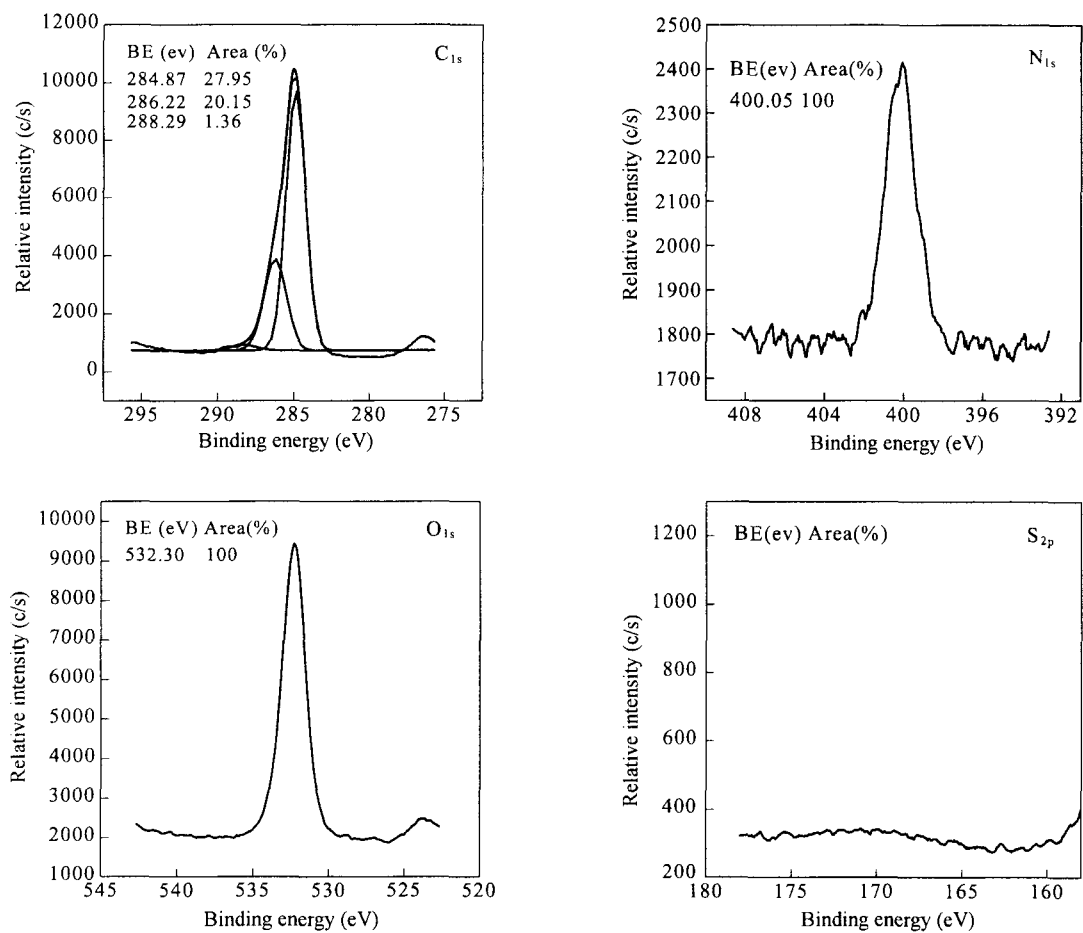


Fig. 5 XPS spectra of blank PU film surface

Analysis

The contact angle measurement results and the water absorption of PU film and grafted PU films are listed in Table 1. The contact angle of treated films decreased greatly in comparison with blank PU films. The water absorption was about 8.2%. In other words, the treated films were more hydrophilic than the original films. Although the reaction involved three steps, the add-on% was as high as 3.3%.

Table 1. Surface properties of PU film and treated sulfobetaine PU films

Materials	Add-on(%)	Water contents (%)	Water contact angle (°)
PU	0	–	78 ± 3
Modified PU	3.3	8.2	63 ± 3

Table 2. Surface elemental composition measured from XPS

Materials	Atomic percentage (%)				Atomic ratio	
	C _{1s}	O _{1s}	N _{1s}	S _{2p}	O _{1s} /C _{1s}	N _{1s} /C _{1s}
PU	76.18	20.57	3.25	0	0.27	0.043
Grafted PU	70.21	25.62	2.99	1.18	0.36	0.043

Table 2 summarizes the atomic percentage of modified surfaces measured by XPS. From the XPS spectra (Figs. 5 and 6), the elemental compositions of the surfaces were calculated; the O/C ratio increased from 0.27 to 0.36. As expected, in Fig. 6, the oxygen content of the modified surfaces increased and the sulfur content of

sulfobetaine was shown to be around 1.18%, resulting from the surface grafting of sulfobetaine chains. The newly appeared peak at a binding energy of 168 eV in Fig. 6(d), which was the characteristic peak of SO_3^- , also confirmed the graft reaction. The N_{1s} peak of PU grafting sulfobetaine consists of two peaks, 400.18 eV for the urethane nitrogen ($\text{N}-\text{COO}$) and 402.22 eV for sulfobetaine ammonium nitrogen $[\text{N}^+(\text{CH}_3)_2\text{CH}_2^-]$. The O_{1s} peak of PU grafting sulfobetaine had a wider binding energy range than that of PU film. It may be the combination of contributions of the ether ($\text{C}-\text{O}-\text{C}$) peak, the urethane oxygen ($\text{N}-\text{COO}$ and $\text{N}-\text{COO}$) and sulfobetaine oxygen ($-\text{SO}_3^-$).

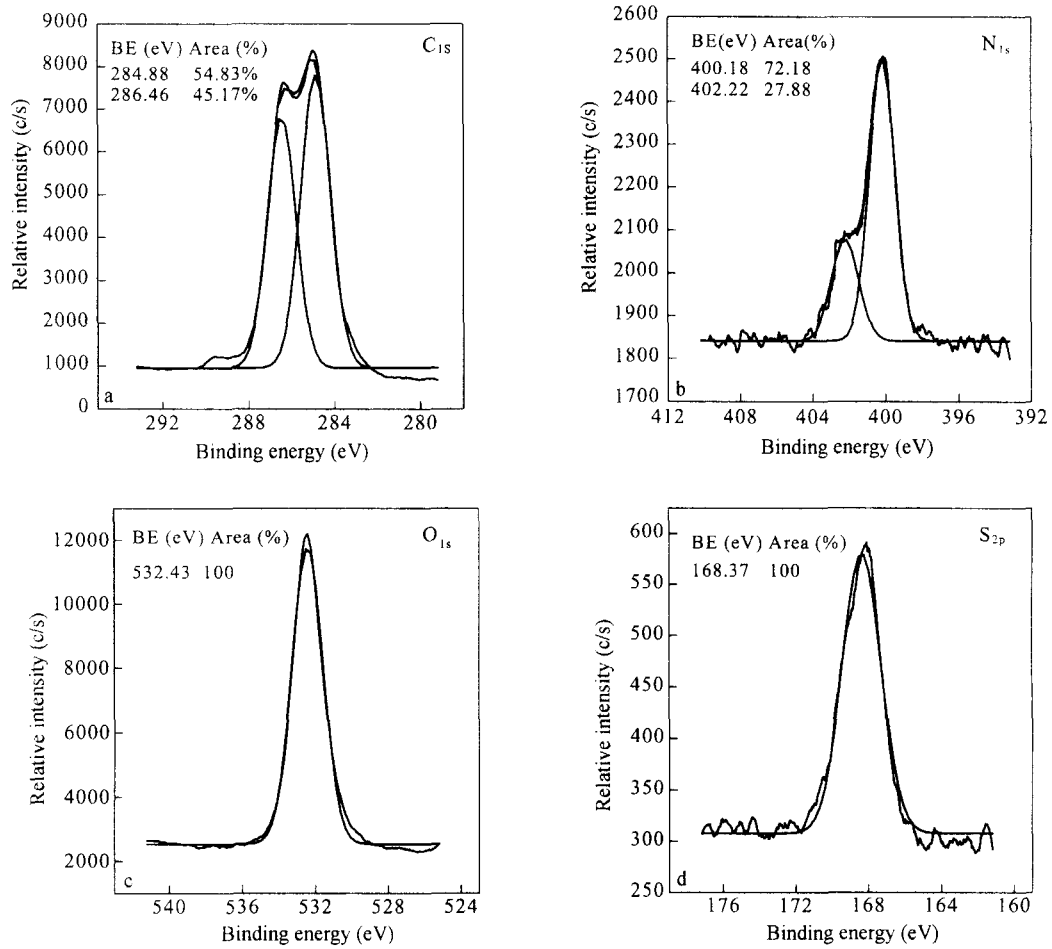


Fig. 6 XPS spectra of grafted PU film surface

Platelet Adhesion

Platelet adhesion results are shown in Fig. 7. PU shows high platelet adhesion, most of the adhered platelets were distorted with pseudopodia. With the longer platelet adhesion time, the number of platelets adhering also increased. However, the surfaces of grafted films have almost no adhering platelets. The platelet adhesion test revealed that films grafted sulfobetaine structure show excellent anti-platelet adhesion. Polymers with sulfobetaine on their surfaces have good antithrombogenicity^[20–22]. Similarly, it is well known that PU with the phosphorylcholine structure on the surface also has good antithrombogenicity. It is considered that the improved antithrombogenicity can be attributed to the zwitterionic structure. In aqueous (blood) medium, the zwitterionic structure molecules not only can not diffuse into the interior of the protein conformation, which is mainly maintained by hydrophobic bonds and hydrogen bonds, to effect the synergetic interaction between main chain and side portions, but also minimize the effect on exterior surface ions of protein, thus contributing to the

maintenance of normal conformation of protein and its assembly^[18]. The results may offer the possibility of use for biomaterial devices, which are in contact with blood.

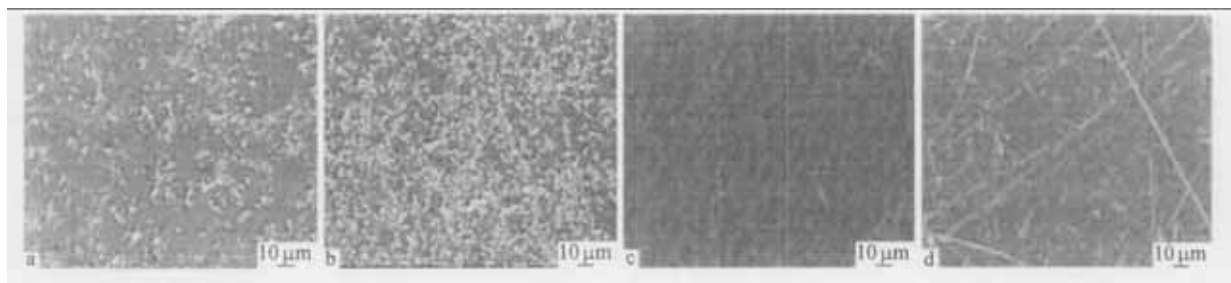


Fig. 7 SEM microphotographs showing the morphologies of platelet rich plasma contacted surfaces
a) PU-1h; b) PU-3h; c) Grafted PU-1h; d) Grafted PU-3h

CONCLUSIONS

In this study, sulfobetaine was successfully grafted onto poly(ether urethane) surface through three reaction steps. Each step was characterized by ATR-IR spectra. The surface composition of films and the hydrophilicity on the poly(ether urethane) surface were investigated by X-ray photoelectron spectroscopy analysis and contact angle measurements, respectively. The blood compatibility of poly(ether urethane) was evaluated by platelet rich plasma contacting experiments and the results were observed by scanning electron microscopy. The platelet adhesion test revealed that films grafting sulfobetaine have excellent blood compatibility.

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