Synthesis and Properties of Phospholipid Polyurethanes with Poly(isoprene) Soft Segment

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SYNOPSIS

Polyurethanes, based on 2-[bis(2-hydroxyethyl)methylammonio]ethylstearylphosphate, alone or together with 1,4-butanediol as the chain extender, poly(isoprene) diol and 4,4'-methylenediphenyl diisocyanate, were prepared. These segmented phospholipid polyurethanes were characterized by IR, elemental analyses, and gel permeation chromatography. The polyurethane, with both phospholipid diol and 1,4-butanediol as the chain extender, was further investigated by differential scanning calorimetry, x-ray diffraction, scanning electron microscopy, plasma contact and clotting time. An x-ray diffraction measurement for the polymer shows a intense scattering at 79.3 Å, corresponding to the length of soft segments, which is hydrophobic poly(isoprene), and a weak diffuse scattering at 5.1 Å, corresponding to the distance between the hydrophobic poly(isoprene) layers. The hemocompatibilities of the polymer were evaluated by platelet rich plasma contacting studies and by scanning electron microscopy using medical grade poly(vinyl chloride) as control. The hot-pressed films of the polymer exhibit a favorable surface in terms of platelet adhesion, and the morphology of adhered platelets undergoes to a relatively lower degree of variation compared to poly(vinyl chloride). Moreover, the clotting time of the polymer in contact with human platelet rich plasma was 220, 100, and 86 s for the phospholipid-based polyurethane, poly(vinyl chloride), and glass, respectively. © 1996 John Wiley & Sons, Inc.

INTRODUCTION

During the past decade, segmented polyurethanes (SPUs) have been widely used for various commercial and experimental blood-contacting applications such as vascular prostheses, endotracheal tubes, pacemaker lead wire insulation, catheters, and artificial hearts due to their generally favorable physical and mechanical properties, together with fairly good biocompatibility and antithrombogenicity characteristics.[1,2] For most cardiovascular products, in which polyurethanes are incorporated as a structural or coating materials, it is essential that the polymers is designed to be not only biocompatible but also stable *in vivo* for a prolonged period.

In order to develop SPUs with biocompatible surfaces, a number of surface modification approaches have been taken. One approach has involved by grafting an alkyl side chain on the poly(ether urethane) backbone, the modified SPUs show a high affinity for albumin adsorption and low platelet reactivity.[3-5] Another approach has involved by introducing long alkyl side chains on to a polyurethane, which has shown to reduce platelet deposition and enhance *in vitro* albumin adsorption.[6-10] More interest and an important approach is that of incorporating phospholipid on to polymers. We have recently developed some new polyurethanes bearing phosphatidylcholine analogs in the main chains[11,12] and in the side chains.[13-15] In addition, segmented phospholipid polyurethaneureas with

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amphiphilic microphase-separated behavior were also synthesized in this laboratory.[16] Our previous studies suggested that phospholipid polyurethanes containing long-chain alkyl groups have excellent hemocompatibilities, and these materials have become very promising candidates for a clinical trial.[15] Some other phospholipid biomaterials have been made either by introducing phosphatidylcholine derivatives as plasticizers into polymers such as poly(vinyl chloride) (PVC) and polyurethane[17] or by copolymerizing phosphatidylcholine monomers into the polymer backbone of polyurethanes and polyesters.[18,19] The phosphatidylcholine polar head groups can be attached on many surfaces by a number of different ways, and large improvements in hemocompatibility have been observed.[20-23]

On the other hand, many approaches to construct polyurethanes with biostability have also been performed. Among them, perhaps the most popular polyurethanes have been segmented poly(ether urethanes), and which have long been considered to have good biostability.[24-26] However, recent investigations have revealed that polyurethanes are subject to significant degradation under certain specific conditions of mechanical or chemical action of implanted devices.[27-30] One of the major causes of degradation is oxidation of the polyether chain. To develop polyurethane biomaterials that are more stable *in vivo,* it may be desirable to prepare SPUs without ether-containing polydiols. Cooper and coworkers[31] used several nonether polydiols such as poly(butadiene), hydrogenated poly(butadiene), and poly(dimethylsiloxane) chain segments to synthesize SPUs. They have demonstrated that the SPUs based on these hydrophobic polydiols showed distinct microphase separation between hard and soft segments. It was concluded that polyurethanes with aliphatic hydrocarbon-based polyol components are stable against oxidative degradation. Moreover, recent investigation shows that SPUs that do not contain the ether linkage in the polyol component do not inhibit metabolic cooperation, and might be less prone to cause tumor formation than polyether-based polyurethane.[32]

Recently, we have successfully synthesized a new phospholipid polyurethane with excellent hemocompatibility and mechanical strength, which is composed of soft segments derived from hydrogenated poly(isoprene) diol and hard segments from 4,4′-methylenediphenyl diisocyanate (MDI), 2-[bis-(2-hydroxyethyl)methylammonio]ethylstearylphosphate (BESP) and 1,4-butanediol (BD).[33] The present article reports the synthesis and characterization of phospholipid polyurethanes with poly(isoprene) soft segment. The characterization of the polymers was based on their IR spectra, elemental analysis, gel permeation chromatography (GPC), differential scanning calorimetry (DSC), and x-ray diffraction measurements. Before characterizing the polymer stabilities in the biological environment, the phospholipid SPU was evaluated by platelet rich plasma (PRP) contacting experiments and by scanning electric microscopy (SEM) observation to obtain a blood-contacting response of the SPU. The hemocompatibility of the SPU was evaluated by describing the platelet state and shape variation for the attached platelets, as well as discussing the results of clotting time measurements.

EXPERIMENTAL

Materials

Poly(isoprene) diol (PIP) with a number average molecular weight of 2520 and 60% of 1,4-structure, 25% of 3,4-structure and 15% of 1,2-structure components was kindly provided by Idemitsu Petrochemical Co. Ltd. Japan. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride to ensure dryness. *N,N*-Dimethylacetamide (DMAc) was dehydrated over calcium hydride for 2 days and then vacuum distilled. Methanol was distilled in the presence of magnesium methoxide to ensure dryness; BD and MDI were commercially obtained and purified by vacuum distillation. All purified solvents were dried over Molecular Sieves 4A (Wako Pure Chemical Industries, Ltd. Japan) and other solvents were of the best commercial grade and used as received, unless otherwise noted. BESP was synthesized by a previously reported method.[15]

Synthesis of Phospholipid SPUs

Two phospholipid SPUs were synthesized according to a three-step addition polymerization procedure outlined in Figure 1 and Table I, which is similar to a conventional two-step solution polymerization procedure under a nitrogen atmosphere.[34]

Polymer SPU**1** was based on 1 : 3 : 2 molar ratio of PIP : MDI : BESP and the reaction was carried out in a 1 : 1 mixture of THF : DMAc without a catalyst. In the first step, 1.19 g of MDI dissolved in 10 mL of the mixed solvent was added to a stirred solution of 4.0 g of PIP and 10 mL of the same mixed solvent under a dry nitrogen atmosphere. After 1 h

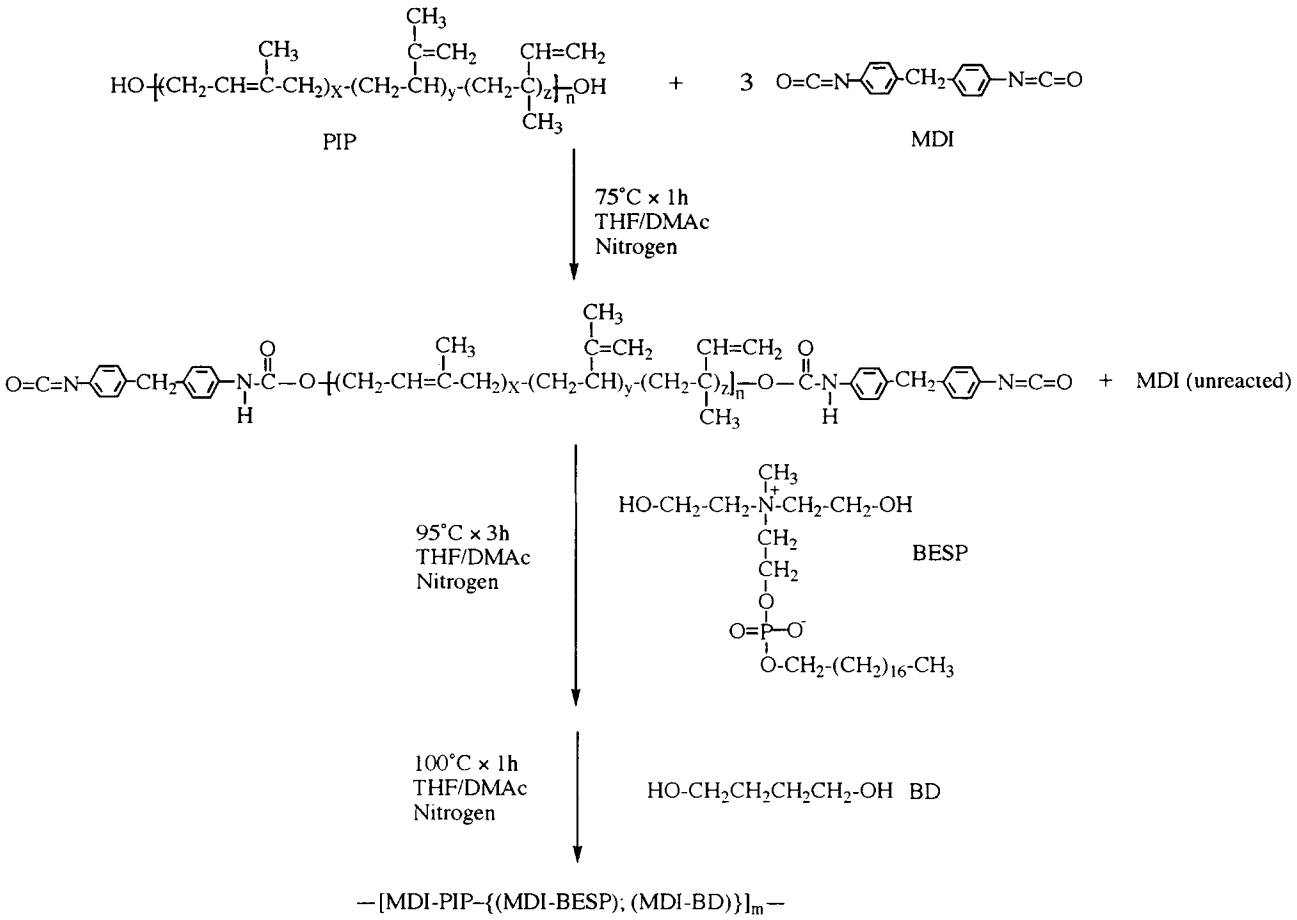

Figure 1 Three-step synthesis of phospholipid SPU.

at 70–75°C the solution was cooled to room temperature slowly. In the second step, 1.574 g of BESP, which was previously dissolved in 15 mL of the mixed solvent, was slowly added into the reaction solution over a 20-min period. Stirring was continued at 90–95°C for 3 h. Finally, the solution was stirred at 100°C for 1 h. The resulting phospholipid polymer was precipitated in methanol. Then the polymer was washed with methanol and the washing procedure was performed three additional times with methanol. The polymer was dried in a vacuum oven at 70°C for at least 48 h. The polymer SPU**1** was obtained in 83% yield as a pale yellow elastomer.

Polymer SPU**2** was based on 1 : 3 : 1 : 1 molar ratio of PIP : MDI : BESP : BD and the reaction was performed by a procedure similar to that described for SPU**1**. Except that at last step, 0.143 g of BD, which was previously dissolved in 15 mL of the same solvent, was added dropwise over another 10-min period to the reaction mixture with stirring. The stirring was then performed at 100°C for 1 h. The SPU**2** was obtained in 88% yield as a pale yellow elastomer.

The characterization of the phospholipid SPUs was based on their IR spectral data, elemental analysis, and GPC measurements, the results are summarized in Table II and Figure 2.

Preparation of Polyurethane Films

In order to obtain a solution suitable for film casting, after briefly drying under vacuum, the resulting SPUs were dissolved in mixed DMAc and THF (volume ratio 1/1) solution by ultrasonic generator. The SPU solutions obtained were defoamed by evaporating in a desiccator and then cast onto glass plates to give films for bulk property measurements. The casting films were first dried in an oven at 70°C under a nitrogen flow for at least 48 h to remove most of the solvents. The final drying stage involved drying the sheet in a vacuum oven at 70°C for at least 48 h.

Table I Synthesis of Phospholipid SPUs[a]

Polymers	First Step			Second Step		Third Step			Yield (%)
	PIP/ Solvent (g/mL)	MDI/ Solvent (g/mL)	Reaction Temperature and Time	BESP/ Solvent (g/mL)	Reaction Temperature and Time	BD/ Solvent (g/mL)	Reaction Temperature and Time	Precipitating Solvent	
SPU**1** (PIP : MDI : BESP)	4.00/10	1.19/10	75°C × 1 h	1.574/15	95°C × 3 h	—	100°C × 1 h	Methanol	83
SPU**2** (PIP : MDI : BESP : BD)	4.00/10	1.19/10	75°C × 1 h	0.787/15	95°C × 3 h	0.143/15	100°C × 1 h	Methanol	88

[a] SPUs: Segmented polyurethanes; PIP: Poly(isoprene) diol; MDI: 4,4'-Methylenediphenyl diisocyanate; BESP: 2-[Bis(2-hydroxyethyl)methylammonio]ethylstearylphosphate; BD: 1,4-Butanediol; Reaction solvent was a mixture of THF: DMAc with volume ratio of 1 : 1. For SPU**1**, on the third step, only the reaction temperature was increased from 95 to 100°C for 1 h but without adding the BD.

Preparation of Hot-Pressed Films

The phospholipid SPU**2** film was placed between two thin polyimide sheets and subjected the materials to a pressure of 100 kg cm^{-2} and a temperature of 150–170°C for 15 min in a mechanical hot press. After cooling and removing the polyimide sheets, SPU**2** gave successful hot-pressed films.

Measurements

GPC measurements were carried out on a HLC802UR GPC instrument with G4000H8 + G2000H8 column; the samples were dissolved in a 1 : 1 mixture of THF : DMAc and polystyrenes were used as standard. IR spectra were recorded on a Jasco A 202 spectrometer. Elemental analysis was performed by Osaka Gas Co. Ltd. (Osaka, Japan). The thermogram was recorded by using a DSC unit (Rigaku thermoflex apparatus DSC-8230B). The sample quantity was 5 mg. Rate of heating was 10°C min^{-1}. An x-ray film diagram was photographed with nickel-filtered CuK_{α} radiation (37.5 kV, 20 mA), using a flat-plate camera of 7.21 cm passage at room temperature. Blood platelet attachment *in vitro* was viewed by SEM: the hot-pressed films of the polymer SPU**2** were washed with saline and incubated at 37°C for 60 min with freshly prepared PRP, which was obtained from the centrifugation of rabbit blood at 1000 rpm for 20 min. The sample was rinsed with saline and treated with 2.5% glutaraldehyde in saline at refrigerated temperatures overnight. The sample was rinsed with saline and dehydrated by systematic immersion in a series of ethanol–water solutions; 60, 70, 80, 90, and 100% v/v. Following critical point drying with carbon dioxide, the sample was coated with gold prior to being observed in a JEOL Hitachi S-2500 SEM operating at an accelerating voltage of 15 or 20 kV. The medical grade PVC (Showa Kasei, Japan, GH-85) was used as control. For clotting time measurements: 10% of phospholipid SPU solution in a 1 : 1 mixture of THF : DMAc were poured into glass vials, after the solution was remained in the vials at 23°C overnight, the excess solutions were poured out, inner surface coated vials were then dried at 70°C overnight under nitrogen atmosphere, following another 30 h drying at 60°C under vacuum. The glass vial coated with 10% of PVC solution in THF and vial without coating were used as control experiments. The fresh PRP was prepared by mixing 8.1 mL of the human blood and 0.9 mL of 3.13% sodium citrate solution, following centrifugation at 1000 rpm for 20 min. The vials were washed with

saline and incubated at 37°C for 10 min. Then, 0.1 *N* calcium chloride solution was added into the vials and clotting time was measured.

RESULTS AND DISCUSSION

Characterization of Phospholipid SPUs

Bulk property characterization data are summarized in Table II. For phospholipid SPU**1**, the stoichiometry of the reaction was 1 : 3 : 2 of PIP : MDI : BESP. The hard segment was composed of MDI and BESP and the designed hard segment was 40.9%; the stoichiometric ratio was 1 : 3 : 1 : 1 (PIP : MDI : BESP : BD) for SPU**2**, with the hard segment (MDI, BESP, and BD) at 34.6%. Moreover, the incorporation of BD in the hard segment has obviously improved the mechanical property, which was observed during the processing of films.

The IR spectra measurements of the polymers were performed on films. Both phospholipid SPUs were related with the inclusion of PIP, MDI, and BESP. This is clear from the IR spectra of both materials (Fig. 2), which show adsorption bands due to —NH— band at 3300 cm^{-1}, —NHCOO— band at 1710 cm^{-1}, —CONH— band at 1540 cm^{-1}, $-CH_2-$ band at 2900, 2840, and 1455 cm^{-1}, aromatic linkage at 1590 cm^{-1}, and P=O at 1230 and $P-O-CH_2-$ at 1060 cm^{-1}, respectively.

The molecular weights of the polymers were characterized by GPC based on polystyrene standard. From the relationship between retention time and molecular weights of standard polystyrenes, the weight average molecular weights of SPU**1** and SPU**2** were calculated as 32,000 and 41,000, respectively. These molecular weights are sufficient for most biomedical applications.

The elemental analysis data were in good agreement with the stoichiometric ratios of PIP : MDI : BESP for SPU**1** and of PIP : MDI : BESP : BD for SPU**2**. This confirmed the proper incorporation of phospholipid groups on the control polyurethane samples.

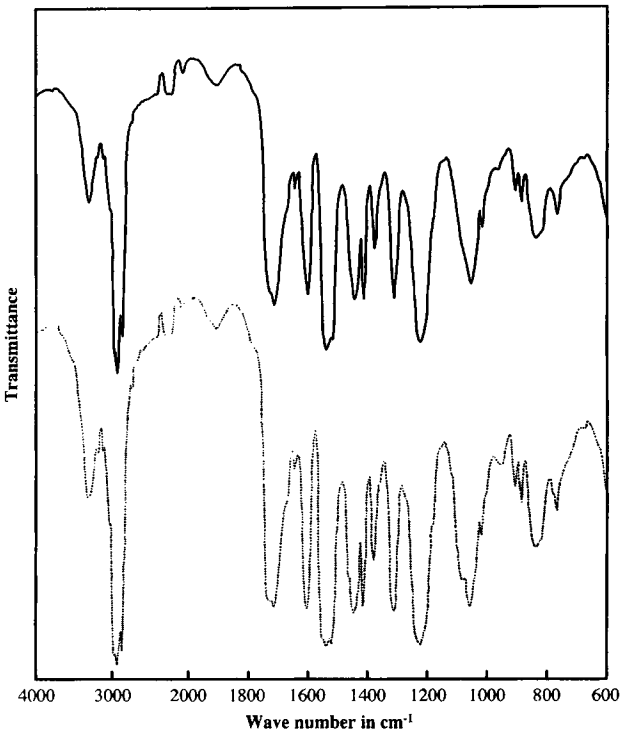

Figure 2 IR spectra of the phospholipid SPUs. SPU**1**(——); SPU**2** (· · · · ·).

Thermal Property

To further investigate the properties and prospective applications of the phospholipid SPUs, we examined SPU**2** further. The DSC thermal transition data were obtained from the first heating of the sample (Fig. 3). The glass transition temperature (T_g) of the polymer was observed near −16°C. Two endotherms occurring at 117.9°C and 203.7°C were also observed. Moreover, when heated to 250°C, the sample was melted, along with the color change of the sample from pale yellow to brown.

Table II Characterization of the Phospholipid SPUs

Polymers	Stoichiometry PIP : MDI : BESP : BD	Hard Segment (%)	Elemental Analysis[a] C	H	N	M_W[b]
SPU**1**	1 : 3 : 2 : 0	40.9	78.3 (78.8)	10.4 (10.2)	2.8 (2.6)	32000
SPU**2**	1 : 3 : 1 : 1	34.6	80.4 (80.6)	10.0 (10.2)	2.7 (2.5)	41000

[a] Values in parentheses are calculated data.
[b] M_W means the weight average molecular weight which was determined by GPC using the polystyrene as standard.

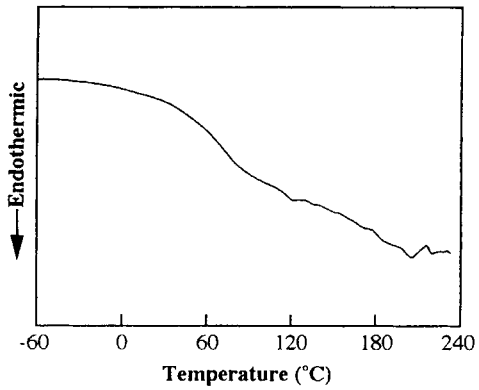

Figure 3 DSC thermogram of the phospholipid SPU2.

X-Ray Diffraction Analysis

An x-ray diffraction analysis for the cast film of SPU2 was carried out with nickel filtered CuK_α radiation. Figure 4 shows the x-ray diffraction pattern of the film. As can be seen from the figure, a ring with strong intensity in the small angle region, together with a weak diffuse scattering in the wide angle region was observed. The 79.3 Å of intense scattering in the small angle region corresponded to the length of soft segment (hydrophobic PIP). This indicates that the PIP hydrophobic layer may be arranged like random coil, because the theoretical length of hydrophobic PIP layer was estimated to be 140.2 Å. On the other hand, the dimension of the weak diffuse scattering in the wide angle region is 5.1 Å. This value should be the distance between two hydrophobic PIP layers, based on our earlier report.[35]

Hemocompatibility Evaluation

The phospholipid SPUs were assessed as biomaterials mainly by the degree and nature of blood platelet adhesion resulting from exposure to PRP. The SPU2 hot-pressed film was exposed to PRP for 60 min and treated for SEM observation. The medical grade PVC was used as control. The typical SEM photographs of the surfaces for the PVC and SPU2 hot-pressed films are shown in Figure 5.

The surface of SPU2 showed some adhered platelets but the very limited shape variation of the adhered platelets. However, medical grade PVC exhibited not only a large number of adhered platelets, but also with larger shape variation of the adhered platelets. Based on the SEM observation, the number of adhered platelets in a area of 10 μm × 10 μm was 1.2 and 3.3 for phospholipid SPU2 and PVC, respectively. Although the shape variation of adhered platelets may affect the accuracy of the estimated number of adhered platelets, it is apparent that the trend of hemocompatibility of the phospholipid SPU2 is better than that of PVC. A further investigation on the clotting time of SPU2 shows the similar results. The clotting time of the materials in contact with human PRP was 220, 100, and 86 s for phospholipid SPU2, PVC, and glass, respectively. This result is consistent with the SEM observation. In addition, preliminary studies show that phospholipid polymer SPU2 film with better mechanical properties compared to SPU1 film, the detail of these studies is still in progress.

CONCLUSION

New phospholipid segmented polyurethanes using poly(isoprene) as the soft segment have been synthesized. The preliminary results suggest that the poly(isoprene) based phospholipid polyurethanes may be regarded as hopeful biomaterials for their

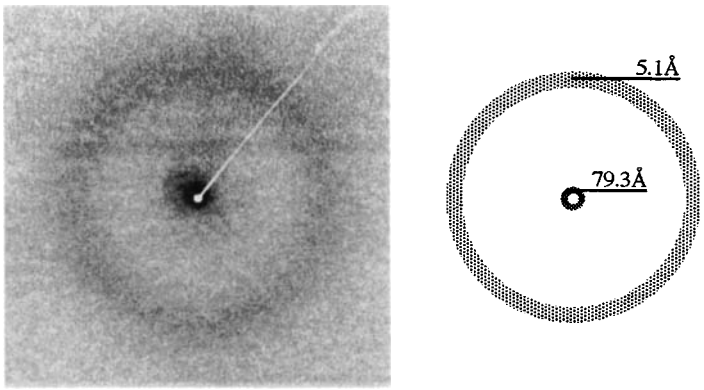

Figure 4 X-ray diffraction pattern of the phospholipid SPU2 obtained with a flat camera by Ni-filtered x-rays of CuK_α (camera length 7.21 cm).

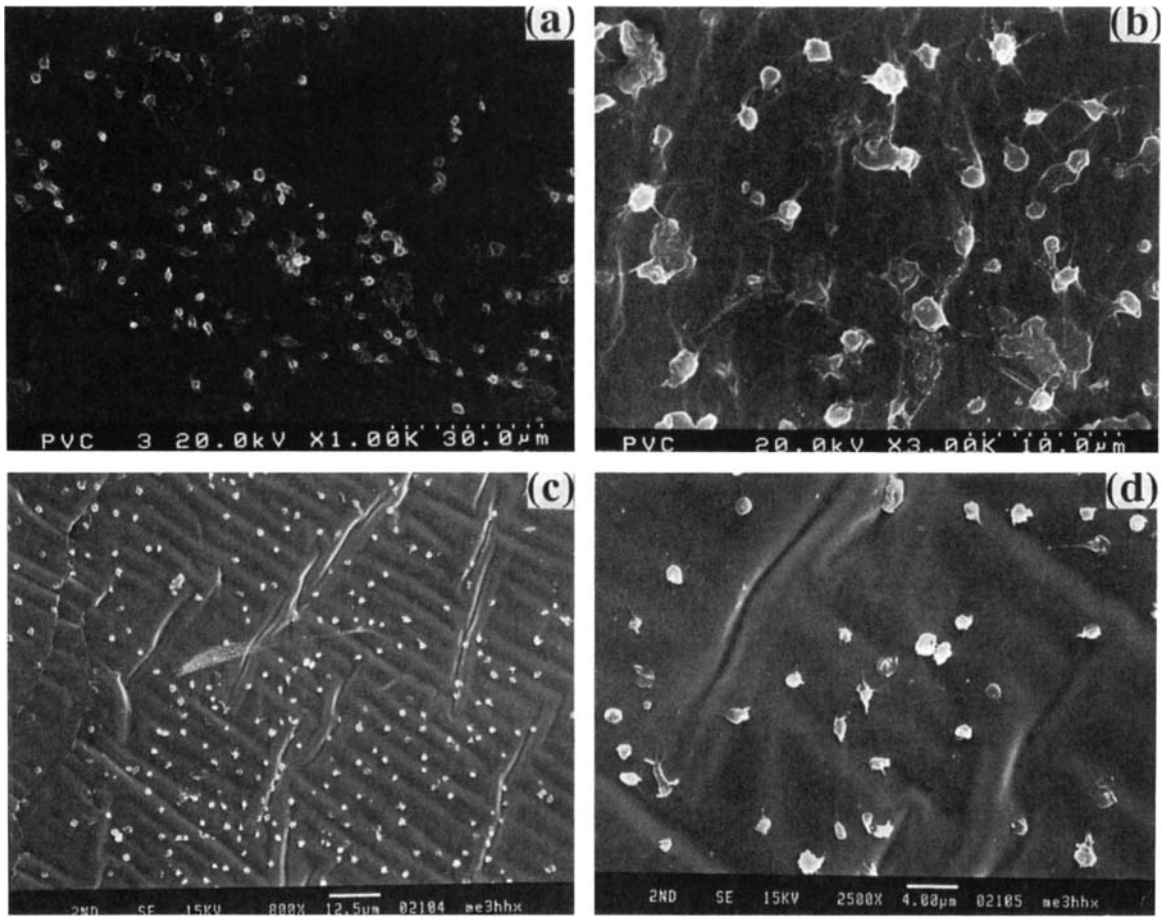

Figure 5 SEM photographs of the surfaces of the medical grade PVC and phospholipid SPU**2** hot-pressed films after 60 min of rabbit PRP exposure. (a) and (b) for PVC; (c) and (d) for SPU**2**. Imaged at original 1000, 3000, 800, and 2500 magnifications for (a)–(d), respectively.

favorable blood compatibilities. This new polyurethane may be useful in biomedical applications such as heart valves, vascular prostheses, pacemaker lead wire insulation, and catheters, etc.

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