Trans. Mat. Res. Soc. Japan 39[4] 411-414 (2014)

Synthesis of Biocompatible Elastic Polyurethane Containing Phospholipid Moiety

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The preparations of segmented polyurethanes containing phosphorylcholine (PC) group and polycarbonate segment were carried out by polyaddition of PC-containing diol monomer, 2-(3,5-bis(2-hydroloxyethoxy)benzoyloxy)ethyl phosphorylcholine, and polycarbonate diol with 4,4'-diphenylmethane diisocyanate. Then, the solubility, the surface property, the blood compatibility and the mechanical strength of the obtained polyurethanes were investigated to reveal the effect of the PC content on the physical and biological properties of polymer films. As a result, the obtained polyurethanes showed the high thermal stability and the elastic property. On the other hand, ultra-thin films (nanosheets) obtained from segmented polyurethanes showed a high adhesive strength and a tendency of shrinking. Furthermore, it was found that the nanosheets-coated surface prevented the adhesion and the activation of platelets.
Key words: biomaterial / polyurethane / elastomer / phosphorylcholine / nanosheet

1. INTRODUCTION

Most of devices used in a medical field are made from polymer materials, such as polyethylene and poly(vinyl chloride), etc. The biocompatibility with the living body is necessary if the materials are used as an implantable artificial organ. Therefore, the development of the materials, which are continuously showing a stable biocompatibility during the long-term use, is desired for the medical devices. In particular, segmented polyurethane (SPU) has been investigated as a biomaterial, due to its favorable physical properties, chemical inertness and biocompatibility [1-3]. The biocompatibility of SPU is thought to arise from the microphase separation of the soft and hard segments, which also derived the elasticity. On the other hand, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer has been developed by Ishihara *et al.* as an excellent biocompatible material, which efficiently reduces the adhesion of cells and proteins on the polymer surface by the effect of the phosphorylcholine (PC) group [4-7]. Ishihara *et al.* have also investigated the IPN composite consisting of segmented polyurethane and MPC polymer, in order to reduce the protein adsorption on to the polymer surface and to improve the biocompatibility of segmented polyurethane [8].

In the recent years, we have investigated the syntheses of novel diamine and diol monomers containing phosphorylcholine (PC) group to prepare the polycondensation- or polyaddition-type polymers which are thermally stable and mechanically strong [9-13]. From the diol monomer, the preparations of polyurethanes and poly(ester-urethane)s containing PC group have been carried out, and it has been found that these polymers exhibited the good biocompatibility and elasticity [11]. However, the solubility of polyurethanes was decreased as the PC content increased, which would be due to the strong interaction between the polar urethane bond and the PC unit. Therefore, in this study, we tried to synthesize the soluble polyurethane having the higher PC content than that of previous polymer, because the higher content of PC unit was expected to derive the higher biocompatibility [11]. Then, the film-forming ability and the physical properties of the obtained polymers were investigated in detail.

By the way, the nanosheet is an ultrathin film consisting of polymers with a thickness less than hundred nm, and known to show the unique properties such as high adhesive strength, flexibility and smoothness of the surface [13]. Then, we attempted to prepare the nanosheets from the obtained polyurethanes and to investigate the physical properties and the biocompatibility of the nanosheet surface.

2. EXPERIMENTAL

2.1 Materials

PC-containing diol monomer, 2-(3,5-bis(2-hydroloxyethoxy)benzoyloxy)ethyl phosphorylcholine (BHPC), was synthesized according to the procedure described in our previous paper [11]. 4,4'-Diphenylmethane diisocyanate (MDI) and poly(carbonate diol) (PCD, *M*n = 1,000, m = 6) were kindly supplied from Nippon Polyurethane Industry Co. Ltd. and Asahi Kasei Corporation, respectively. Other chemical reagents were used without further purification. Bionate®, a commercially available poly(carbonate-urethane), was kindly supplied by Asahi Kasei Corporation as a reference sample for the mechanical property.

2.2 Syntheses of polyurethanes containing PC group (SPUPC-1, SPUPC-2 and SPUPC-3)

Under an argon atmosphere, BHPC (1.63 g, 3.62 mmol), MDI (2.26 g, 9.04 mmol) and PCD (5.42 g, 5.42 mmol) were dissolved in 16.0 mL, 15.0 mL and 6.00 mL of DMSO, respectively. Then, the BHPC solution and the MDI solution were mixed and stirred at 70 °C for 15 min. Then, the PCD solution were added in the mixture and stirred at 70 °C for 1 h. The mixture was poured into excess methanol to precipitate the polymer, and it was purified by reprecipitation from its DMF solution to excess methanol. Finally, the product was dried *in vacuo*

to obtain SPUPC-2 as a brown solid. Yield: 6.35 g (67.7 %).
^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 1.29 (-$OCH_2CH_2C\underline{H_2}C\underline{H_2}CH_2CH_2OCO$-, m), 1.55 (-$OCH_2C\underline{H_2}CH_2CH_2C\underline{H_2}CH_2OCO$-, m), 3.07 (-$N^+(C\underline{H_3})_3$, s), 3.55 (-$POCH_2C\underline{H_2}N$-, m), 3.76 (-$PhC\underline{H_2}Ph$-, s), 4.02 (-$OC\underline{H_2}CH_2OPh$-, -$OC\underline{H_2}CH_2CH_2CH_2CH_2C\underline{H_2}$-OCO-, m), 4.15 (-$COOCH_2C\underline{H_2}OP$-, m), 4.25 (-$PO\text{-}C\underline{H_2}CH_2$-N, m), 4.37 (-$OCH_2C\underline{H_2}OPh$-, -$COO\text{-}C\underline{H_2}CH_2OP$-, m), 6.86 (-Ph-, m), 7.07 (-Ph-, m), 7.11 (-Ph-, m), 7.33 (-Ph-, m), 9.47 (-N$\underline{H}$COO-, s), 9.68 (-N$\underline{H}$COO-, s).

SPUPC-1 and SPUPC-3 were synthesized by the same procedure as described in our previous report [11].

2.3 Syntheses of polyurethane (SPU-1 and SPU-2)

Under an argon atmosphere, 3,5-bis(2-hydroloxyethoxy)benzene (BHE, 0.317 g, 1.60 mmol), MDI (1.00 g, 4.00 mmol) and PCD (2.40 g, 2.40 mmol) were dissolved in 1.6 mL, 12 mL and 2.4 mL of DMSO, respectively. Then, the MDI solution and the PCD solution were mixed and stirred at 70 °C for 2 h. The BHE solution were added in the mixture and stirred at 70 °C for 1 h. The mixture was poured into excess methanol to precipitate the polymer, and it was purified by reprecipitation from its DMF solution to excess methanol. Finally, the product was dried *in vacuo* to obtain SPU-2 as a white solid. Yield: 2.59 g (71.7 %).
^{1}H-NMR, δ (400 MHz, DMSO-d_6, ppm): 1.28 (-$OCH_2CH_2C\underline{H_2}C\underline{H_2}CH_2CH_2OCO$-, m), 1.55 (-$OCH_2C\underline{H_2}CH_2CH_2C\underline{H_2}CH_2OCO$-, m), 3.76 (-$PhC\underline{H_2}Ph$-, s), 4.00 (-$OC\underline{H_2}CH_2OPh$-, -$OC\underline{H_2}CH_2CH_2CH_2CH_2C\underline{H_2}OCO$-, m), 4.17 (-$COOCH_2C\underline{H_2}OP$-, m), 4.36 (-$OCH_2C\underline{H_2}OPh$-, m), 6.52 (-Ph-, m), 7.05 (-Ph-, m), 7.32 (-Ph-, m), 9.46 (-N$\underline{H}$COO-, s), 9.68 (-N$\underline{H}$COO-, s).

SPU-1 was synthesized by the same procedure as above fixing the different molar ratio of BHE and PCD as shown in Table 1.

2.4 Characterizations

^{1}H-NMR spectra were conducted with a JEOL NM-TH5SK 400MHz FT-NMR spectrometer, and chemical shifts were estimated in units of ppm with tetramethylsilane (TMS) as an internal standard. The molecular weights of polymers were estimated with the two gel permeation chromatography (GPC) systems. One of them is a Tosoh HLC-8320 equipped with three columns of TSK gels, Super Multipore HZ-H using THF as an eluent. The second is a Tosoh GPC system equipped with four columns of TSK gels, Multipore HXL-M, and RI detector of RI-8010 using DMF as an eluent. The average molecular weights were calibrated based on polystyrene standards.

Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) were carried out on Seiko Instruments DSC-6200 and TG/DTA-6200, respectively, at a heating rate of 10 °C/min under a nitrogen atmosphere.

2.5 Preparation of polymer films

The synthesized polymers were dissolved in DMF, and the solution was poured on a Teflon sheet. The solvent was removed at 50 °C for 3 days. The obtained films were then dried *in vacuo* at 80 °C for overnight, and the self-standing films were obtained.

2.6 Measurements of stress-strain behavior

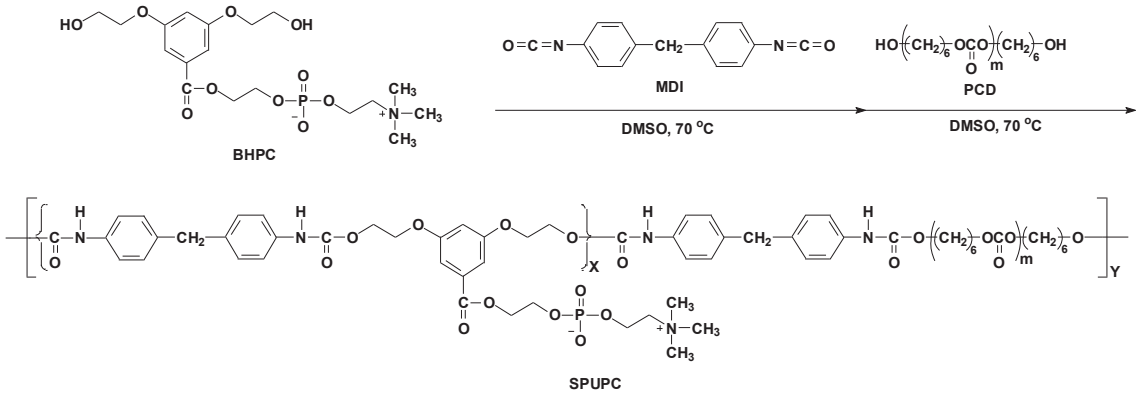
The polymer films were cut into rectangular strips with a length of 40 mm, a width of 10 mm and a thickness of *ca.* 0.10 mm. Stress-strain curves were obtained on a JSV-H1000L equipped with HF-10 (Japan Instrumentation System Co., Ltd), where the gauge length was 20 mm and the crosshead speed was 0.2 mm sec^{-1}.

2.7 Fabrication of polymer nanosheets

SPUPC-1 and SPUPC-2 were dissolved in chloroform and DMSO, respectively. Preparations of polymer nanosheets were carried out by the method of Okamura *et al.* [13], and the self-standing nanosheets were obtained. Measurements of thickness and adhesive strength were carried out by Kosaka Laboratory Ltd ET200 and scratch tester for thin films (model CSR-2000, Rhesca Co., Tokyo, Japan) , respectively.

2.8 Platelet adhesion test

The obtained polymer nanosheets were coated on the circle PET plates. The nanosheet-coated PET plates were immersed in phosphate buffered solution (PBS) for overnight and in 500 μL of human platelet-rich plasma (PRP) prepared from healthy donor. Then, the films were incubated for 2 h at 37 °C, and PRP was removed and the plates were washed with PBS. The surfaces of the plates were observed by a scanning electron



Scheme 1 Synthesis of polyurethane containing PC group.

Table 1 Compositions and molecular weights of polymers.

Code	Molar ratio of diol monomers BHPC/BHE/PCD[a)]	PC content[b)] (mol %)	(wt.%)	$M_n \times 10^{-3}$	M_w/M_n
SPUPC-1	30/00/70	33.8	22.3	13.6[c)]	1.69[c)]
SPUPC-2	40/00/60	41.2	28.2	44.3[d)]	4.25[d)]
SPUPC-3	50/00/50	-	-	-	-
SPU-1	00/30/70	0	0	25.0[c)]	1.69[c)]
SPU-2	00/40/60	0	0	16.6[d)]	1.89[d)]

a) PCD: Polycarbonatediol (*Mn*=*c.a.*1000), BHE: 1,3-Bis(2-hydroxyethoxy)benzene, BHPC: 2-(3,5-Bis(2-hydroxyethoxy)benzoyloxy)ethyl phosphorylcholine
b) PC content was calculated from the ratio of peak intensities in H-^{1}NMR spectra.
c) Number-average and Weight-average molecular weights (*M*w and *M*n) were estimated by GPC using THF as eluent.
d) *M*w and *M*n were estimated by GPC using DMF as eluent.

Table 2 Solubility of polymers.

Code	Solubility [a)]							
	Water	Ethanol	Chloroform	THF	NMP	DMAc	DMF	DMSO
SPUPC-1	×	×	○	△	○	○	○	○
SPUPC-2	×	×	×	×	○	○	○	○
SPUPC-3	×	×	×	×	△	△	△	△
SPU-1	×	×	○	○	○	○	○	○
SPU-2	×	×	○	○	○	○	○	○

a) ○: Soluble, △: Partially soluble, ×: Insoluble (10 mg/mL).

microscope (SEM) using FE-SEM S-4800, Hitachi High-technologies, Co., Tokyo, Japan, where the acceleration voltage was 3 kV and the magnification was 2000-folds.

3. RESULTS AND DISCUSSION

3.1 Preparations of segmented polyurethanes containing PC group

As shown in Scheme 1, the syntheses of segmented polyurethanes containing PC group and polycarbonate segment with different contents (SPUPC-1, SPUPC-2 and SPUPC-3) were carried out by polyaddition of BHPC and PCD with MDI. On the other hand, segmented polyurethanes without PC group (SPU-1 and SPU-2) were prepared from BHE instead of BHPC to compare the physical properties with the polyurethanes containing PC group.

The compositions and the molecular weights of the obtained polymers are summarized in Table 1. The chemical structures of these polymers were confirmed by ^{1}H-NMR. The contents of PC group in these polymers were determined from the ratio of the peak intensities of the ammonium protons (3.07 ppm) in the PC unit and the methylene protons (3.76 ppm) in the diphenylmethane unit of the MDI component. The observed PC content in mol % was in good agreement with the molar ratio of BHPC and PCD in the polymerization. SPUPC-2 (BHPC: 40 mol%) was first

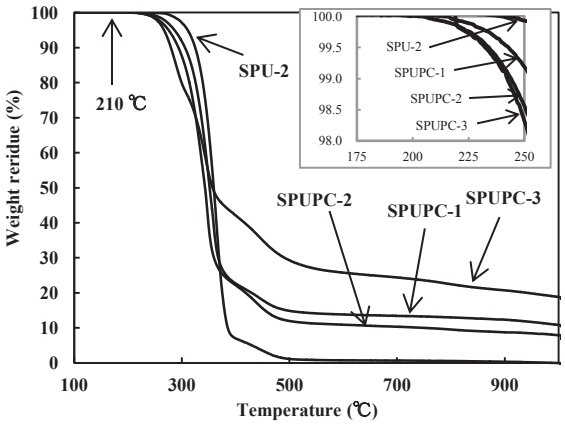


Fig. 1 TGA curves of polymers.

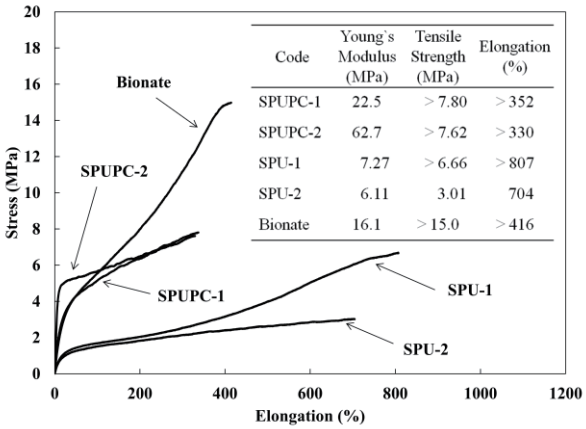


Fig. 2 Stress-strain behavior of polymer films.

synthesized as an intermediate composition between SPUPC-1 and 3, which were already reported in our previous paper [11].

Then, the obtained SPUPC-2 containing 41.2 mol% of PC unit was completely soluble in aprotic polar solvents such as DMF, NMP and DMSO, whereas SPUPC-3 was partially soluble in these solvents. Therefore, it was confirmed that the solubility of polyurethanes was decreased as the PC content increased, and the limited PC unit for the solubility in this copolymer system was almost 40 mol%.

3.2 Thermal property of polymers

The thermal property of the obtained polyurethanes was investigated by DSC and TGA. From the results of TGA measurements, it was found that the weight loss of all of the SPUPC series started at *ca*. 210 °C, whereas that of SPU-2 started at nearly 300 °C, as shown in Fig. 1. Therefore, the thermal degradation of PC-containing polyurethanes would be initiated by the degradation of PC component. This tendency was similar to the case of other PC-containing polymers [9-12]. The heat resistance of PC-containing polymers until 200 °C would be enough for the thermal sterilization process over 150 °C.

In the DSC thermograms, the glass transition temperature (*T*g) was observed for all of the polymers at around -30 °C. It is considered that the observed *T*g would be derived from the glass transition of polycarbonate soft segment. Actually, *T*g of PCD was confirmed at -20 °C by the measurement of DSC. The other transition and the melting were not observed in the range between 0 °C and 250 °C for each polymer, which suggested that the glass transition of the hard segment in these polyurethanes was over 250 °C.

3.3 Mechanical property of polymers

The mechanical properties of the films of SPUPC-1, SPUPC-2, SPU-1 and SPU-2 were evaluated to reveal the effect of introduction of PC unit on the mechanical property. SPUPC-3 film could not be obtained because of the insolubility in any solvent. Fig. 2 shows the stress-strain behaviors of the polymer films as compared with Bionate® film, where the Young's modulus, the tensile strength and the elongation to break are summarized. As seen in Fig. 2, the obtained polymers showed the elasticity derived from soft segments of polycarbonate

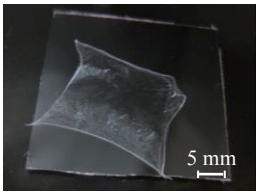


Fig. 3 A self-standing nanosheet composed of SPUPC-2 suspended in water.

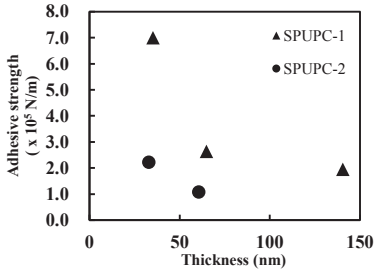


Fig. 4 The relationship between the thickness and the adhesive strength of nanosheets.

component. In addition, SPUPC series exhibited the higher Young's modulus than SPU-1, SPU-2 and Bionate®, which would be due to the strong aggregation of the polar PC groups.

3.4 Property of nanosheets

Polymer nanosheets obtained from SPUPC-1 and SPUPC-2 showed a flexible property and a tendency of shrinking as shown in Fig. 3. Fig. 4 shows the relationship between the thickness and the adhesive strength of nanosheets. The obtained nanosheets show high adhesive strength, and the adhesive strength of nanosheets was increased as the thickness became thin.

On the other hand, SPUPC-2 showed the lower adhesive strength than SPUPC-1. It was thought that this result would relate to the Young's moduli of SPUPC-1 and SPUPC-2, and the more flexible SPUPC-1 caused the higher adhesive strength than SPUPC-2.

3.5 Platelet adhesion on the surface

The blood compatibility of the nanosheet-coated PET plates was evaluated by contacting the plates with a human PRP. Fig. 5 shows the SEM images of the PET plate and those coated with SPU-1, SPUPC-1 and SPUPC-2. Abundant platelets were nonspecifically adhered on the surface of PET plate and activated. Similarly, the platelets adhered on SPU-2 surface were activated. On the contrary, the polymer surfaces containing PC group, SPUPC-1 and SPUPC-2, prevented the adhesion and the activation of the platelets. Especially, in the case of SPUPC-2, platelets hardly adhered, where almost no platelet was observed on the surface. From these results, it is considered that SPUPC series has the superior blood compatibility than the normal segmented polyurethane.

4. CONCLUSION

Copolyurethane having the higher PC content than previously reported polymers was synthesized, and it was found that the limited PC unit for the solubility in this copolymer system was almost 40 mol%. It was also confirmed that the obtained polyurethanes showed the high thermal stability up to 210 °C and the self-standing tough films were prepared by casting, which showed the elastic property. Furthermore, the nanosheets could be successfully fabricated from the solutions of copolyurethanes, where the nanosheets showed a high adhesive strength and a tendency of shrinking. Furthermore, the polymer nanosheets obtained from copolyurethane containing 40 mol% of PC unit exhibited the significant blood compatibility. Therefore, such nanosheets are expected as a new coating agent to construct a biocompatible surface.

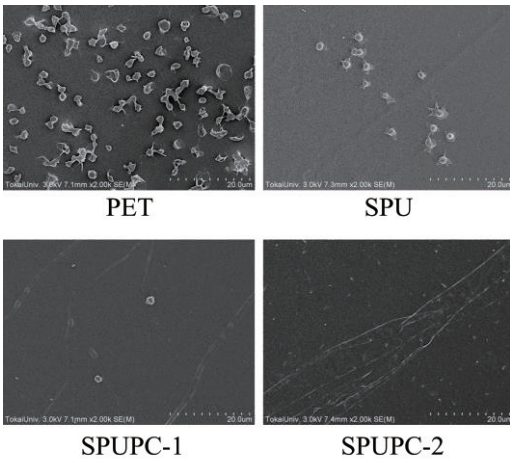


Fig. 5 SEM images of surfaces with and without PC unit after contact with human PRP for 2 h at 37 ℃.

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(Received April 25, 2014; Accepted August 22, 2014)