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Syntheses and properties of elastic copoly(ester-urethane)s containing a phospholipid moiety and the fabrication of nanosheets

Wariya Sirithep[a], Kohei Morita[a], Atsushi Iwano[a], Takuya Komachi[a], Yosuke Okamura[b] and Yu Nagase[a]*

[a]*Course of Industrial Chemistry, Graduate School of Engineering, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259 1292, Japan;* [b]*Institute of Innovative Science and Technology, Tokai University, 4-1-1 Kitakaname, Hiratsuka, Kanagawa 259 1292, Japan*

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In these years, we have investigated the syntheses of novel diamine and diol monomers containing phosphorylcholine (PC) group to obtain biocompatible polymers, the backbone components of which were thermally stable and mechanically strong. In this study, the preparations of elastic copoly(ester-urethane)s containing PC group and polycarbonate segment were carried out by polycondensation and polyaddition using a diol monomer containing PC group and polycarbonate diol. It was found that the obtained polymers exhibited the high-thermal stability up to 200 °C and the elasticity derived from the soft segment. The introduction of PC group was effective to improve the resistance to the adhesions of proteins and platelets on the polymer films, which was the result of surface properties derived from the PC moiety. In addition, we tried to prepare ultra-thin polymer films composed of copoly(ester-urethane)s, so-called nanosheets. As a result, the desired nanosheets were successfully fabricated and the obtained nanosheets exhibited the high adhesive strength, indicating that the nanosheets could conform closely to the desired surfaces due to their exquisite flexibility and low roughness.

Keywords: phosphorylcholine; diol monomer; poly(ester-urethane); biocompatibility; nanosheet

1. Introduction

Segmented polyurethanes have been widely used in practical applications for medical devices due to their high mechanical strength and biocompatibility.[1,2] The several studies of surface or chemically modified segmented polyurethanes have been conducted to improve biostability by reducing the adhesion of cells and proteins.[3–7] In addition, the phosphorylcholine (PC) group is a polar component of phospholipid molecules, which cover the surface of cell membranes, and it has been well known that synthetic polymer materials containing PC group exhibit biocompatibility including non-thrombogenicity. Firstly, Ishihara et al. have been developed 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer as an excellent biocompatible material, which efficiently reduces the adhesion of cells and proteins to the polymer surface.[8,9] Then, in recent years, the MPC polymer has been widely applied in biological and medical fields. Ishihara et al. have also investigated a polymer composite consisting of segmented polyurethane and MPC polymer to reduce protein adsorption to the polymer surface and

*Corresponding author. Email: yunagase@tokai-u.jp

to improve the biocompatibility of segmented polyurethane.[10–15] In addition, Adhikari et al. have reported the biodegradable polyurethanes containing PC moiety which was prepared from a new monomer, dihydroxypolycaprolactone phosphorylcholine.[16] It was described that a slow degradation and a very mild inflammatory response were observed for the implantation of these polyurethanes into rats.

In these years, we have developed the syntheses of novel diamine and diol monomers containing PC group to prepare the thermally stable and mechanically strong polymers rather than MPC polymers. From these monomers, the preparations of polyamides, poly(urethane-urea)s, polyesters, and segmented polyurethanes containing PC group have been carried out by polycondensation or polyaddition,[17–23] and it has been found that these polymers exhibited the good biocompatibility and physical properties.[20] In fact, the amounts of platelets and proteins adhered on these polymer films efficiently reduced as the increase of the content of PC unit in these polymers. On the contrary, it was also found that the solubility of polyurethanes obtained from PC-diol monomer and polycarbonate diol with diphenylmethane diisocyanate became poor as the PC content increased.[22] It would be due to the high-molecular interaction between the PC group in the side chain and the polar urethane bond in the main chain. Accordingly, we have found that the introduction of ester bond in the main chain was effective to improve the solubility of the obtained polymers.[23]

Therefore, in this study, the polycondensation of PC-diol monomer and polycarbonate diol with terephthaloyl chloride (TPC) was carried out followed by the reaction with 4,4′-diphenylmethane diisocyanate (MDI) to obtain copoly(ester-urethane)s having the higher PC content. Recently, Khan et al. reported that a potential application of poly (carbonate-urethane) was as a long-term biomedical implant material due to its resistance to biodegradation and its biocompatibility,[24] therefore, we selected polycarbonate diol to construct the soft segment of copoly(ester-urethane)s. The effect of the main chain structure on the solubility, the film-forming ability, and the physical properties of the obtained polymers was investigated in detail.

On the other hand, the development of practical biomaterials will desire the collaborations among chemists, biologists, and material scientists. We focused in the field of nanotechnology, especially the processing for free-standing ultra-thin films consisting of polymers with a thickness less than 100 nm (often called nanosheets), which exhibited the unique properties such as high adhesive strength, flexibility, transparency, and smoothness.[25] Using these properties, we have demonstrated that biodegradable nanosheets act as a novel wound dressing instead of traditional suture operation in surgery. Recently, we have also revealed that fragmented nanosheets suspended in water can adhere to not only flat surfaces but also to uneven surfaces.[26] If the nanosheets could be fabricated from such PC-containing polymers, the applications as new biomaterials would be significantly advanced, e.g. aqueous coating materials to provide blood compatible surfaces. Then, we attempted to prepare the nanosheets from the obtained copoly(ester-urethane)s and to investigate the physical properties and the biocompatibility of the nanosheet surface.

2. Materials and methods

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 report.[22] MDI, polycarbonate diol (PCD, $M_n = 1000$, $m = 6$), were kindly supplied from Nippon Polyurethane Industry Co., Ltd (Tokyo, Japan) and Asahi Kasei Co. (Tokyo, Japan), respectively. TPCs and 1,3-bis(2-hydroxyethyl)benzene (BHE) were purchased from Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), and triethylene glycol (TEG) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). The monomer compounds, BHPC, PCD, BHE, and TEG were dried *in vacuo* at 80 °C for 6 h before the polymerization reactions. Anhydrous *N*-methylpyrrolidone (NMP) was purchased from Sigma-Aldrich Japan as a solvent for polymerization, and used without further purification. Fibrinogen from bovine plasma and poly(ethylene terephthalate) (PET) plate for cell culture were purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Poly(vinyl alcohol) (PVA, $M_w = 22{,}000$, 89% hydrolyzed) was obtained from Kanto Chemical Co. (Tokyo, Japan).

2.2. Synthesis

2.2.1. Preparation of homopolyester (HPEPC)

Under an argon atmosphere, BHPC (0.80 g, 1.78 mmol) and terephthaloyl chloride (0.36 g, 1.78 mmol) were mixed in 8.0 mL of NMP at −78 °C. The mixture was stirred for 67 h with increasing the temperature to room temperature (rt). Then, the mixture was poured into excess tetrahydrofuran (THF) to precipitate the polymer, and it was filtered and purified by reprecipitation from its NMP solution to excess THF. Finally, the product was dried *in vacuo* to obtain HPEPC as a brown solid. Yield: 1.01 g (87.0%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 3.09 (–N^+($C\underline{H}_3$)$_3$, m), 3.57 (–POCH$_2$C$\underline{H}_2$N–, m), 4.11 (–OC$\underline{H}_2$CH$_2$OPh–, m), 4.22 (–COOCH$_2$C$\underline{H}_2$OP–, m), 4.37 (–POC$\underline{H}_2$CH$_2$ N–,m), 4.59 (–OCH$_2$C$\underline{H}_2$OPh–, –COO–C$\underline{H}_2$CH$_2$OP–, m), 6.94 (–Ph–, m), 7.11 (–Ph–, m), 8.03 (–Ph–, m).

IR, υ (KBr, cm^{-1}): 2955, 2880, 1720 (C=O), 1597, 1493, 1450, 1408, 1373, 1269 (P=O), 1165, 1126, 1099, 729.

2.2.2. Preparations of copoly(ester-urethane)s (PEUPC-a, PEU-1)

Under an argon atmosphere, BHPC (0.97 g, 2.16 mmol), PCD (2.16 g, 2.16 mmol), and TPC (0.44 g, 2.16 mmol) were mixed in 6.0 mL of NMP at −78 °C. The mixture was stirred for 24 h with increasing the temperature to rt. Then, the solution containing MDI (0.54 g, 2.16 mmol) in 2.5 mL of NMP was gradually added to the mixture at rt. After the mixture was stirred at 50 °C for 38 h. The mixture was poured into excess methanol to precipitate the polymer, and it was filtered and purified by reprecipitation from its chloroform solution to excess methanol. Finally, the product was dried *in vacuo* to afford PEUPC-a as a brown solid. Yield: 2.43 g (59.7%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 1.28 (–OCH$_2$CH$_2$C$\underline{H}_2$C$\underline{H}_2$CH$_2$CH$_2$OCO–, m), 1.55 (–OCH$_2$C$\underline{H}_2$CH$_2$CH$_2$C$\underline{H}_2$CH$_2$OCO–, m), 3.08 (–N^+($C\underline{H}_3$)$_3$, m), 3.42 (–POCH$_2$C$\underline{H}_2$N–, m), 3.75 (–Ph–C$\underline{H}_2$–Ph–, bs), 4.01 (–OC$\underline{H}_2$CH$_2$OPh–, –OC$\underline{H}_2$CH$_2$CH$_2$CH$_2$CH$_2$C$\underline{H}_2$–OCO–, m), 4.15 (–COOCH$_2$C$\underline{H}_2$OP–, m), 4.24 (–PO–C$\underline{H}_2$CH$_2$N–, m), 4.36 (–OCH$_2$-C$\underline{H}_2$OPh–, –COOC$\underline{H}_2$CH$_2$–OP–, m), 6.83 (–Ph–, m), 7.08 (–Ph–, m), 7.33 (–Ph–, m), 8.04 (–Ph–, m), 9.48 (–N$\underline{H}$COO–, bs), 9.70 (–N$\underline{H}$COO–, bs).

IR, υ (Film, cm^{-1}): 3500 (N–H), 2938, 2868, 2310, 1734 (C=O), 1716, 1597, 1541, 1375, 1346 (P=O), 1242, 1115, 1067, 876, 729.

In the above polymerization reaction, BHE was used instead of BHPC, and the similar reactions and the reprecipitation from its THF solution to excess methanol were carried out to afford PEU-1 as a white powder. Yield: 6.54 g (80.8%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 1.28 (–OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 1.54 (OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 3.75 (–Ph–CH$_2$–Ph–, bs), 4.01 (–OCH$_2$CH$_2$OPh–, –OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 4.36 (–OCH$_2$CH$_2$OPh–, m), 6.54 (–Ph–, m), 7.06 (–Ph–, m), 7.35 (–Ph–, m), 8.04 (–Ph–, m), 9.45 (–NHCOO–, bs), 9.67 (–NHCOO–, bs).

IR, υ (Film, cm^{-1}): 3342 (N–H), 2937, 2862, 1736 (C=O), 1718, 1597, 1531, 1458, 1240, 1184, 1066, 1018, 961, 790.

2.2.3. Preparation of copoly(ester-urethane) (PEUPC-b)

Under an argon atmosphere, BHPC (1.04 g, 2.32 mmol), PCD (1.85 g, 1.85 mmol), BHE (91.9 mg, 0.464 mmol), and TPC (0.471 g, 2.32 mmol) were mixed in 7.0 mL of NMP at −78 °C. The mixture was stirred for 24 h with increasing the temperature to rt. Then, the solution containing MDI (0.640 g, 2.32 mmol) in 2.3 mL of NMP was gradually added to the mixture at rt. After the mixture was stirred at 50 °C for 42 h. The mixture was poured into excess methanol to precipitate the polymer, and it was filtered and purified by reprecipitation from its chloroform solution to excess methanol. Finally, the product was dried *in vacuo* to afford PEUPC-b as a brown solid. Yield: 3.41 g (86.0%).

^{1}H NMR, δ (400 MHz, DMSO-d_6, ppm): 1.29 (–OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 1.56 (–OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 3.08 (–N$^+$(CH$_3$)$_3$, m), 3.54 (–POCH$_2$CH$_2$N–, m), 3.77 (–Ph–CH$_2$–Ph–, bs), 4.03 (–OCH$_2$CH$_2$OPh–, –OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$–OCO–, m), 4.17 (–COOCH$_2$CH$_2$OP–, m), 4.27 (–PO–CH$_2$CH$_2$N–, m), 4.38 (–OCH$_2$-CH$_2$OPh–, –COOCH$_2$CH$_2$–OP–, m), 4.62 (–OCH$_2$CH$_2$OPh–, m), 6.53 (–Ph–, m), 6.83 (–Ph–, m), 7.08 (–Ph–, m), 7.34 (–Ph–, m), 8.05 (–Ph–, m), 9.49 (–NHCOO–, bs), 9.71 (–NHCOO–, bs).

IR, υ (Film, cm^{-1}): 3447 (N–H), 2976, 2857, 2625, 2315, 1699 (C=O), 1341, 1319 (P=O), 1126, 1109, 1041, 874, 837.

2.2.4. Preparations of copoly(ester-urethane)s (PEUPC-c, PEU-2)

Under an argon atmosphere, BHPC (1.21 g, 2.67 mmol), PCD (2.14 g, 2.14 mmol), TEG (80.4 mg, 0.535 mmol), and TPC (0.543 g, 2.67 mmol) were mixed in 8.0 mL of NMP at −78 °C. The mixture was stirred for 24 h with increasing the temperature to rt. Then, the solution containing MDI (0.669 g, 2.67 mmol) in 2.7 mL of NMP was gradually added to the mixture at rt. After the mixture was stirred at 70 °C for 24 h. The mixture was poured into excess methanol to precipitate the polymer, and it was filtered and purified by reprecipitation from its chloroform solution to excess methanol. Finally, the product was dried *in vacuo* to afford PEUPC-c as a brown solid. Yield: 2.54 g (56.2%).

^{1}H NMR, δ (500 MHz, DMSO-d_6, ppm): 1.28 (–OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 1.54 (–OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$OCO–, m), 3.15 (–N$^+$(CH$_3$)$_3$, m), 3.60 (–POCH$_2$CH$_2$N–, m), 3.75 (–Ph–CH$_2$–Ph–, bs), 4.01 (–OCH$_2$CH$_2$OPh–, –OCH$_2$CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$–OCO–, –OCH$_2$CH$_2$O–, m), 4.15 (–COOCH$_2$CH$_2$OP–, m), 4.24 (–PO–CH$_2$CH$_2$N–, m), 4.36 (–OCH$_2$CH$_2$OPh–, –COOCH$_2$CH$_2$–OP–, m), 6.83 (–Ph–, m), 7.08 (–Ph–, m), 7.33 (–Ph–, m), 8.04 (–Ph–, m), 9.50 (–NHCOO–, bs), 9.70 (–NHCOO–, bs).

IR, υ (Film, cm^{-1}): 3313, 3294 (N–H), 2932, 2857, 2309, 1734 (C=O), 1717, 1595, 1595, 1348, 1341 (P=O), 1308, 1171, 1061, 731, 683.

In the above polymerization reaction, BHE was used instead of BHPC, and the similar reactions and the reprecipitation from its THF solution to excess methanol were carried out to afford PEU-2 as a white powder. Yield: 6.60 g (84.6%).

^{1}H NMR, δ (500 MHz, DMSO-d_6, ppm): 1.28 (–$OCH_2CH_2C\underline{H_2}C\underline{H_2}CH_2CH_2OCO$, m), 1.54 ($OCH_2C\underline{H_2}CH_2CH_2C\underline{H_2}CH_2OCO$–, m), 3.75 (–$PhC\underline{H_2}$–Ph–, bs), 4.01 (–$OC\underline{H_2}$-$CH_2OPh$–, –$OC\underline{H_2}CH_2CH_2CH_2CH_2C\underline{H_2}OCO$–, –$OC\underline{H_2}C\underline{H_2}O$–, m), 4.36 (–$OCH_2$-$C\underline{H_2}OPh$–, m), 6.54 (–Ph–, m), 7.06 (–Ph–, m), 7.35 (–Ph–, m), 8.04 (–Ph–, m), 9.45 (–N$\underline{H}$COO–, bs), 9.67 (–N$\underline{H}$COO–, bs).

IR, υ (Film, cm^{-1}): 3337 (N–H), 2938, 2862, 1736 (C=O), 1707, 1597, 1530, 1404, 1240, 1184, 1161, 1069, 1018, 963, 853.

2.3. *Characterizations of polymers*

^{1}H NMR spectra were conducted with a JEOL NM-TH5SK FT NMR spectrometer (400 MHz, JEOL Ltd, Tokyo, Japan) or a Bruker Advance AV500 FT NMR spectrometer (500 MHz, Bruker BioSpin Co., Kanagawa, Japan), and the chemical shifts were estimated in ppm units with tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded with a Shimadzu FTIR-8400 spectrometer (Shimadzu Co., Kyoto, Japan). The molecular weights of polymers were estimated with a Tosoh gel permeation chromatography (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 DSC-6200 and TG/DTA-6200 (Seiko Instruments Inc., Chiba, Japan), respectively, at a heating rate of 10 °C/min under a nitrogen atmosphere. The surfaces of the polymer films were analyzed with a Quantum 2000 X-ray photoelectron spectroscope (ULVAC-PHI Inc., Kanagawa, Japan), where the take-off angle of photoelectrons was adjusted to be 90°.

2.4. *Measurements of stress–strain behavior*

The obtained polymers were dissolved in chloroform or THF and then the solution (8–10 wt.%) was poured on Teflon sheet. The solvent was removed at rt for 3 days under its vapor atmosphere. The obtained films were then dried *in vacuo* at 70 °C for overnight, and the self-standing films were obtained. Then, the polymer films were cut into rectangular strips with a length of 40 mm, a width of 10 mm, and a thickness of 0.15–0.20 mm. Stress–strain curves were obtained on JSV-H1000L equipped with HF-10 (Japan Instrumentation System Co., Ltd, Nara, Japan), where the gauge length was 20 mm and the cross-head speed was 0.2 mm/s. The three films of each sample were used to determine the average values of Young's modulus, tensile strength, and elongation to break.

2.5. *Quantitative analysis of protein adsorbed to the coated films*

The polymer solutions dissolved in DMSO (10 mg/mL) were spin coated three times on both the sides of PET plate with a diameter of 14 mm. The polymer-coated PET

plates were immersed in phosphate-buffered saline (PBS, pH 7.4) at rt for 15 h to equilibrate the surface, and incubated at 37 °C for 2 h in PBS solution containing fibrinogen (5 mg/mL). After 2 h, the plates were removed from the solution and rinsed in excess distilled water to remove non-adsorbed proteins. The plates were then immersed in a 1.0 wt.% aqueous solution of sodium dodecyl sulfate (SDS) to remove the adsorbed proteins. The concentration of fibrinogen in the SDS solution was estimated with a Micro BCA™ Protein Assay kit (Thermo Fisher Scientific Co., Ltd, Kanagawa, Japan), and the amount of adsorbed proteins on the polymer-coated PET plates was determined based on the absorbance at a wavelength of 550 nm in a protein dilution system using a Benchmark microplate reader (Bio-Rad Laboratories, Inc., Tokyo, Japan).

2.6. Platelet adhesion test

A 10% volume of 3.8% (w/v) sodium citrate solution was added to blood withdrawn from healthy volunteers according to the guidelines approved by the Ethical Committee of the Tokai University. The blood was centrifuged (120 *g*, 15 min, rt) and platelet-rich plasma (PRP) in the supernatant was collected. PET plates (14 mmϕ in diameter) coated with the polymers were incubated overnight in PBS (pH 7.4). The PET plates were immersed into 0.5 mL of PRP and incubated at 37 °C for 2 h. Finally, PRP was removed and the substrates were gently washed with PBS three times. The surface of the plates was observed by a scanning electron microscope (SEM, FE-SEM S-4800, Hitachi High-technologies, Co., Tokyo, Japan), where the acceleration voltage was 3 kV and the magnification was 2000-folds.

2.7. Fabrication of polymer nanosheets

Polymer nanosheets were essentially fabricated as described previously.[24] In order to produce a water-soluble sacrificial layer, an aqueous solution of 100 mg/mL PVA was dropped on a silicon oxide (SiO_2) substrate (KST World Co., Fukui, Japan) and then spin coated at 4000 rpm for 20 s, followed by a drying step at 70 °C for 90 s. Following the same procedure, the PVA-coated SiO_2 substrate was then coated with 10 mg/mL polymer solutions dissolved in chloroform or THF. The substrates were immersed in distilled water at rt to obtain free-standing nanosheets. Then, the obtained nanosheets were washed several times with distilled water to completely remove the PVA.

2.8. Measurement of adhesive strength of nanosheet

The adhesive strength of nanosheets was measured by a scratch tester for thin films (model CSR-2000, Rhesca Co., Tokyo, Japan). The procedure in brief was as follows: a diamond tip at a radius of curvature of 25 μm was continuously and vertically loaded at a rate of 10 mN/min, and used to horizontally scratch the polymer nanosheets readsorbed on the SiO_2 substrate (scratch length: 100 μm, scratch rate:10 μm/s). The signal of frictional vibration just after detaching the nanosheet was detected (designated a critical load). Then, the critical loads of nanosheets were measured with changing thickness from 40 to 600 nm and corrected the critical loads divided by the thickness. The critical loads were measured in triplicate.

3. Results and discussion

3.1. Syntheses and characterizations of copoly(ester-urethane)s

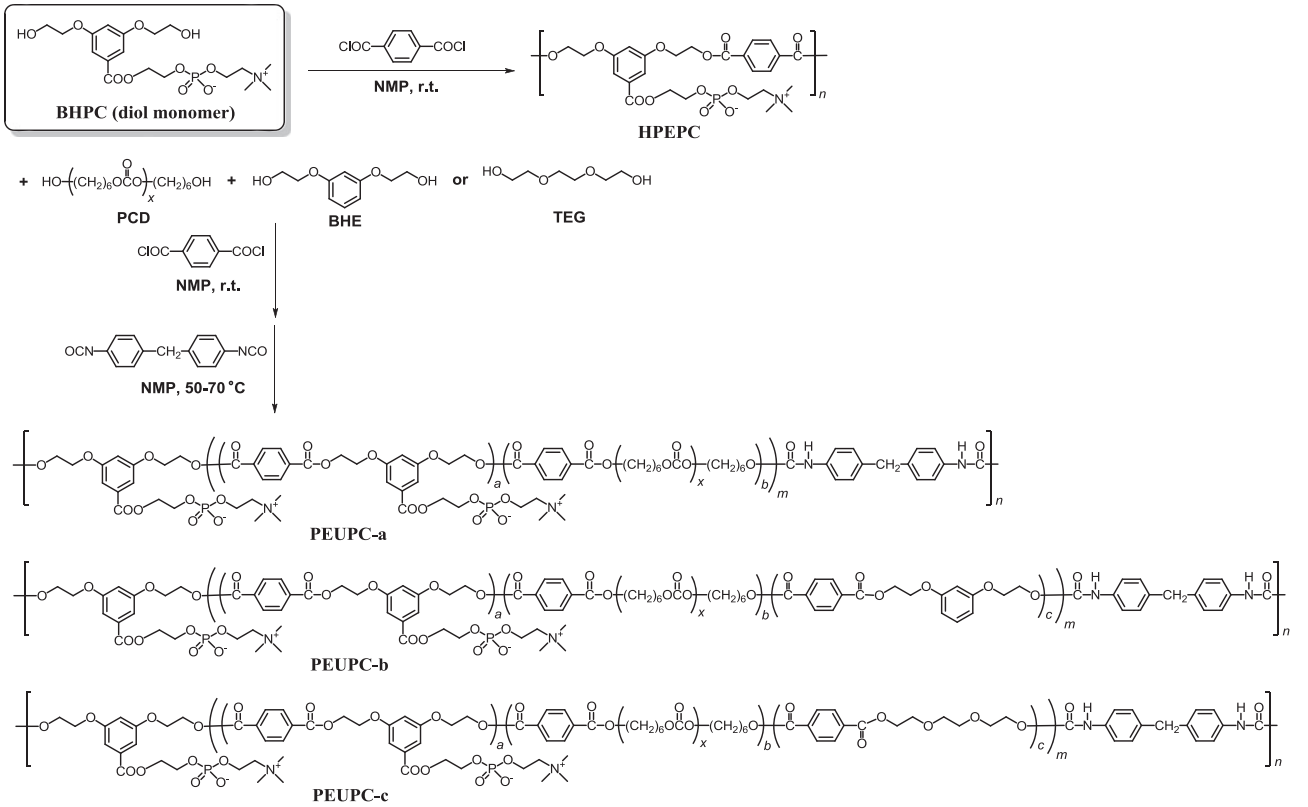
The syntheses of copoly(ester-urethane)s were carried out by the polycondensation of BHPC, PCD, and the another diol monomer with TPC to obtain the oligomer of polyester in the first step, followed by the addition of MDI as a chain extender. Three different types of copoly(ester-urethane)s were prepared, one of which consisted of two monomer units derived from BHPC and PCD (PEUPC-a), and the others of which consisted of three monomer units derived from BHPC, PCD, and BHE or TEG (PEUPC-b and PEUPC-c), as shown in Scheme 1. In these polymerizations, the monomer ratio of BHPC was fixed at 50 mol%, because it was found that the suitable content of the PC unit was nearly 30 wt.% for the biocompatibility, in the case of polyurethanes containing PC unit obtained by the polyaddition of BHPC and PCD with MDI.[21] Furthermore, the copoly(ester-urethane)s, PEU-1 and PEU-2, without PC unit were prepared as reference samples by the similar polymerization of PCD, BHE, and TEG with TPC and MDI. On the other hand, the homopolyester, HPEPC, was prepared as a reference polymer according to the same procedure in our previous paper.[22]

The compositions and molecular weights of the obtained copoly(ester-urethane)s are summarized in Table 1. The chemical structures of these copolymers were confirmed by ^{1}H NMR and IR spectra, as described in the experimental section. Compositions of the PC units in copolymers were determined from the ratio of the peak intensities of the methyl protons of ammonium group in the PC moiety (3.08–3.15 ppm) and methylene protons of polycarbonate unit (1.28–1.54 ppm), which existed in each monomer component. The ratio of ester and urethane bonds in the main chain was determined from the ratio of the peak intensities of the protons on the aromatic ring of TPC component (8.04–8.05 ppm) and the protons on the phenylene unit of MDI component (7.33–7.35 ppm). As shown in Table 1, the mass contents of the PC units in the copolymers could be controlled in the range of 27–28 wt.%. On the other hand, the molecular weights of the obtained polymers seemed to be very high, most of which were over 4×10^5 as listed in Table 1. It is considered that these values must be higher than the real molecular weights. It was assumed that the molecular chain of these copolymers would be fully extended in DMF and result in a very large free volume of molecules in such a polar solvent, although the absolute molecular weight should be measured by, for example, a light scattering method. The similar tendency of the high molecular weights was also observed in the GPC results of segmented polyurethanes containing PC component.[22]

The solubility of copolymers is listed in Table 2. The obtained polymers were insoluble in ethanol and water, and PEUPC-a, PEUPC-b, and PEUPC-c exhibited good solubility in chloroform and aprotic polar solvents, such as DMSO, NMP, and DMF. On the other hand, PEU-1 and PEU-2 were insoluble in chloroform but soluble in THF. The PEUPC series showed the better solubility than HPEPC, which was soluble only in NMP and DMSO. It was speculated that a large content of the polar PC group in the side chain would have a strong interaction with the polar bond in the main chain, which would make a polycondensation-type polymer insoluble even in aprotic polar solvents.

3.2. Thermal property of polymers

The thermal properties of these copolymers were evaluated by DSC and TGA. The DSC curves of the copolymers are shown in Figure 1. In the DSC thermograms, the glass transition temperature (Tg) was observed in the range between −38 and −35 °C



Scheme 1. Syntheses of polyester and poly(ester-urethane)s containing PC group.

Table 1. Results of polymerizations.

Code	Molar ratio of monomers BHPC/PCD/BHE/TEG[a]	PC content[b] (mol%)	PC content[b] (wt.%)	M_n[c] × 10^{-3}	M_w/M_n[c]
HPEPC	100/0/0/0	100	100	–	–
PEUPC-a	50/50/0/0	49	27	421	1.66
PEUPC-b	50/40/10/0	49	28	65.7	2.00
PEUPC-c	50/40/0/10	50	28	767	1.73
PEU-1	0/50/50/0	0	0	501	1.61
PEU-2	0/40/50/10	0	0	615	1.44

[a]BHPC: 2-(3,5-bis(2-hydroxyethoxy)benzoyloxy)ethyl phosphorylcholine, PCD: polycarbonate diol (M_n = *ca.* 1000), BHE: 1,3-bis(2-hydroxyethoxy)benzene, TEG: triethylene glycol.
[b]PC content was calculated from the ratio of peak intensities in ^{1}H NMR spectra.
[c]Number-average and weight-average molecular weights (M_w and M_n) were estimated by GPC using DMF as an eluent.

Table 2. Solubility of polymers.

Code	Solubility[a] Water	Ethanol	Chloroform	THF	NMP	DMAc	DMF	DMSO
HPEPC	×	×	×	×	○	×	×	○
PEUPC-a	×	×	○	○	○	○	○	○
PEUPC-b	×	×	○	×	○	○	○	○
PEUPC-c	×	×	○	×	○	○	○	○
PEU-1	×	×	×	○	○	○	○	○
PEU-2	×	×	×	○	○	○	○	○

[a]The concentration of the solubility test was 1.0 wt.% in each solvent.
Note: Symbol: ○: Soluble, ×: Insoluble.

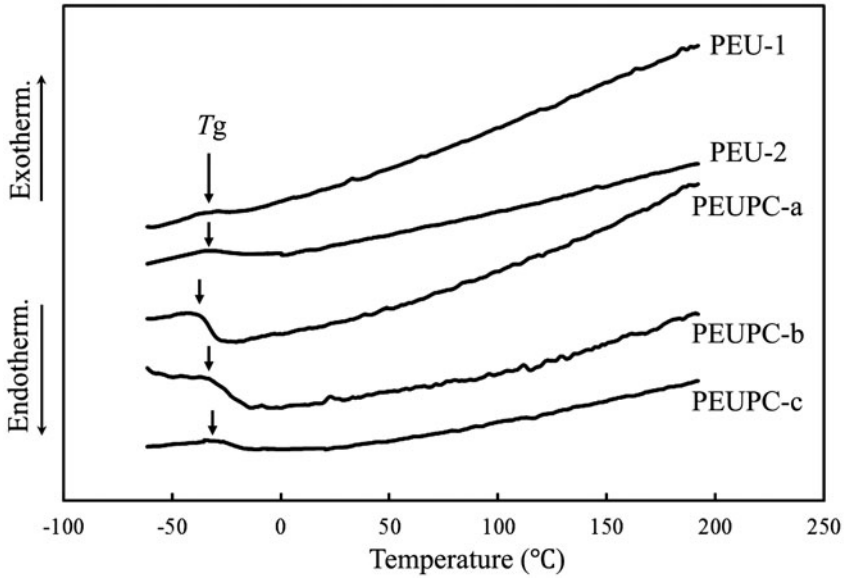


Figure 1. DSC curves of the second heating scan of copolymers at a heating rate of 10 °C/min in N_2 flow.

for the every copolymers in the PEUPC and PEU series. It would be due to the glass transition of the soft segment, polycarbonate unit, in these copolymers.[27,28] The other transition or the melting temperature was not observed in the range between 0 and 200 °C for every copolymers, which suggested that the glass transition of the main chain component in these copoly(ester-urethane)s was over 200 °C. On the contrary, in the case of HPEPC, the melting temperature (T_m) was observed at around 96 °C.

From TGA curves of these polymers, it was found that the weight loss of HPEPC and PEUPC series started at 200–230 °C as shown in Figure 2, whereas that of PEU-1 started over 300 °C. Therefore, it was speculated that the weight loss of the PC-containing copolymers would derived from the degradation of the polymer side chain, which contained PC groups or spacer moieties. The heat resistance of these PC-containing copoly(ester-urethane)s until 200 °C would be sufficient for the use in medical devices, for example, for thermal sterilization processes above 150 °C. These results demonstrate that PC-containing copoly(ester-urethane)s possess similar thermal stability to PC-containing polyamides and polyurethanes, the properties of which were reported in our previous papers.[20–23]

3.3. *Physical properties of polymer films*

3.3.1. *Surface property*

The surface structures of polymer films were analyzed by X-ray photoelectron spectroscopy (XPS). The XPS spectra based on P_{2p} and C_{1s} of the PC-containing polymers, HPEPC, PEUPC-a, PEUPC-b, and PEUPC-c, are shown in Figure 3. Then, the atomic percentages of the polymer film surfaces based on the XPS analysis are summarized in Table 3. As shown in this table, it was found that the P_{2p} percentage of HPEUPC surface (0.97%) was obviously higher than those of PEUPC series, where the P_{2p} percentages of PEUPC-a, b, and c were almost same at 0.40–0.46%. Therefore, it was confirmed that the PC concentrations on the film surfaces depended on the PC contents of the polymers.

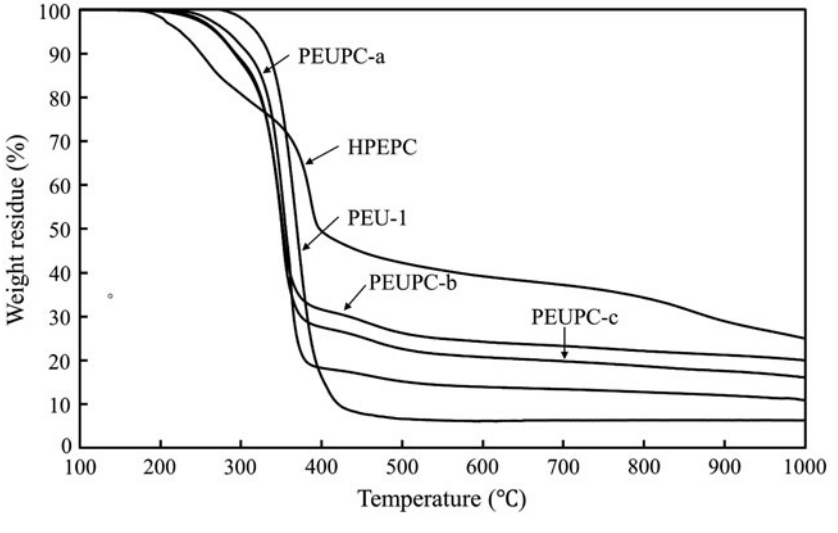


Figure 2. TGA curves of polymers at a heating rate of 10 °C/min in N_2 flow.

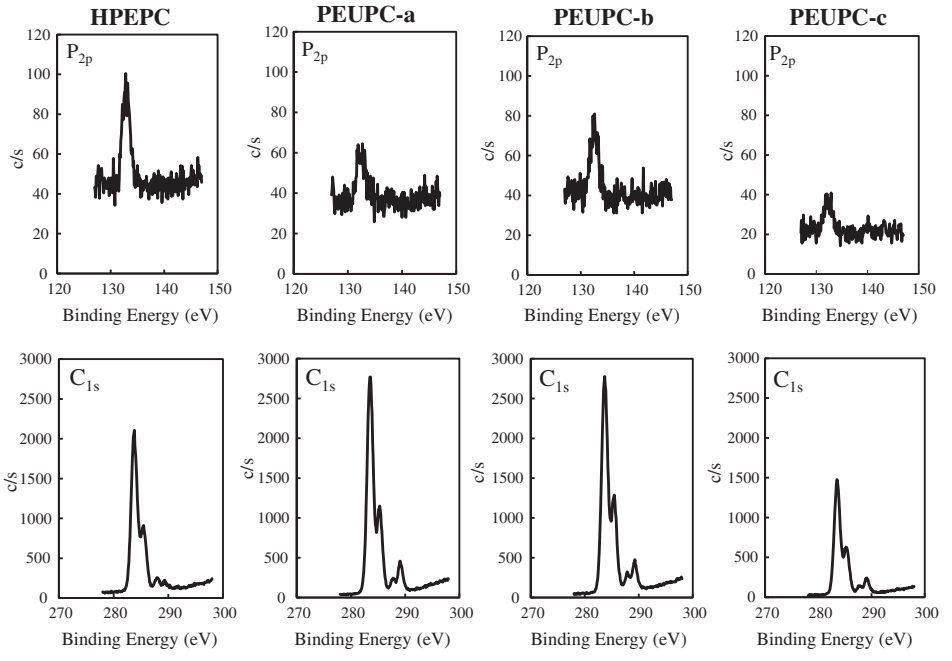


Figure 3. XPS spectra of the films composed of HPEPC, PEUPC-a, PEUPC-b and PEUPC-c.

Table 3. Atomic percentages of the polymer film surfaces determined by XPS analysis.

	C_{1s}	N_{1s}	O_{1s}	P_{2p}
HPEPC	65.9	0.58	32.6	0.97
PEUPC-a	69.1	1.03	29.4	0.46
PEUPC-b	68.8	2.91	27.9	0.46
PEUPC-c	69.9	0.80	28.9	0.40
PEU-1	73.3	1.09	25.5	–
PEU-2	71.9	0.81	27.3	–

On the other hand, the static contact angles of water on the film surfaces of HPEPC, PEUPC-a, PEUPC-b, and PEUPC-c were observed at 51°, 88°, 91°, and 91°, respectively, whereas that of the PEU-1 film was observed at 101°. HPEPC film was prepared by coating its DMSO solution on the PET substrate, because the casting film of HPEPC was brittle and inflexible. Obviously, the contact angle of water on the HPEPC surface was smaller than those of PEUPC-a, PEUPC-b, and PEUPC-c, which would be due to the higher content of the polar PC groups in the HPEPC film surface rather than PEUPC series. Therefore, it was suggested that the PC moiety exerts to some extent on the film surfaces of the HPEPC and PEUPC series.

3.3.2. Mechanical property

The stress–strain behaviors of copoly(ester-urethane) films were evaluated to reveal the effect of the main chain structure and the composition in these copolymers on the

mechanical properties. For these experiments, PEUPC-a, PEUPC-b, PEUPC-c, PEU-1, and PEU-2 were used as film samples. Figure 4 shows the typical stress–strain behavior of the copolymer films, and the Young's modulus, the tensile strength, and the elongation to break are summarized in Table 4, which are the average values of each three films.

As shown in Figure 4, these copolymer films underwent a large elongation from 400 to 900%, which appeared to have rubber-like elasticity. These stress–strain behaviors were similar to those of polyurethanes containing polycarbonate as the soft segment, which was described in the literature.[27–30] In particular, the large elongations were observed for the films of PEUPC-a and PEU-1, which would be due to the high content of soft segments of polycarbonate units above 70 wt.%. The films of PEUPC-b and PEUPC-c possessed the higher Young's moduli than PEUPC-a film. Therefore, the Young's modulus increased with the addition of comonomer component, BHE or TEG, which resulted in the decrease of the soft polycarbonate component. Thus, the introduction of a *m*-phenylene or tri(ethylene oxide) unit in the main chain instead of soft segments induced the higher Young's moduli and the tensile strength in these films. The effects of composition of the copolymers on the mechanical properties are in good agreement with our previous results of poly(urethane-urea)s and polyurethanes containing PC moieties.[19–22]

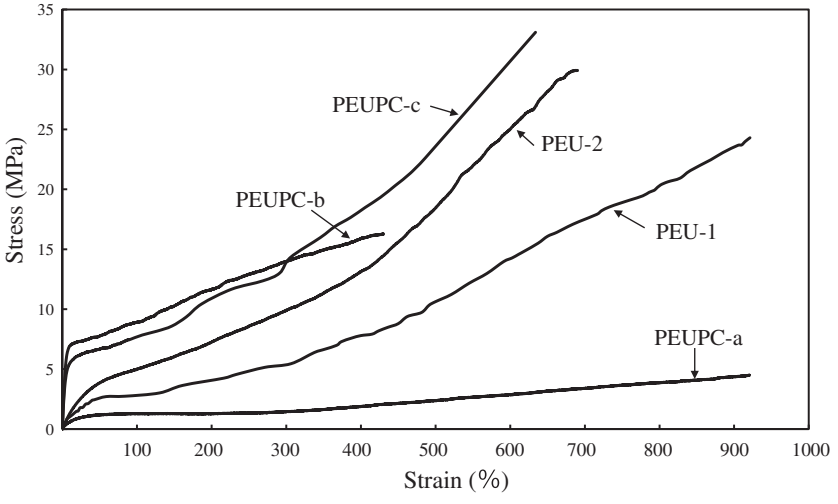


Figure 4. Stress–strain curves of polymer films.

Table 4. Mechanical properties of polymer films.

Code	Young's modulus (MPa)	Tensile strength (MPa)	Elongation (%)
PEUPC-a	5.87 ± 0.14	4.43 ± 0.26	878 ± 100
PEUPC-b	106 ± 22	15.0 ± 2.36	371 ± 31
PEUPC-c	101 ± 35	32.8 ± 3.51	645 ± 92
PEU-1	10.0 ± 1.39	23.8 ± 1.13	870 ± 98
PEU-2	12.9 ± 0.21	30.5 ± 0.85	737 ± 65

3.4. Biocompatibility of polymer surface

For the preliminary evaluation of biocompatibility, we tested whether fibrinogen, which is involved in platelet thrombus formation,[31] adsorbs onto the copoly(ester-urethane) films coated on PET plates. To this end, the films (HPEPC, PEUPC-a, PEUPC-b, and PEUPC-c) were immersed in the solutions of fibrinogen for 2 h and the amount of adsorbed fibrinogen was quantified as shown in Figure 5. As reference samples, PEU-1 and PEU-2 without PC moieties, and PET plate were used.

As shown in Figure 5, the protein adsorption was significantly suppressed on the film surfaces of the HPEPC and PEUPC series compared with PEU-1, PEU-2, and PET due to the effect of PC moieties in the polymers. We expected at first that the protein adsorption on the surface of HPEPC would be the lowest because of its highest content of PC unit. However, it was found that the protein adsorption of the PEUPC-a surface was lower than that of the HPEPC surface, and those of PEUPC-b and PEUPC-c were higher than that of HPEPC. Therefore, the reduction effects of protein adsorptions of these PC-containing polymers would be in the similar level. In addition, it was considered that the surface morphology of PEUPC-a film would be suitable for the reduction of protein adsorption in these PC-containing polymers, where the highest reduction effect of PEUPC-a related to the higher intensity of XPS spectrum of P_{2p} peak in PEUPC-a as compared with those in PEUPC-b and PEUPC-c. Furthermore, it was considered that the difference in the amount of absorbed protein on PEUPC-b and PEUPC-c films related to that on PEU-1 and PEU-2 films.

The blood compatibility of these polymers was also evaluated by contacting the copolymers-coated films on PET plates with human PRP for 2 h. The results are shown in Figure 6. In the case of HPEPC, PEUPC-a, PEUPC-b, and PEUPC-c, the adhered platelets and the fibrin clotting clearly decreased as compared with PEU-1, PEU-2, and PET plate. In particular, it was estimated that the blood compatibility of PEUPC series was similar to that of HPEPC, because the amounts of adhered platelets on these

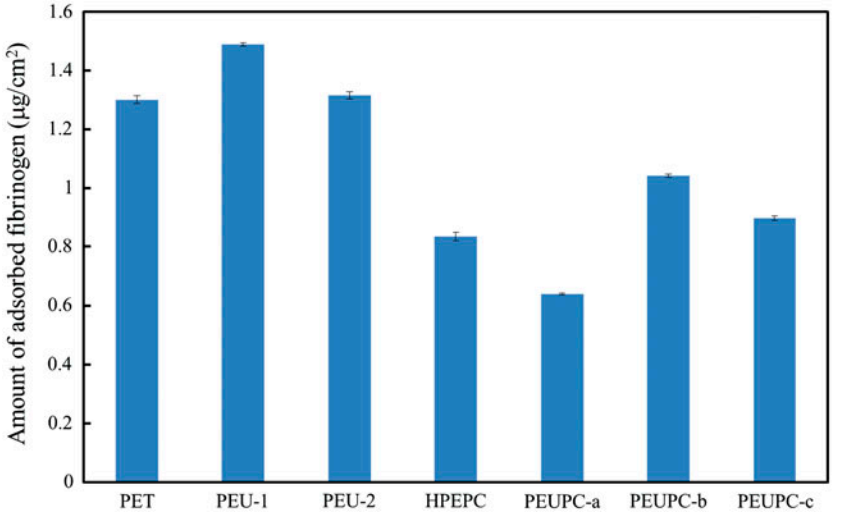


Figure 5. Amount of adhered fibrinogen on polymer films after contact with fibrinogen solution for 2 h.

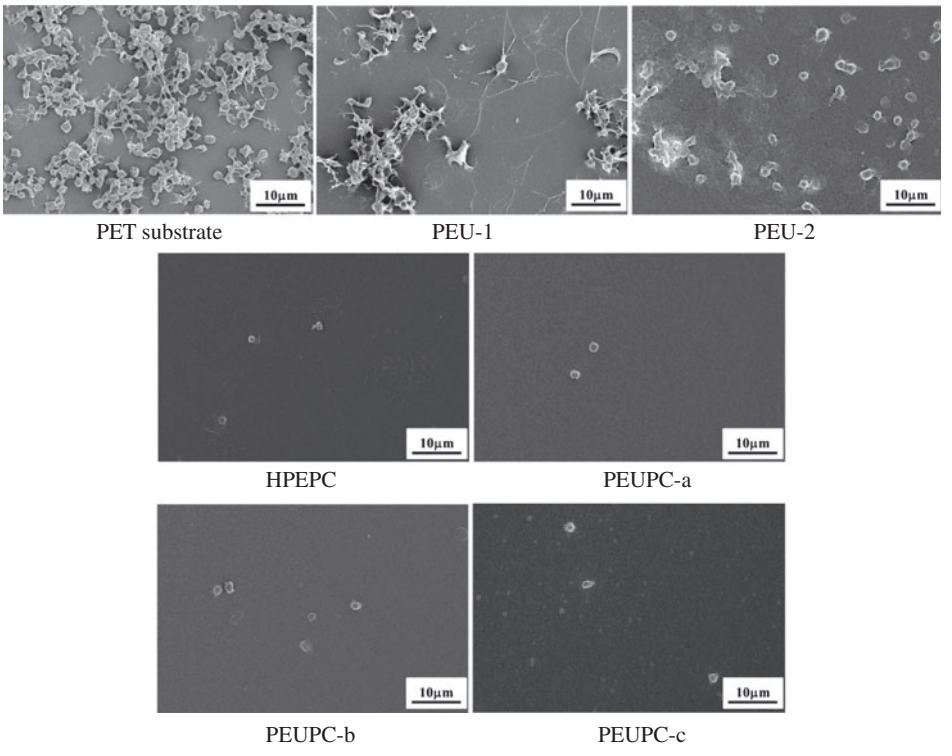


Figure 6. SEM images of polymer surfaces after contact with human PRP for 2 h at 37 °C.

PC-containing polymer surfaces were almost same as shown in Figure 6. The tendency of the platelet adhesion of these polymers was in good agreement with the results of protein adsorption and the peak intensity in the XPS analysis. Consequently, it was assumed that a PC content of *ca.* 30 wt.% would be sufficient to reduce the adhesion of protein and platelets for these PC-containing copoly(ester-urethane)s. The biocompatibility of these polymers was very similar to that of PC-containing segmented polyurethanes as described in our previous paper.[22]

3.5. Fabrications of ultra-thin films (nanosheets)

We attempted to prepare ultra-thin films, so-called nanosheets, from PC-containing copoly(ester-urethane)s using the procedure reported by Okamura et al. [25]. The fabrication of a polymer nanosheet is illustrated in Figure 7(A). PVA was used as a water-soluble sacrificing film, and a polymer solution dissolved in chloroform was cast on the substrate previously spin coated with PVA solution and dried. Then, the double-layered substrate was immersed in water to obtain easily a self-standing nanosheet after PVA was dissolved in water. The macroscopic images of the obtained nanosheets of PEU-1, PEU-2, PEUPC-a, PEUPC-b, and PEUPC-c are shown in Figure 7(B). The resulting films were flexible and transparent. Especially, PEUPC-a nanosheet was more flexible than PEUPC-b and PEUPC-c nanosheets, which was the similar tendency as described in the stress–strain behaviors of these films. As shown in the images of Figure 7(B), these nanosheets composed of the elastic polymers had a tendency to

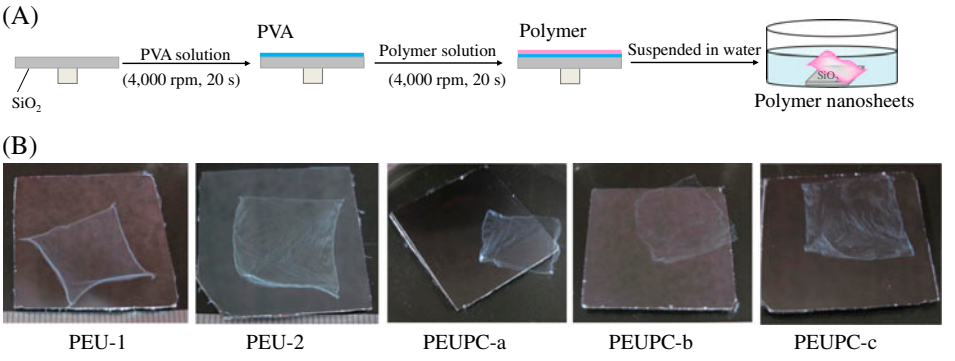


Figure 7. Fabrication of free-standing nanosheet. (A) Fabrication of a PEUPC series nanosheet by the simple combination of spin-coatings of PVA and polymers. (B) Macroscopic images of the nanosheets composed of PEU-1, PEU-2, PEUPC-a, PEUPC-b, and PEUPC-c with a thickness of 45, 42, 35, 46, and 45 nm, respectively, which were suspended in water. (Size of SiO_2 substrate: 3×3 cm^2.)

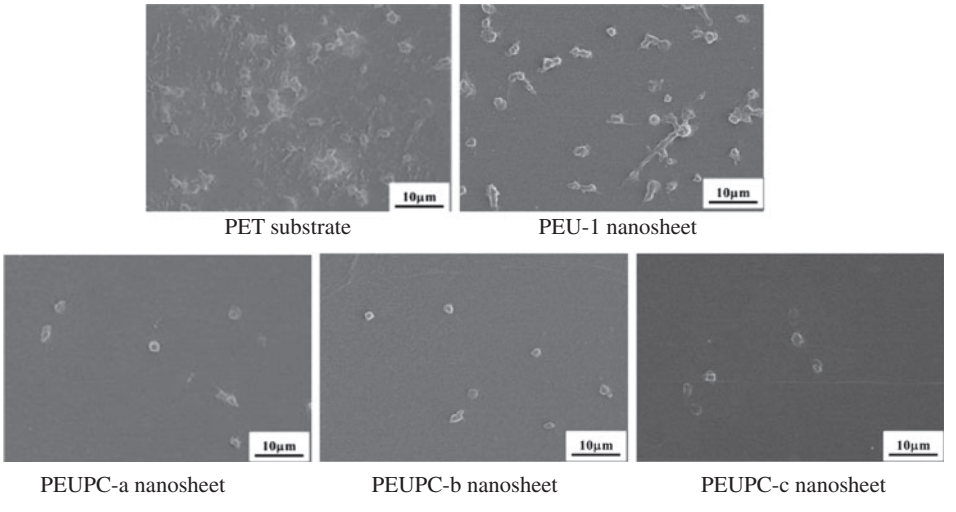


Figure 8. SEM images of nanosheet-coated surfaces on PET substrates after contact with human PRP for 2 h at 37 °C.

shrink after immersing the spin-coated substrates in water. Probably, the polymer thin films would be extended by the centrifugal force in the spin coating, and the films shrunk by their elasticity after released from the substrate. This tendency was not observed in the fabrications of nanosheets from non-elastic polymers.[25]

In this procedure, the thickness of the obtained nanosheets could be controlled by changing the concentration of polymer solution. For example, the PEUPC-a nanosheets with the thickness of 35, 125, and 588 nm were obtained from its chloroform solutions with the concentration of 10, 20, and 40 mg/mL, respectively. Then, we explored the adhesive strength attached on SiO_2 substrate using a novel scratch tester for thin films.[32] When the thickness was below 100 nm, the adhesion strength was significantly increased, which indicates that the nanosheet with a large contact area could conform to the substrate surface because of its exquisite flexibility and low roughness

as described in the literature.[25] The adhesive strengths of the nanosheets prepared from PEUPC-a, PEUPC-b, PEUPC-c, PEU-1, and PEU-2 were in the values of 1.70×10^5 N/m (thickness: 35 nm), 7.50×10^4 N/m (thickness: 46 nm), 9.55×10^4 N/m (thickness: 45 nm), 3.20×10^4 N/m (thickness: 72 nm), and 8.30×10^4 N/m (thickness: 62 nm), respectively. Consequently, the greatest benefit of the nanothickness is the high potential to adhere.

Furthermore, the blood compatibility of the nanosheet-coated surfaces on PET plates was evaluated as shown in Figure 8. Obviously, the reduction of platelet adhesion was observed in these images, where few platelets are adhered. Therefore, it was confirmed that the blood compatibility of the PC-containing copoly(ester-urethane)s was maintained in the surface of the ultra-thin films.

4. Conclusions

Copoly(ester-urethane)s containing PC group and polycarbonate segment were successfully synthesized, and it was found that the obtained polymers exhibited the high thermal stability up to 200 °C and the elasticity derived from the soft segment. The heat resistance of these polymers would be sufficient for the thermal sterilization processes. It was also found that the introduction of comonomers to construct the hard segment was effective to improve the mechanical strength and the elasticity of polymer films. The introduction of such a polar phospholipid group was effective to improve the resistance to the adhesions of proteins and platelets on the polymer film, which was the result of surface properties derived from the PC moiety. On the other hand, the ultra-thin films (nanosheet) could be successfully fabricated from the PC-containing copoly(ester-urethane)s by spin-coating method. It was revealed that the obtained nanosheets showed the flexibility, the high adhesive strength, and the blood compatibility. On the other hand, Okamura et al. have reported that the dispersion of fragmented nanosheets in water was useful for a new patchwork coating on the several shapes of devices.[26] Therefore, if the fragmented nanosheets of the PC-containing polymers could be successfully fabricated, a new aqueous coating material is expected to be developed to construct a biocompatible surface on the several medical devices, which is currently in progress.

Acknowledgments

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References

[1] Lelah MD, Cooper SL. Polyurethanes in medicine. Boca Raton (FL): CRC Press; 1986.
[2] Eisenbach CD, Fischer K, Hayen H, Nefzger H, Ribbe A, Stadler E. Polyurethane elastomers, segmented (non-hydrogen bonding systems). Polym. Mater. Encycl. Boca Raton (FL): CRC Press. 1996;9:6957–6968.
[3] Kang IK, Kwon OH, Kim MK, Lee YM, Sung YK. *In vitro* blood compatibility of functional group-grafted and heparin-immobilized polyurethanes prepared by plasma glow discharge. Biomaterials. 1997;18:1099–1107.
[4] Mathur AB, Collier TO, Kao WJ, Wiggins M, Schubert MA, Hiltner A, Anderson JM. *In vivo* biocompatibility and biostability of modified polyurethanes. J. Biomed. Mater. Res. 1997;36:246–257.

[5] Flemming RG, Proctor RA, Cooper SL. Bacterial adhesion to functionalized polyurethanes. J. Biomater. Sci., Polym. Ed. 1999;10:679–697.
[6] Roh HW, Song MJ, Han DK, Lee DS, Ahn JH, Kim SC. Effect of cross-link density and hydrophilicity of PU on blood compatibility of hydrophobic PS/hydrophilic PU IPNs. J. Biomater. Sci., Polym. Ed. 1999;10:123–143.
[7] Lee JH, Ju YM, Kim DM. Platelet adhesion onto segmented polyurethane film surfaces modified by addition and crosslinking of PEO-containing block copolymers. Biomaterials. 2000;21:683–691.
[8] Ishihara K, Ueda T, Nakabayashi N. Preparation of phospholipid polymers and their properties as polymer hydrogel membranes. Polym. J. 1990;22:355–360.
[9] Ueda T, Oshida H, Kurita K, Ishihara K, Nakabayashi N. Preparation of 2-methacryloxyethyl phosphorylcholine copolymers with alkyl methacrylates and their blood compatibility. Polym. J. 1992;24:1259–1269.
[10] Ishihara K, Hanyuda H, Nakabayashi N. Synthesis of phospholipid polymers having a urethane bond in the side chain as coating material on segmented polyurethane and their platelet adhesion-resistant properties. Biomaterials. 1995;16:873–879.
[11] Ishihara K, Tanaka S, Furukawa N, Kurita K, Nakabayashi N. Improved blood compatibility of segmented polyurethanes by polymeric additives having phospholipid polar groups. I. Molecular design of polymeric additives and their functions. J. Biomed. Mater. Res. 1996;32:391–399.
[12] Ishihara K, Shibata N, Tanaka S, Iwasaki Y, Kurosaki T, Nakabayashi N. Improved blood compatibility of segmented polyurethane by polymeric additives having phospholipid polar group. II. Dispersion state of the polymeric additive and protein adsorption on the surface. J. Biomed. Mater. Res. 1996;32:401–408.
[13] Iwasaki Y, Aiba Y, Morimoto N, Nakabayashi N, Ishihara K. Semi-interpenetrating polymer networks composed of biocompatible phospholipid polymer and segmented polyurethane. J. Biomed. Mater. Res. 2000;52:701–708.
[14] Ishihara K, Iwasaki Y. Biocompatible elastomers composed of segmented polyurethane and 2-methacryloyloxyethyl phosphorylcholine polymer. Polym. Adv. Technol. 2000;11: 626–634.
[15] Morimoto N, Iwasaki Y, Nakabayashi N, Ishihara K. Physical properties and blood compatibility of surface-modified segmented polyurethane by semi-interpenetrating polymer networks with a phospholipid polymer. Biomaterials. 2002;23:4881–4887.
[16] Adhikari R, Danon SJ, Bean P, Le T, Gunatillake P, Ramshaw JAM, Werkmeister JA. Evaluation of in situ curable biodegradable polyurethanes containing zwitterion components. J. Mater. Sci. – Mater. Med. 2010;21:1081–1089.
[17] Nagase Y, Oku M, Iwasaki Y, Ishihara K. Preparations of aromatic diamine monomers and copolyamides containing phosphorylcholine moiety and the biocompatibility of copolyamides. Polym. J. 2007;39:712–721.
[18] Horiguchi K, Shimoyamada N, Nagawa D, Nagase Y, Iwasaki Y, Ishihara K. Syntheses of a novel diamine monomer and aromatic polyamides containing phosphorylcholine group. Trans. Mater. Res. Soc. Jpn. 2008;33:1261–1264.
[19] Nagase Y, Nakajima S, Oku M, Iwasaki Y, Ishihara K. Synthesis and properties of segmented poly(urethane-urea)s containing phosphorylcholine moiety in the side-chain. Polym. J. 2008;40:1149–1156.
[20] Nagase Y, Horiguchi K. Biomedical engineering – frontiers and challenges. Croatia: InTech; 2011. Chapter 11, Biocompatible polyamides and polyurethanes containing phospholipid moiety; pp. 217–232.
[21] Narita Y, Sirithep W, Okamura Y, Nagase Y. Syntheses and biocompatibility of elastomers containing phospholipid polar groups. Kobunshi Ronbunshu. 2013;70:199–208.
[22] Sakagami Y, Horiguchi K, Narita Y, Sirithep W, Morita K, Nagase Y. Syntheses of a novel diol monomer and polyurethane elastomers containing phospholipid moieties. Polym. J. 2013;45:1159–1166.
[23] Sirithep W, Narita Y, Nagase Y. Syntheses and physical properties of polyester and poly (ester-urethane) containing phosphorylcholine moiety. Trans. Mater. Res. Soc. Jpn. 2013;38:473–476.

[24] Khan I, Smith N, Jones E, Finch DS, Cameron RE. Analysis and evaluation of a biomedical polycarbonate urethane tested in an in vitro study and an ovine arthroplasty model. Part I: materials selection and evaluation. Biomaterials. 2005;26:621–631. Part II: in vivo investigation. 633–643.
[25] Okamura Y, Kabata K, Kinoshita M, Saito D, Takeoka S. Free-standing biodegradable poly (lactic acid) nanosheet for sealing operations in surgery. Adv. Mater. 2009;21:4388–4392.
[26] Okamura Y, Kabata K, Kinoshita M, Miyazaki H, Saito A, Fujie T, Ohtsubo S, Saito D, Takeoka S. Fragmentation of poly(lactic acid) nanosheets and patchwork treatment for burn wounds. Adv. Mater. 2013;25:545–551.
[27] Tanaka H, Kunimura M. Mechanical properties of thermoplastic polyurethanes containing aliphatic polycarbonate soft segments with different chemical structures. Polym. Eng. Sci. 2002;42:1333–1349.
[28] Eceiza A, Martin MD, de la Caba K, Kortaberria G, Gabilondo N, Corcuera MA, Mondragon I. Thermoplastic polyurethane elastomers based on polycarbonate diols with different soft segment molecular weight and chemical structure: mechanical and thermal properties. Polym. Eng. Sci. 2008;48:297–306.
[29] Kojio K, Furukawa M, Motokucho S, Shimada M, Sakai M. Structure−mechanical property relationships for poly(carbonate urethane) elastomers with novel soft segments. Macromolecules. 2009;42:8322–8327.
[30] Chung YC, Choi JW, Shin SJ, Chun BC. Laterally crosslinked polyurethane copolymers with polycarbonate as a soft segment. Fibers Polym. 2012;13:815–822.
[31] Shattil SJ, Newman PJ. Integrins: dynamic scaffolds for adhesion and signaling in platelets. Blood. 2004;104:1606–1615.
[32] Baba S, Midorikawa T, Nakano T. Unambiguous detection of the adhesive failure of metal films in the microscratch test by waveform analysis of the friction signal. Appl. Surf. Sci. 1999;144:344–349.