Surface Modification and *In Vitro* Blood Compatibilities of Polyurethanes Containing *z*-Lysine Segments in the Main Chain

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ABSTRACT: Polyurethanes containing *z*-lysine segments in the main chain (PULL) were synthesized from 4,4′-diphenylmethyl diisocyanate, poly(tetramethylene glycol), and *z*-lysine oligomer as a chain extender. PULL film was treated with a 10% HBr–acetic acid solution and subsequently with a saturated sodium bicarbonate aqueous solution to produce a primary amine group on the surface. Heparin-immobilized PULL (PULL-H) was prepared by the coupling reaction of PULL surface amine groups and heparin carboxylic acid groups. The surface-modified PULLs were then characterized by attenuated total reflection–Fourier transform infrared spectroscopy, electron spectroscopy for chemical analysis, and a contact angle goniometer. The concentration of amine groups and heparins introduced on the PULL surfaces were about 2.1 μmol/cm^2 and 1.9 μg/cm^2, respectively. To assess *in vitro* blood compatibility, the interaction of modified PULLs with plasma proteins and platelets was examined. In the experiments with plasma proteins, plasma recalcification time and activated partial thromboplastin time were significantly prolonged on PULL-H compared to those on the other surfaces. The percentage of serotonin released from platelets adhering on PULL-H was less than that on the other surfaces, demonstrating a good *in vitro* blood compatibility. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 1959–1969, 2003

Key words: films; oligomers; polyurethanes; surfaces; ATR-FTIR

INTRODUCTION

Blood compatibility is one of the most important factors for material to be used in artificial organs. Coagulation often happens when blood flows in artificial organs. The interaction of materials with blood components can be reduced when introducing blood-compatible molecules to the surface of artificial organs.[1]

Segmented polyurethanes (PUs) have been widely used in a number of blood-contacting devices, such as catheters, heart assist pumps, and artificial hearts, and their relatively good blood compatibility and physical properties such as strength, flexibility, flexural endurance, and fatigue resistance make them primary candidates for cardiovascular implants.[2,3] The antithrombogenicity of PU, however, needs to be significantly improved for further biomedical applications.[4–6]

Different chemicals,[7,8] bioactive ligands,[9–11] and methods[12–14] have been investigated to improve the hemocompatibility of surfaces when they come into contact with blood. One of the methods for surface modification is the immobilization of natural glycosaminoglycans,[15,16] such as heparin,[17,18] which inhibit the transformation of fibrinogen into fibrin and promote low levels of platelet adhesion,[19] and chondroitin sulfate and hyaluronic acid, which reduce platelet adhesion.[20] There are two general methods to develop thromboresistant polymeric materials using heparin. One is the chemical immobilization of heparin onto a polymer surface by ionic bonding, covalent bonding, and plasma glow discharge treatment. The other is a heparin-releasing system that mixes heparin with polymer and allows heparin to be released out of the polymeric matrix.[21]

In previous work,[22] we prepared poly(ether urethane) containing diester groups in the side chain, and introduced carboxylic acid groups to the film surface by hydrolysis reaction for use as a reaction site with heparin. The amount of heparin immobilized on the film surface was 0.84–0.92 μg/cm^2, which is lower than that (1.16–1.3 μg/cm^2) obtained by plasma glow discharge treatment as reported previously.[23] Kim et al.[24] attempted a novel method to increase the reaction site for heparin immobilization. They grafted a polyfunctional compound on a PU surface through diisocyanate groups, introduced a poly(ethylene oxide) (PEO) spacer onto the grafted surface, and then covalently immobilized heparin onto the end group of the grafted PEO.

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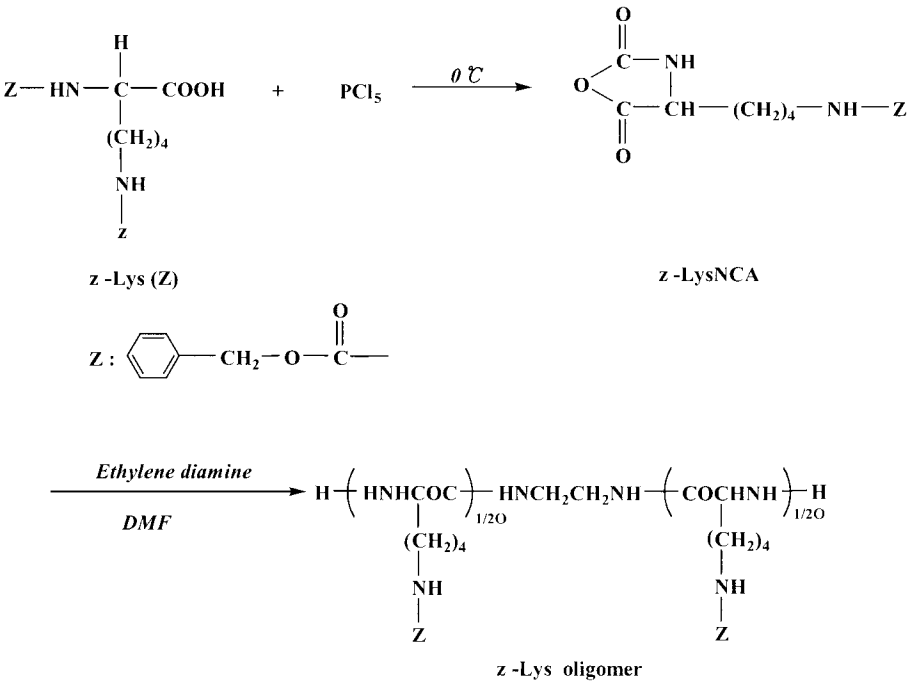


Scheme 1 Synthesis of *z*-lysine oligomer.

The aim of this work was to introduce primary amines on the PU surface and couple them with heparin. To achieve this, polyurethanes containing *z*-lysine segments in the main chain (PULL) were synthesized by the reaction of isocyanate-terminated polyurethane prepolymers and amine-terminated poly(ϵ-benzyloxycarbonyl L-lysine) oligomers. Heparin-immobilized PULL films were prepared with HBr–acetic acid followed by washing with citric acid. Subsequently, this caused a reaction with heparin. The surface-modified PULLs were characterized by attenuated total reflection–Fourier transform infrared (ATR-FTIR) spectroscopy, electron spectroscopy for chemical analysis (ESCA), and a contact angle goniometer. To evaluate the *in vitro* blood compatibility of the modified PULLs, the interaction of surface-modified PULLs with blood components such as plasma proteins and platelets was also examined.

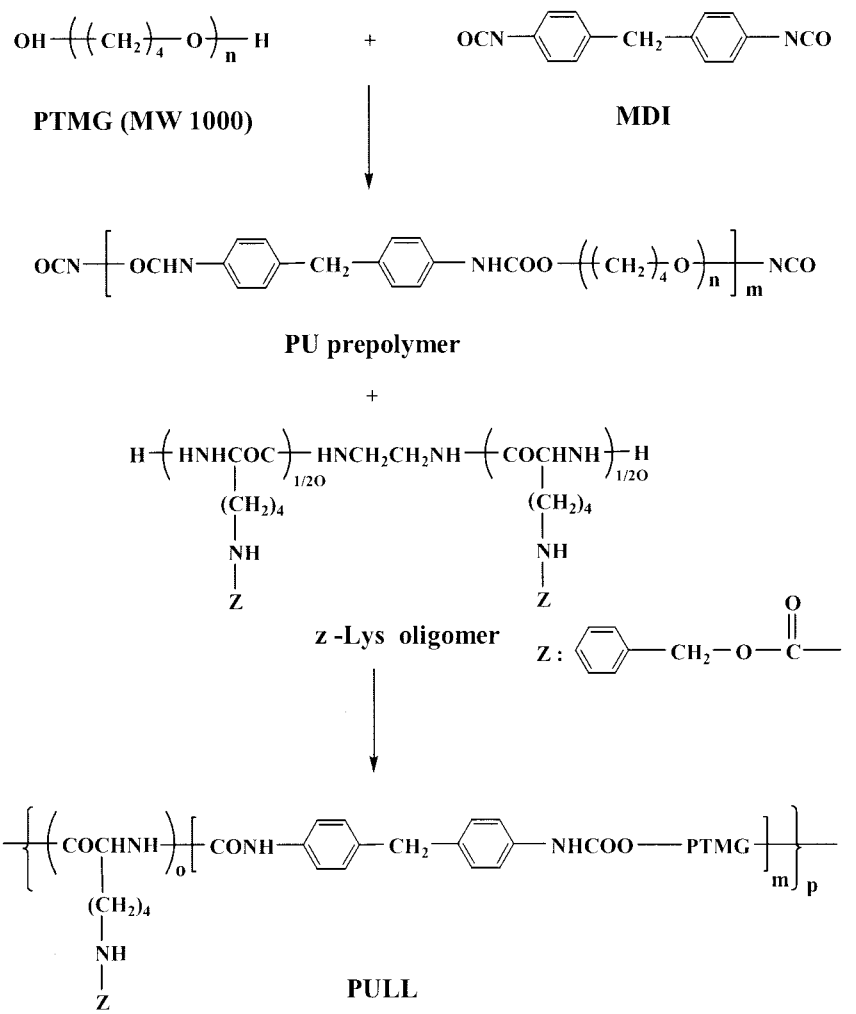
EXPERIMENTAL

Synthesis of PULL

A solution of L-lysine hydrochloride (75.3 g, 410 mmol, TCI Co., Tokyo, Japan) and sodium hydroxide (80 g, 2 mol) dissolved in distilled water (500 mL) was cooled to 0°C and benzyloxycarbonylchloride (*z*-Cl, 196 g, 1.14 mol, Wako Chemical Co., Osaka, Japan) was added drop by drop, followed by vigorously stirring. After 2 h, the solution was extracted with diethyl ether, unused *z*-Cl was removed, and the aqueous layer acidified. The oil was isolated with diethyl ether. N^{α},N^{ϵ}-Dicarbobenzoxy-L-lysine [*z*-Lys (*z*)] was extracted from the reaction solution using benzene and evaporated to produce white crystals. The crystal mixture was dried under reduced pressure.[25] Yield: 89%.

z-Lysine *N*-carboxyanhydride (*z*-LysNCA) was synthesized using phosphorus pentachloride, as shown in **Scheme 1**. Briefly, *z*-lysine (*z*) (20 g) was mixed with diethyl ether (100 mL) in a flask and the mixture was suspended using a magnetic stirrer. Phosphorus pentachloride (12 g) was then added to the mixed solution and stored at 0°C for 30 min. The reaction solution was precipitated in *n*-hexane (300 mL) and kept at 0°C. The precipitates were filtrated using a glass filter and dried at room temperature for 24 h. The samples were then recrystallized using ethyl acetate to produce *z*-LysNCA and dried at room temperature under reduced pressure. The *z*-lysine oligomer was synthesized by ring-opening polymerization. Briefly, *z*-LysNCA (14.5 g, 0.0475 mol) was dissolved in dimethylformamide (DMF, 10 mL) and mixed with ethylene diamine (0.33 mL, 0.0049 mol). The solution was kept at room temperature for 2 h to produce primary amine-terminated *z*-lysine oligomers.

The PU prepolymer was prepared by reacting 4,4′-diphenylmethane diisocyanate (MDI; Wako Chemical) with poly(tetramethylene glycol) (PTMG, M_n: 1000, Polyscience Co., Warrington, PA) at the molar ratio of 1.15 : 1. *z*-Lysine oligomers dissolved in DMF (20 mL) were then added drop by drop to a flask containing PU prepolymer, to produce polyurethanes containing *z*-lysine segments in the main chain (PULL), as shown in **Scheme 2**. The reaction solution was diluted using DMF (10 mL) when its viscosity



Scheme 2 Synthesis of poly(ether urethane)s containing *z*-lysine segments (PULL).

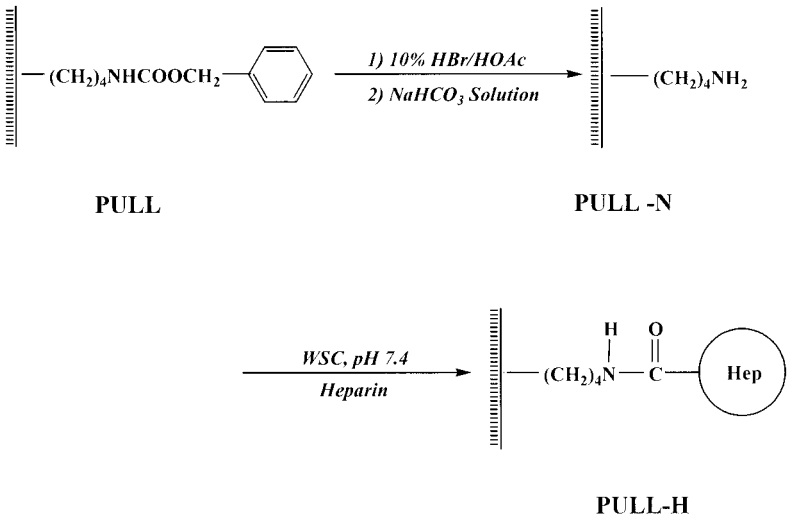
increased. Finally, butylamine diluted by DMF (10 mL) was added to the reaction solution to stop further reaction with the terminal isocyanate group. The reaction solution was then precipitated in distilled water and the precipitates were washed with methanol three times and dried under reduced pressure at 60°C for 24 h. The PU prepolymer was also chain-extended with ethylene diamine to produce polyetherurethaneurea (PU). This was used as the control.

Preparation of PULL film

A 10 wt % dimethylformamide solution of PULL was used for the preparation of the sample film. The solution was spread onto a glass plate (6 × 6 cm^2) and the solvent was evaporated at room temperature. The concentrated PULL solution was then irradiated by an infrared lamp to evaporate the residual solvent. The glass plate was immersed in distilled water to separate the film from the plate. The film was finally dried under reduced pressure at room temperature for 48 h.

Surface modification

To introduce amine groups to the surfaces, the PULL film was immersed in a 10% HBr–acetic acid solution for 30 s at room temperature and subsequently washed with diethyl ether, ethanol, saturated sodium bicarbonate solution, and deionized water, as shown in **Scheme 3**. The film was then washed with 0.01 wt % Triton X-100 aqueous solution, with distilled water, and dried under reduced pressure for 24 h at 30°C. The concentrations of amine groups introduced to the surfaces were determined using the modified fluorescamine interaction method previously reported.[26] For the preparation of the dye solution, fluorescamine (10 mg, Aldrich Chemical, Milwaukee, WI) was dissolved in 100 mL of acetone. Amine group–introduced PULL (PULL-N, 3 × 6 cm^2) was soaked in the dye solution for 1 min and removed. The initial dye solution and the residual dye solution were mixed separately with the excess amount of *n*-butylamine to produce the fluorescamine–amine complexes. The different de-



Scheme 3 Surface modification of PULL film.

grees of absorbance of both complexes at 495 nm were measured using a spectrophotometer (Shimadzu UV-2100, Kyoto, Japan). The concentrations of amino groups introduced to the surfaces were calculated from the calibration curve between a decrease in absorbance of fluorescamine–amine complexes at 495 nm and the concentration of *n*-butyl amine in the dye reagent.

Mechanical properties

The mechanical properties of hydrolyzed films (0.04 $\times$ 1.5 $\times$ 6 cm^3) were measured using a tensilometer (Model No. 4465; Instron, Canton, MA) at a speed of 50 mm/min.

Heparin immobilization

Heparin sodium salt (170 USP units/mg; Sigma Chemical, St. Louis, MO) was dissolved in a 0.075*M* sodium citrate solution (pH 4.7, 80 mL) to convert sodium carboxylate into carboxylic acid and was activated by the addition of water soluble carbodiimide (WSC, 80 mg). The PULL-N film (6 $\times$ 6 cm^2) was then immersed in the heparin solution for 24 h at 4°C to produce heparin-immobilized PULL (PULL-H). After heparin immobilization, the film was washed with citric acid and 0.1% Triton X-100 aqueous solution and subsequently rinsed with distilled water in an ultrasonic cleaner for 10 min. The amount of heparin immobilized on the surfaces was determined by the toluidine blue method previously reported.[22]

Toluidine blue (25 mg; Fluka Chemie, Milwaukee, WI) was dissolved in a 0.01*N* hydrochloric acid containing 0.2 wt % NaCl. A known amount of aqueous heparin solution (2 mL) was added to the toluidine blue solution (3 mL). The solution was then agitated using a Vortex mixer. *n*-Hexane (3 mL) was then added, and the mixture was shaken so that the toluidine blue–heparin complex was extracted to the organic layer. The amount of unextracted toluidine blue remaining in an aqueous phase was determined with the absorption at 631 nm. The linear relationship between the absorbance of residual toluidine blue at 631 nm and the concentration of heparin in an aqueous solution was obtained and used as the calibration curve to determine the amount of immobilized heparin. To determine the amount of immobilized heparin, toluidine blue solution (3 mL) was mixed with an aqueous solution (2 mL) and the PULL-H film (1 $\times$ 1 cm^2) was immersed in the mixed solution for 30 min. *n*-Hexane (3 mL) was then added to ensure uniformity in treatment. The mixture was then shaken. After removal of the PULL-H–dye complex, the aqueous layers of the solution were sampled. The absorbance at 631 nm was then measured and the amount of immobilized heparin was calculated from the previously constructed calibration curve.

Surface characterization

The surface-modified PULLs were analyzed using ESCA (ESCALAB MKII, V.G. Scientific Co., East Grinstead, UK) equipped with Al–K_α at 1487 eV and 300 W power at the anode. Survey scan spectra were taken and surface elemental compositions relative to carbon were calculated from peak heights with a correction for atomic sensitivity.[27] The surfaces of modified PULLs were investigated using a Midac spectrophotometer equipped with a ZnSe reflection element. The water contact angles of the modified surfaces were also measured at room temperature using a contact angle goniometer (Model 100-0, Ramé–Hart, Moun-

tain Lakes, NJ) to evaluate the wettability of the samples.[28]

Thrombus formation

Blood (30 mL) from a healthy volunteer was collected and mixed with an aqueous solution containing anhydrous D-glucose (0.136*M*), sodium citrate dihydrate (0.075*M*), and citric acid monohydrate (0.0004*M*) (ACD, 3 mL). The surface-modified PULL film (3 × 3 cm^2) was attached to a watch glass (diameter 7.5 cm) and subjected to a thrombus formation test. The test was carried out according to a previously reported method.[29] Briefly, the ACD-blood (200 μL) was added to the sample and incubated at 37°C in a constant temperature bath. The clotting reaction was initiated by adding a 0.1*M* aqueous $CaCl_2$ solution (20 μL) to the blood. The sample was shaken gently by hand to homogeneously mix the chemicals and blood. Distilled water (5 mL) was then added to stop the reaction after 20 min of incubation. The thrombus formed was then removed with a spatula and transferred into distilled water (5 mL) in a watch glass, left for 5 min at room temperature, and then placed in a 37°C aqueous formaldehyde solution (5 mL) to fix the thrombus. The thrombus was dried at a reduced pressure until the sample weight remained unchanged. Finally, the resulting weight of the thrombus relative to that formed on the glass was calculated. The experiment was carried out in quadruplicate and a mean value was recorded.

Plasma recalcification time (PRT)

Human blood containing 10% ACD was centrifuged at 3000 *g* for 10 min to separate the blood cells. The resulting platelet-poor plasma (PPP) was used for the PRT experiment. PPP (300 μL) was placed on a sample film (3 × 3 cm^2) attached to a watch glass (diameter 4 cm), and incubated statically at 37°C. Thereafter, a 0.025*M* $CaCl_2$ aqueous solution (300 μL) was added to the PPP and the plasma solution was monitored for clotting by manually dipping a stainless-steel wire hook coated with silicone into the solution to detect any fibrin threads. Clotting times were recorded at the first sign of fibrin formation on the hook.[26] The experiment was carried out in triplicate and a mean value was recorded.

Activated partial thromboplastin time (APTT)

The sample film (3 × 3 cm^2) was attached to a glass (diameter 4 cm, height 1.5 cm) and preswelled with distilled water (2 mL). The plasma solution (100 μL) and an actin-activated cephaloplastin reagent (100 μL; Baxter Diagnostics Inc., Deerfield, IL) were then added to the sample film, followed by the addition of a 0.025*M* $CaCl_2$ solution (100 μL) after 5 min of incubation.[30] The clotting time of the plasma solution was monitored as described in the PRT experiment. The experiment was carried out in triplicate and a mean value was recorded.

Adhesion and activation of platelets

ACD-blood was centrifuged at 180 *g* for 10 min to obtain platelet-rich plasma (PRP). The PRP (100 μL) was then placed on surface-modified PULLs (3 × 3 cm^2) at 37°C for 30 and 60 min, respectively. Phosphate-buffered saline (PBS, 6 mL) was then added to the PRP and incubated for 1 min to stop further platelet adhesion. The number of platelets adhering to the samples was determined by measuring the lactate dehydrogenase (LDH) activity of the cells lysed with Triton X-100. A linear relationship was obtained between the LDH activity of the aliquots of the cell suspension and the number of platelets counted with a hemocytometer. The LDH activity was determined by measuring the initial rate of nicotinamide adenine dinucleotide hydride (NADH) oxidation in the presence of pyruvate. The assay was carried out in a solution containing sodium phosphate (50 m*M*), NADH (0.06 m*M*, Sigma), and pyruvate (0.2 m*M*) at pH 7.5 in the presence of Triton X-100 (0.02 wt %; Sigma). According to a Shimadzu UV-2100 spectrophotometer, the NADH oxidation was followed by a decrease in absorbance at 340 nm.[30]

The serotonin released from the adhering platelets was measured as follows: from preliminary tests using surface-modified PULLs, the serotonin amount released was basically uninfluenced by the presence of imipramine. The following platelet experiment, therefore, was carried out in the absence of imipramine. A surface-modified PULL (diameter 1.5 cm) was attached to a 24-well plate and kept at 37°C. PRP (500 μL) was then placed on the sample. After 30 or 60 min of incubation, 0.02*M* ethylenediamine tetraacetic acid (EDTA, 500 μL) was added to stop any further platelet release reaction. The platelet and plasma layers were then transferred to different containers after centrifugation at 18,000 *g*. Thereafter, 200% trichloroacetic acid (TCA, 200 μL; TCI Co.) was added to both the platelet and plasma layers to aggregate the proteins. The proteins aggregated by the TCA were removed by centrifugation and the resulting protein-free samples were transferred to a glass tube containing 2 mL of an *o*-phthalaldehyde solution (10 mL of 0.5% *o*-phthalaldehyde ethanol + 100 mL of 8*N* hydrochloric acid) and subsequently incubated at 100°C for 10 min. The excess TCA was extracted with chloroform (2 mL) and the fluorescent intensity of the aqueous layer phase at 475 nm was measured using a Shimadzu RF-5000 spectrophotometer. The amount of serotonin remaining in both the platelet and plasma layers was calcu-

lated from a standard calibration curve, constructed according to the relationship between fluorescent intensity and the known concentration of serotonin creatinine sulfate.[31]

Scanning electron microscopy analysis

The platelets adhering to the surface-modified PULLs were dipped into 0.1% glutaraldehyde aqueous solution (pH 7.2) at 37°C for 30 min. The films were then dehydrated in a graded series of ethanol, dried in a Hitachi model HCP-2 (Hitachi, Ibaraki, Japan) critical point drier using liquid CO_2 as the transition liquid, and finally sputter-coated with platinum. These samples were observed with a Hitachi S-510 scanning electron microscope (SEM).[31]

Statistical analysis

Differences between the PU control and the surface-modified PULLs were analyzed statistically using the two-sample *t*-test. The differences observed between samples were considered significant for p values not greater than 0.05.

RESULTS AND DISCUSSION

Preparation and characterization of surface-modified PULLs

Polyurethanes containing *z*-lysine segments in the main chain (PULL) were obtained by the reaction of a *z*-lysine oligomer and an isocyanate-terminated polyurethane prepolymer (see **Scheme 2**). The intrinsic viscosity of polyurethane prepolymer and PULL, as measured in DMF solution, were 0.52 and 0.68 dL/g, respectively. Figure 1 shows the infrared spectra of

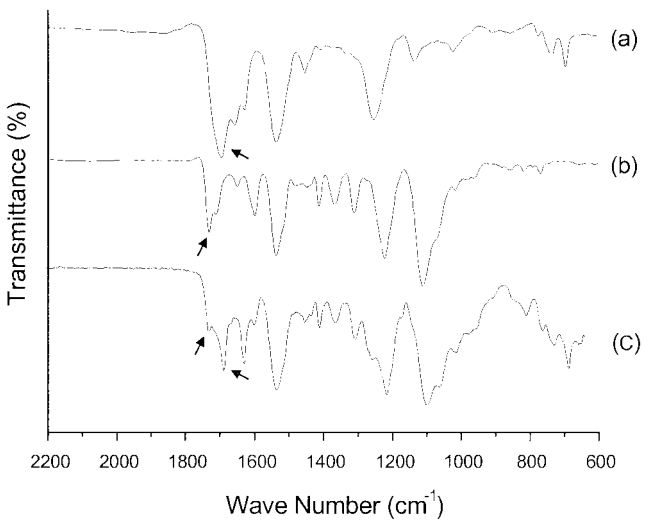


Figure 1 FTIR spectra of (a) *z*-lysine oligomer, (b) PU prepolymer, and (c) PULL.

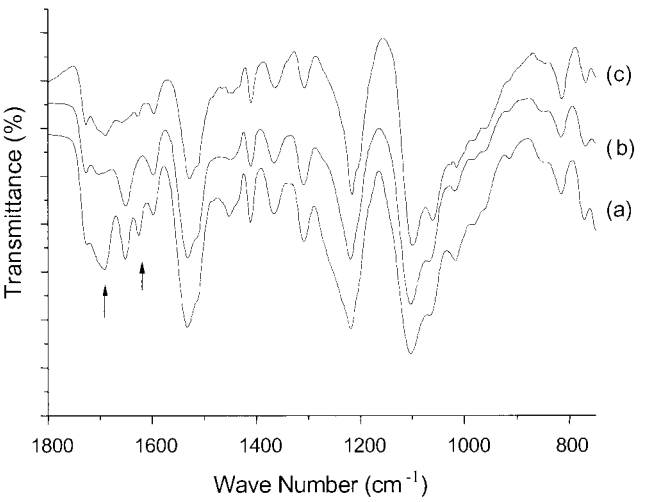


Figure 2 ATR-FTIR spectra of (a) PULL, (b) PULL-N, and (c) PULL-H.

z-lysine oligomer (a), PU prepolymer (b), and PULL (c). The absorption of urethane bond (–O-*CO*NH–) of a *z*-lysine oligomer and a PU prepolymer appeared at 1695 and 1729 cm^{-1}, respectively. After the reaction of *z*-lysine oligomers with PU prepolymers, both peaks at 1695 and 1729 cm^{-1} appeared, as shown in Figure 1(c), indicating the successful synthesis of PULL. The PULL film, obtained by the solvent-casting method, was treated with 10% HBr–acetic acid and subsequently immersed in a heparin aqueous solution in the presence of water-soluble carbodiimide to immobilize heparins on the surfaces. Figure 2 shows the ATR-FTIR spectra of surface-modified PULL films. The ATR-FTIR spectrum of PULL [Fig. 2(a)] showed absorptions at 1630 and 1650 cm^{-1}, based on the conformation of peptide bonds. The absorption at 1690 cm^{-1}, possibly attributed to the urethane bond of the side chain in PULL, largely decreased after surface hydrolysis, as shown in Figure 2(b). The spectrum of heparin-immobilized PULL (PULL-H), however, was not much different from that of PULL-N.

To evaluate the wettability of surface-modified PULLs, the water contact angles of the surfaces were measured using the sessile droplet method, results of which are shown in Table I. The PULL water contact angle of 69° decreased after the introduction of an amino group (PULL-N, 42°) and heparin immobiliza-

TABLE I
Water Contact Angle of PU and Surface-Modified PULL Films

Sample	Water contact angle[a] (°)
PU	72 ± 3
PULL	69 ± 2
PULL-N	42 ± 5
PULL-H	38 ± 4

[a] Measured by water droplet method.

TABLE II
Mechanical Properties of PULL and Hydrolyzed PULL Film[a]

Sample	Young's modulus (kgf/mm^2)	Stress at break (kgf/mm^2)	Strain at break (%)
PU	1.2	5.3	610
PULL	1.9	5.9	420
PULL-0.5[b]	2.1	5.8	408
PULL-1	2.3	5.8	400
PULL-2	3.5	4.6	362
PULL-3	4.5	3.5	308
PULL-5	5.2	2.3	303

[a] Sample was treated with 10% HBr/acetic acid for different times.
[b] Number means time (in min) used for hydrolysis reaction.

tion (PULL-H, 38°). The hydrolysis reaction of PULL films was carried out using 10% HBr–acetic acid to introduce primary amino groups to the surfaces and the effect of hydrolysis time on the mechanical properties of PULL film was examined. As shown in Table II, the PULL strain (420%) was smaller than that of PU (610%). A decrease in the PULL strain may be attributed to the peptide bond rigidity in the main chains. As the hydrolysis time increases, the PULL stress and strain slightly decrease while the Young's modulus increases.

Changes in the chemical structure of surface-modified PULLs were investigated using ESCA. Figure 3 shows ESCA survey scans of the (a) PULL, (b) PULL-N, and (c) PULL-H surfaces. The surface-modified PULLs show three peaks corresponding to C1s (binding energy, 285 eV), N1s (binding energy, 400 eV), and O1s (binding energy, 532 eV), as expected. The chemical compositions of the surface-modified PULLs calculated from the ESCA survey scan spectra are shown in Table III. The oxygen content (24.8%) of the PULL surface slightly increased after hydrolysis (28.8%), probably because of the chain rearrangement on the surfaces. On the other hand, sulfur (0.7%) was found on the PULL-H surface, indicating successful heparin immobilization. The amount of immobilized heparin, as determined by the toluidine blue method, was about 1.9 $\mu g/cm^2$.

TABLE III
Chemical Composition of Surface-Modified PULLs Calculated from ESCA Survey Scan Spectra

Sample	Atomic percentage (%)			
	C	O	N	S
PU	76.8	21.2	2.0	
PULL	72.4	24.8	2.8	
PULL-N	69.2	28.8	2.0	
PULL-H	68.2	25.9	5.1	0.7

Activation of plasma proteins

Figure 4 shows the amount of thrombus formed on the surface-modified PULLs after 20 min of incubation.

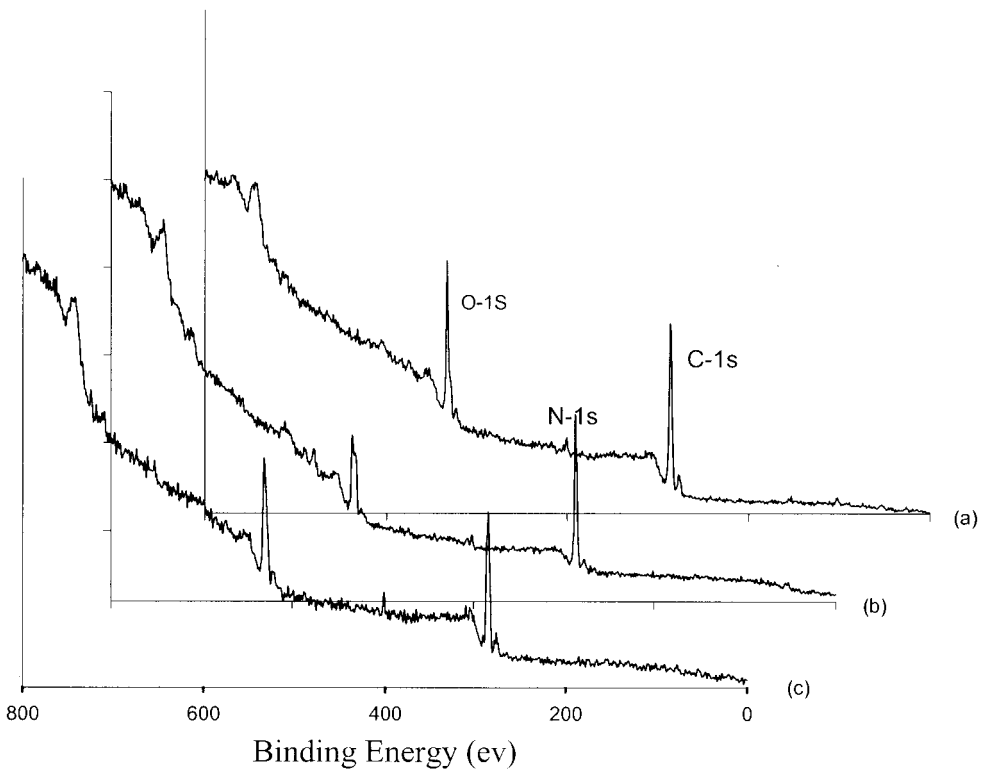


Figure 3 ESCA survey scan spectra of (a) PULL, (b) PULL-N, and (c) PULL-H.

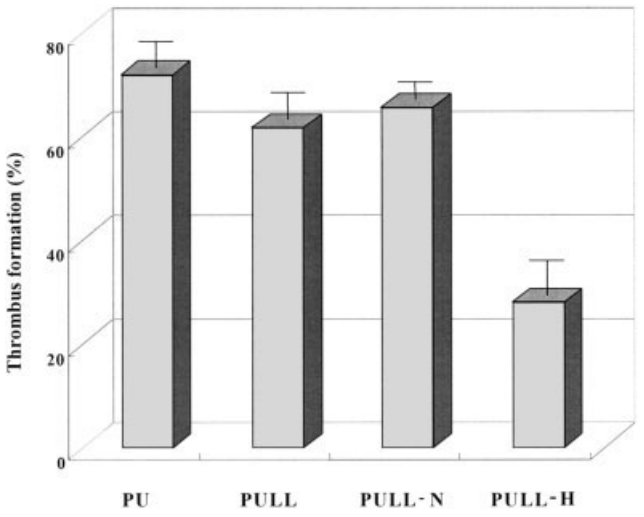


Figure 4 Thrombus formation on PULL and surface-modified PULLs after 20-min incubation.

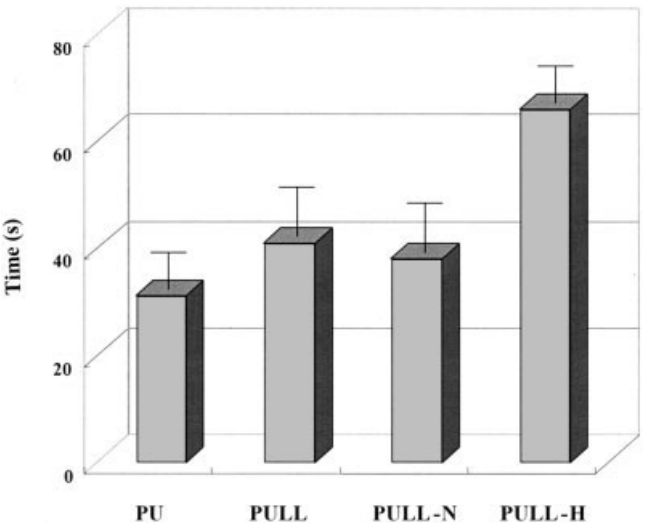


Figure 6 Activated partial thromboplastin time of PULL and surface-modified PULLs.

The thrombus formation rate on the PULL was about 72% as that on glass. The thrombus amount on PULL (59 ± 4%), however, slightly decreased with the introduction of amine (PULL-N, 63 ± 3%, $p < 0.02$). It further decreased through the immobilization of heparin (PULL-H, 26 ± 2%, $p < 0.008$). To elucidate the antithrombogenicity of surface-modified PULLs, the interaction of the samples with plasma proteins and platelets was examined.

The plasma recalcification time (PRT) of surface-modified PULLs is shown in Figure 5. The PRT of PULL (408 s) was longer than that of PU (247 s, $p < 0.005$) but not much different from that of PULL-N (388 s, $p < 0.008$). However, it was significantly elongated on PULL-H (762 s, $p < 0.007$). The activated partial thromboplastin times (APTTs) of the surface-modified PULLs are presented in Figure 6. The APTT of PULL (39 s) was almost the same as that of PU (29 s) within a standard deviation. The APTT of PULL was not prolonged by the introduction of amine groups (PULL-N, 36 s, $p < 0.05$) but was significantly prolonged by heparin immobilization (PULL-H, 64 s, $p < 0.009$). The elongation of APTT of PULL-H indicates that antithrombin III (ATIII) was bound to the immobilized heparins, thus leading to a suppression of thrombin activity.[24]

Activation of platelets

Figure 7 shows the amount of platelets that were adhered to PU control and modified PULLs after 30 and 60 min of incubation. The amount of adhering

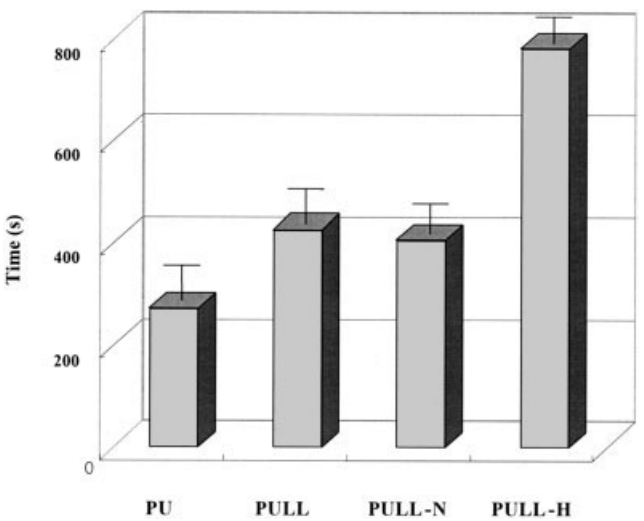


Figure 5 Plasma recalcification time of PULL and surface-modified PULLs.

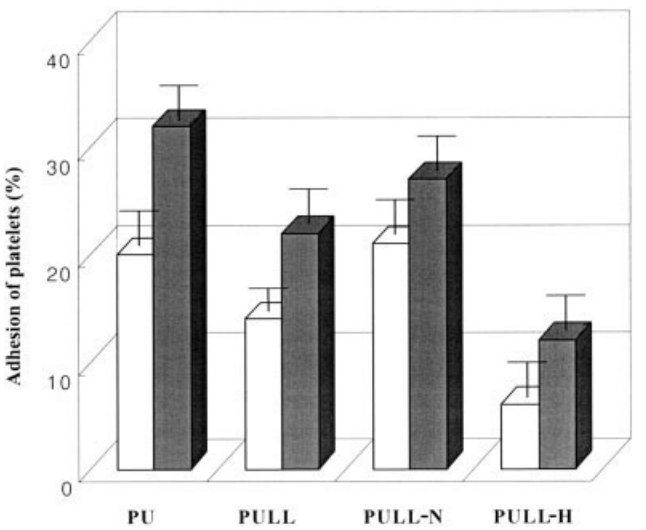


Figure 7 Relative percentage of platelets adhering to the surface of modified PULLs after (□) 30- and (■) 60-min incubation.

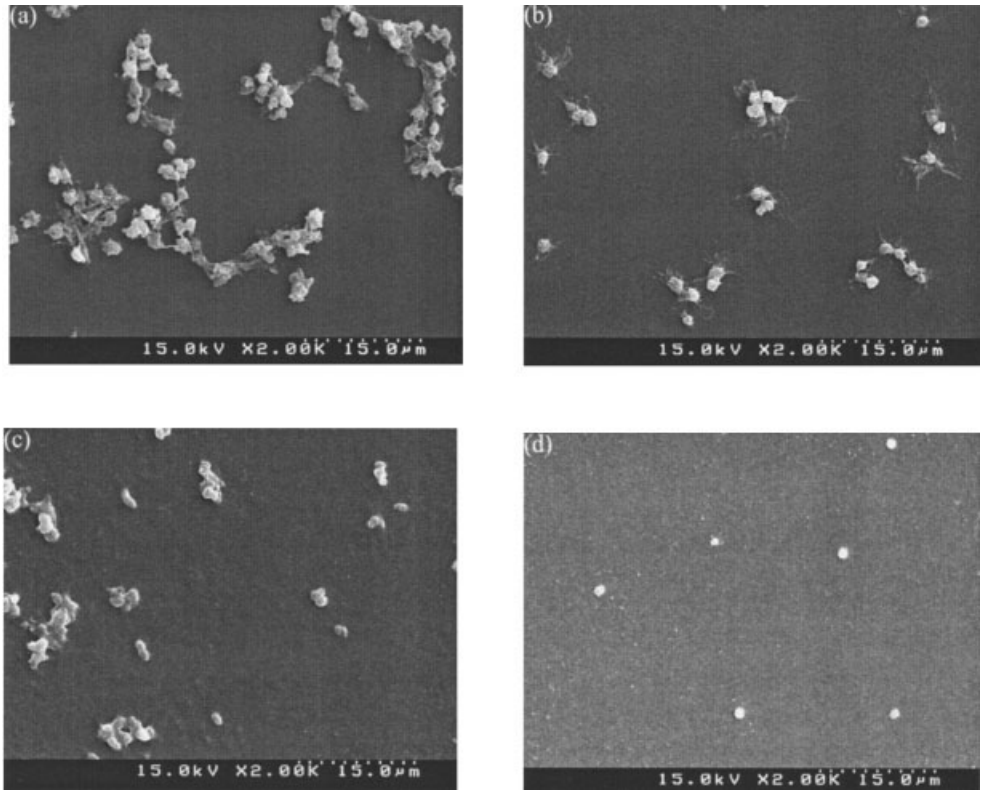


Figure 8 SEM micrographs of platelets adhering to (a) PU, (b) PULL, (c) PULL-N, and (d) PULL-H.

platelets increased when changing the incubation time from 30 to 60 min, irrespective of the kind of sample. Platelet adhesion on PULL (21%) after 60 min of incubation was lower than that on PU (31%, $p < 0.006$). It slightly accelerated, however, with the introduction of amine groups (PULL-N, 26%, $p < 0.04$). It is interesting to note that it was significantly suppressed by the immobilization of heparin (PULL-H, 11%, $p < 0.005$). As shown in Table IV, the serotonin release increased with an increase in platelet incubation time on the surface-modified PULLs. The percentage of serotonin released from the platelets that were adhered to PULL (38%) was not much different from that on PULL-N (45%) when incubating for 60 min. Serotonin release, however, was significantly suppressed on PULL-H (21%) compared to that on the other substrates. Figure 8 shows SEM micrographs of platelets adhering to the surface-modified PULLs after 60 min of incubation. Platelets were aggregated on PU, PULL, and PULL-N. However, they scarcely aggregated at all on PULL-H.

L-Lysine, which has two amino groups (α- and ϵ-amino) and a carboxyl group, is often used as a coupling reagent when designing drug carriers and biomaterials. Ito et al.[33] prepared carboxyl group–protected L-lysine and used it as a chain extender for the preparation of polyurethanes. They introduced carboxyl groups to the PU film surface by hydrolysis reaction and there was a subsequent reaction with heparins. McClung et al.[34] introduced benzophenone and L-lysine to the pendant groups of polyacrylamides and photochemically immobilized them on the surface of polyurethanes. They reported that plasminogen is selectively adsorbed to the polyurethane surface containing lysine residues and adsorbed plasminogen is readily converted to plasmin in the presence of a tissue-plasminogen activator. In this work, we synthesized *z*-lysine oligomers with the ring-opening polymerization of *z*-lysine *N*-carboxyanhydride and directly reacted with PU prepolymers to produce PU containing L-lysine segments in the main chain (PULL).

Up to the present, it has been extremely difficult to obtain a matrix with physiological and biomechanical properties similar to those of natural living tissue. To date, many experiments have been conducted to elucidate the potential of poly(ether urethane) for bio-

TABLE IV
Release of Serotonin from Adhering Platelets on Surface-Modified PULLs[a]

	Serotonin release (%)	
Sample	After 30 min	After 60 min
PU	34 ± 4	47 ± 3
PULL	27 ± 3	38 ± 4
PULL-N	32 ± 3	45 ± 5
PULL-H	13 ± 2	21 ± 3

[a] Measured by *o*-phthalaldehyde method.

medical applications.[33] Phua et al.[34] pointed out that, because enzymes have the characteristic ability to reduce the activation energy of chemical reactions, a degradation process that usually takes place only at high temperature or in the presence of UV light may conceivably take place under physiological conditions in the presence of the proper enzymes. Tiwari et al.[35] developed a compliant graft (trade name MyoLink) based on poly(carbonate-urea)urethane chemistry that has compliance similar to that of the human artery. The graft has undergone *in vitro* degradation tests and has been in a dog model for 36 months, demonstrating very high biostability.[36]

The PULL prepared in this study may have the potential to be degraded hydrolytically or enzymatically because it consists of polyether and peptide segments. The aim of this work was to provide basic information on both the synthesis of polyurethane containing peptide segments in the main chain and the immobilization of heparin on the surfaces. By treating the PULL film with 10% HBr–acetic acid, ϵ-amino groups could be introduced to the surface at a concentration of 2.1 μmol/cm^2. As known from the mechanical property experiment (Table II), PULL showed larger Young's modulus and lower strain than those of PU. The lower strain of PULL seems attributable to the incorporation of rigid PLL segments into the elastic PU backbone.

Heparin has been frequently used as an immobilizing biomolecule for the surface modification of polymeric materials to attain high blood compatibility. In this study, heparins were coupled with the amino groups on the PULL surface, and the amount of immobilized heparin was about 1.9 μg/cm^2. This value is larger than that obtained either by plasma glow discharge treatment of PU (1.16–1.3 μg/cm^2) or by hydrolysis reaction of PU containing diester groups in the side chain (0.84–0.92 μg/cm^2) as previously reported.[22,23] This result suggests that *z*-lysine oligomer segments are very useful for the covalent immobilization of heparin on the surface.

From the APTT experiment it was found that the activation of plasma proteins was significantly suppressed on PULL-H (64 s) compared to the other substrates (Fig. 6). This result suggests that ATIII was bound to the immobilized heparin, thereby leading to a suppression of thrombin activity. Rosenberg et al.[37] reported the overall mechanism by which thrombin is activated by ATIII. They showed that ATIII neutralizes thrombin by forming a 1 : 1 stoichiometric complex by a reactive site (arginine)–active center (serine) interaction. The complex formation occurs at a relatively slow rate in the absence of heparin. However, heparin binds to lysine residues on ATIII, thereby accelerating the inhibition of thrombin.[38] McClung et al.[34,39] prepared a polymeric coating reagent containing both pendant lysine groups and pendant benzophenone groups and coated the reagent onto polyurethane surfaces using photochemical methods. They reported that these surfaces, when placed in contact with plasma, adsorb only plasminogen and adsorbed plasminogen is readily converted to plasmin in the presence of a tissue-plasminogen activator.

It has been generally thought that platelets play an important role in thrombus formation. When platelets are stimulated with an agonist, they initiate a release reaction, which was named the "basic platelet reaction" by Holmsen.[40] He reported that platelets respond to a variety of stimuli, causing shape change, aggregation, prostaglandin/thromboxane synthesis, secretion from dense granule, and secretion from α-granule. *In vitro* platelet activation is induced by a variety of stimuli such as thrombin, collagen, prostaglandin G_2, adrenaline, ADP, and serotonin. It has been reported that platelets release serotonin and reabsorb released serotonin.[40] From the results of a preliminary test using PULL, it was found that the amount of serotonin released from adhering platelets remains almost unchanged in the presence of imipramine. The degree of serotonin release on PULL (38%) for 60 min of incubation was smaller than that on PU (47%, $p < 0.02$) and PULL-N (45%, $p < 0.02$), as shown in Table IV. Serotonin release, however, was highly suppressed on PULL-H (21%, $p < 0.002$). Thus, it could be inferred from the results of Figure 6 and Table IV that the serotonin release was accelerated by a surface to which more platelets easily adhered. Similar results using polypeptide derivatives with different degrees of wettability were reported previously.[41]

CONCLUSIONS

Poly(ether urethane)s containing *z*-lysine segments in the main chains (PULL) were prepared using 4,4′-diphenylmethane diisocyanate, poly(tetramethylene glycol), and amino-terminated L-lysine oligomer as the chain extender. Heparin-immobilized PULL (PULL-H) was synthesized by a hydrolysis reaction of PULL with a hydrobromide–acetic acid solution, followed by a coupling reaction with heparin. The activation of plasma proteins and platelets was significantly suppressed on PULL-H, thus leading to good *in vitro* antithrombogenicity.

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