Synthesis and Blood compatibility of New Segmented Polyurethanes containing Phospholipid-like moieties

Lee Mi-kyung, Park Soo-min, Nakaya Tadao*
Pusan National University, Dept. of Textile Eng.,
**Osaka City University, Dept. of Bio-applied Chemistry*

1. Introduction

Segmented poly(urethanes)(SPU) are linear multiblock copolymers which consists of polyols soft segments and an alternating sequence of poly(urethanes) hard segments combining of diisocyanates and low molecular chain extenders. They are extensively used in medicine, especially in the labrication of desirable mechanical properties, as a results of their microphase separated structures, and unsurpassed patency rates, still warrants their extensive usage as blood contacting synthetic biomaterials.

On the other hand, phospholipids are the main components of the biomembrane and interesting substanes in biological and biomedical field[1, 2]. Recently, the phospholipid membranes have been used as a drug carrier, sensor, separation membrane[3]. However these phospholipid membranes were unstable physically and chemically, because the phospholipids constituting membranes do not bond covalently and have high mobility.

To improve the mechanical strength and the blood compatibility of the phospholipid membranes, we synthesized the prepolymers based on poly(butadieneglycol)(PBG) and hexamethylene diisocyanate (HDI), and then new segmented polyurethanes with phospholipid analogues and 1,4-butanediol(BD) as chain extenders. The polyurethanes were characterized by IR spectra, ^{1}H NMR spectra and X-ray electron spectroscopy. The blood compatibility of the polymers was evaluated by measurements of blood platelet attachment *in vitro* and by determining the degree of activation of the adhered platelets by scanning electron microscopy.

2. Experimental

2.1 Materials

Ethylene glycol, phosphorus trichloride, dichloromethane, benzene, triethylamine(TEA), tetrahydrofuran(THF), N,N-dimethylformamide(DMF), acetone, chloroform, diethyl ether, methanol, and 1,4-butanediol were commercially obtained and purified by vacuum distillation. N-methyldiethanolamine and hexamethylene diisocyanate(HDI) were used without further purification. Poly(butadieneglycol) (Mn=2840) was served by Nippon Yushi.

2-Cholro-1,3,2-dioxaphospholane[4] was prepared by the reaction of ethylene glycol with

phosphorus trichloride in dichloromethane, according to the method of Lucas et al., and oxidized to 2-chloro-2-oxo-1,3,2-dioxaphospholane with oxygen according to the procedure of Edmundson[5].

2.2 Preparation of Polyurethanes

2.2.1 Synthesis of 2-[Bis(2-hydroxyethyl)methylammonio]ethyl stearyl phosphate(SPD)

Into a thorouguly dried 500cm^3 three-necked round-bottomed flask, equipped with a mechanical stirrer, drying tube, and dropping funnel, were placed 10.00g of stearyl alcohol and 4.15g of triethylamine in 150cm^3 dry THF. After cooling with ice/water(10 ℃), 5.27g of 2-chloro-2-oxo-1,3,2-dioxaphospholane was added slowly to the stirred solution over a period of 1hr. The reaction mixture was maintained at 10℃ for 1hr with stirring and then allowed to warm to 15~20℃. After being kept at this temperature for 1.5hr, the precipitate formed was filtered off and washed with 30cm^3 of dry THF. The filtrate and the THF solution were evaporated in vacuum in a stream of nitrogen. To the residue, 50cm^3 of dry THF were added. The mixture was shaken for 30s and then filtrated with a glass filter to remove a small amount of triethylamine hydrochloride. The filtrate was evaporated in vacuum in a stream of nitrogen for about 1.5hr to give 2-stearyloxy-2-oxo-1,3,2-dioxaphospholane (3) as a white solid.

Into a 300cm^3 glass pressure bottle were placed 12.0g of (3) and 100cm^3 of dry DMF. 5.72g of N-methyldiethanolamine was rapidly added to the solution. The pressure bottle was closed and the shaken in a thermostst maintained at 75℃ for 20hr. After the bottle was opened, the solvent was evaporated in vacuum in a stream of nitrogen. To the residue, 100cm^3 of dry acetone were added. After the mixture was shaken for 1min, the solvent was discarded by decantation. The residue was collected and dried in vacuum to give a crude product which was dissolved in dry methanol and reprecipitated from dry diethyl ether. The reprecipitation procedure was repeated three times to give pure SPD (4) as a pale yellow solid.

2.2.2 Oleyl-2-(N-methyldiethanolammonium)ethyl phosphate (OPD)

In the similar manner of (3) 2-oleyloxy-2-oxo-1,3,2-dioxaphospholane was prepared from oleyl alcohol and 2-chloro-2-oxo-1,3,2-dioxaphospholane in THF in the presence of triethylamine at -20 to -15℃, given as a pale brown semisolid. OPD was synthesized in the manner similar to SPD

2.2.3 Synthesis of Segmented Polyurethanes

All polyurethanes were prepared by a two-step polymerization. Into a 100cm^3 round-bottomed flask, equipped with a reflux condenser and a magnetic stirring bar, were placed PBG and HDI in DMF under a nitrogen atomosphere. The mixture was stirred at 90~100℃ for 1hr. After the solution had cooled to room temperature, the synthesized phospholipid diols and 1,4-butanediol were added slowly to the solution. The polymerization was carried out at 100~110℃ for 4hr and the products was purified by precipitation in acetone.

These polyurethanes solutions of 10% concentration in DMF were cast on glass plate,

dried in dry-oven at 65℃ for 1 day, and then dried in vacuum at 65℃ for 1 day to produce the polyurethane films.

2.3 Characterization

IR spectra of all samples were measured by a Jasco A 202 spectrometer and ^1H NMR spectra were recorded on a 400 M Hz alpha FT NMR spectrometer(JNM-A-400) using tetramethylsilane (TMS) as an internal standard.

X-ray photoelectron spectroscopy (XPS) was carried out for surface analysis. All samples were vacuum-dried for 24hr at room temperature and stored in a desiccator.

2.4 Evaluation of Thrombogenicity

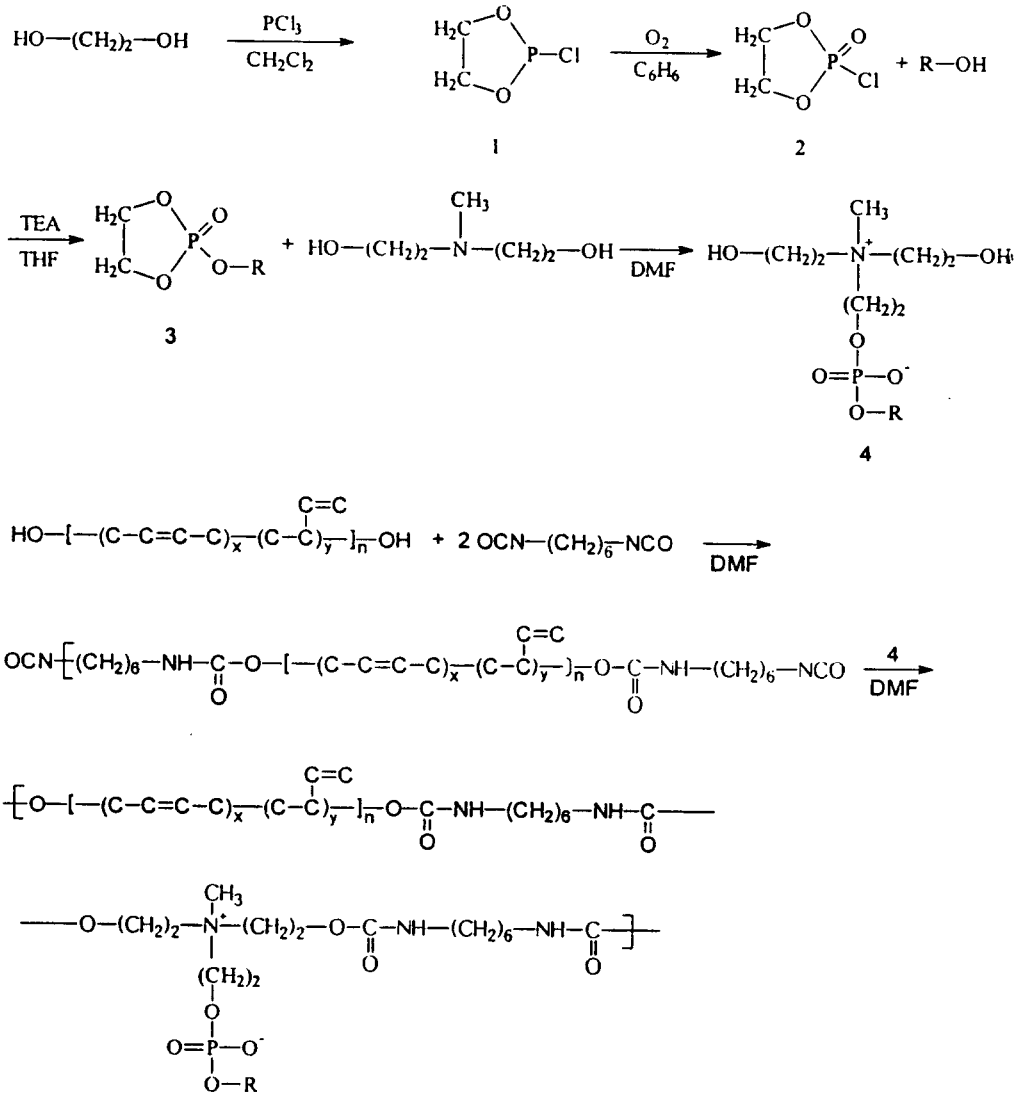
The polymer films were washed with saline and incubated at 37℃ for 1hr with freshly prepared, platelet rich plasma(PRP) which was obtained from the centrifugation of rabbit blood. The samples were rinsed with saline and treated with 2.5% glutaraldehyde in saline at refrigerated temperature overnight. This treament fixes all platelets attached to the surface of the polymers. The samples were rinsed with saline and dehydrated by systematic immersion in a series of ethanol-water solutions ; 60, 70, 80, 90 and 100% v/v. Following critical point drying with CO_2, the samples were coated with gold for the analysis by scanning electron microscopy(SEM).

3. Results and Discussion

3.1 Polymer preparation and characterization

The new diols and segmented polyurethanes were prepared according to Fig. 1. The ^1H NMR spectrum of SPD($CDCl_3$) has following chemical shift : δ = 0.88(C-CH_3, 3H), 1.26(-$(CH_2)_{16}$)-, 32H), 1.7~2.1(PO-CH_2-C, 2H), 3.6~4.4(-OCH_2CH_2-, 4H) and the ^1H NMR spectrum of OPD : δ = 0.88(-CH_3, 3H), 1.26(-CH_2, 28H), 3.12(N^+-CH_3, 3H), 3.70~4.55(-OCH_2-, N^+-CH_2, 14H), 5.2(-CH=CH-, 2H), 5.8~6.1(-OH, 2H). The structures of SPD and OPD were indentified by these characteristic peaks. The polymerizations of segmented polyurethanes were characterized by the IR spectra. The polymers were indentified by the characteristic peaks 1700~1710cm^{-1} (-CONH-), at 1260cm^{-1} (-P=O-), at 1080cm^{-1} (-PO-CH_2-), and at 965 and 905 cm^{-1} (-CH=CH-).

The XPS surface elemental analysis of polyurethane films was carried out. The air-side surface of PBG-HDI-SPD and PBG-HDI-OPD films shows peaks from phosphorus at 133 eV, which was not present at PBG-HDI-BD. The glass side surface of PBG-HDI-SPD films shows the same peak of phosphorus as the air side surface of the film. On the other hand, the peak of phosphorous was not present at the glass side surface of PBG-HDI-OPD film. It means that phospholipid chains of PBG-HDI-OPD was present or near the surface of the polymer film.



Polyurethanes	Molar ratio	Chain extenders
PBG-HDI-BD	1:2:1	BD (1,4-butanediol)
PBG-HDI-SPD	1:2:1	SPD (R=-$(CH_2)_{17}$-CH_3)
PBG-HDI-OPD	1:2:1	OPD (R=-$CH_3(CH_2)_7CH$=$CH(CH_2)_8$)

Fig. 1. Synthesis of Segmented Polyurethanes

3.2 Nonthrombogenicity of the film surfaces

Thrombogenicity of the film surface was evaluated with PRP by *in vitro* adhesion test. The results of platelets retention at the surface with varying segmented polyurethanes and PVA film are shown in Fig. 2. Platelet adhesion was minimized at

the surfaces of segmented polyurethanes. On the contrary, no such suppressive effect on platelet adhesion was observed for PVA film. This significant differences in platelet adhesion behavior between PVA and segmented polyurethanes films strongly suggest that the formation of microdomain structure is a determinative factor for the antithrombogenic feature of segmented polyurethanes.

Futhermore, platelet adhesion was effectively reduced at the segmented polyurethanes surfaces with containing phospholipid-like moieties. In case of the film surface of polyurethane with OPD, platelets were less adhered to the surface. It suggests that platelets recognize the pattern of phospholipid structure and the adhesion is effectively suppressed at the surface of the segmented polyurethane.

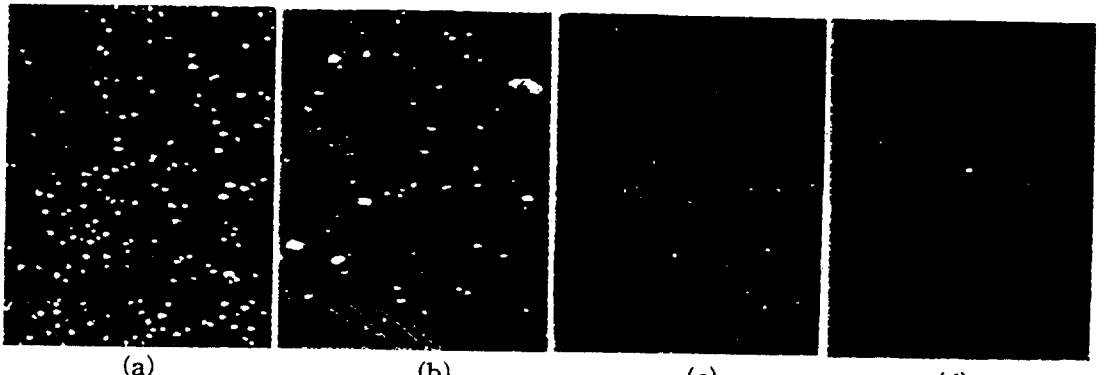


Fig. 2. SEM photographs of film surfaces after contactiog with PRP
(a) PVA (b) PBG-HDI-BD (c) PBG-HDI-SPD (d) PBG-HDI-OPD

4. Conclusion

New segmented polyurethanes with phospholipid-like moieties were synthesized and their blood compatibilities were investigated. The platelet adhesions were effectively suppressed at the surface of PBG-HDI-OPD film. This study suggests that synthesized segmented polyurethanes with phospholipid-like moieties are very exciting as new biometerials and their prospective imfortance for biomedical applications.

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