

SYNTHETIC STUDIES ON BLOOD COMPATIBLE BIOMATERIALS 13: A NOVEL SEGMENTED POLYURETHANE CONTAINING PHOSPHORYLCHOLINE STRUCTURE: SYNTHESIS, CHARACTERIZATION AND BLOOD COMPATIBILITY EVALUATION*

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Abstract A new phosphorylcholine, (6-hydroxy)hexyl-2-(trimethylammonio)ethyl phosphate (HTEP), was synthesized and characterized. Segmented polyurethane (SPU) containing phosphorylcholine structure was synthesized based on diphenylmethane diisocyanate (MDI), soft segment polytetramethylene glycol (PTMG) and HTEP, with 1,4-butanediol (BD) as a chain extender. The existence of phosphorylcholine structure on the surface of SPU was revealed by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray photoelectron spectroscopy (XPS) and water contact angle measurements. The blood compatibilities of the polymers were evaluated by hemolytic testing and a platelet-rich plasma (PRP) adhesion experiment, which was viewed by scanning electron microscopy (SEM) with polyurethane as a reference. The novel segmented polyurethane containing phosphorylcholine structure showed improved blood compatibility.

Keywords Platelet adhesion, Phosphorylcholine, Zwitterionic structure, Blood compatibility, Segmented polyurethane

INTRODUCTION

Polymeric materials are widely used in biomedical fields for artificial organs, medical devices and disposable clinical apparatus^[1]. In use, these polymeric materials are in contact with living organisms. To obtain polymers with biocompatible surfaces, many approaches have been explored. One of these approaches is to construct segmented polyurethanes (SPUs)^[2, 3]. Although their blood compatibilities are fair, they are not adequate for the more demanding applications. A possible approach to improve the blood compatibility of polyurethanes involves the covalent attachment of key molecular structure on their surfaces.

Since the first report in 1977^[4], phosphorylcholine has been shown to enhance the blood compatibility^[5–7]. In recent years, Nakaya and co-workers reported a series of polyurethanes containing phospholipid moieties in the side chains and in the main chains^[8–10] and they showed significant blood compatibilities. In this paper, we report the synthesis, characterization and blood compatibility evaluation of a novel kind of polyurethane which was prepared from a new derivative of phosphorylcholine, (6-hydroxy)hexyl-2-(trimethylammonio)ethyl phosphate (HTEP, Scheme 1).

EXPERIMENTAL

Materials and Methods

Acetonitrile (A.R.) was distilled over phosphorus pentoxide. Tetrahydrofuran (THF, A.R.), 1,6-hexanediol (A.R.) and triethylamine (TEA, A.R.) were distilled from calcium hydride (CaH₂). Anhydrous methanol (A.R.) was

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obtained by distillation in the presence of magnesium and iodine. *N,N*-dimethylformamide (DMF, A.R.), dimethyl sulfoxide (DMSO, A.R.) and methyl isobutyl ketone (MIBK, A.R.) were dehydrated over CaH_2 and vacuum distilled. 1,4-Butanediol (BD) was available from Shanghai Chemical Reagent Co. Ltd. and purified by vacuum distillation. Diphenylmethane diisocyanate (MDI) and 1,6-diisocyanatohexane (HDI) were obtained from Acros and purified by vacuum distillation. Polytetramethylene glycol (PTMG, $M_n = 1000$) was dehydrated under reduced pressure. 2-Chloro-2-oxo-1,3,2-dioxaphospholane (COP) [b.p. 89–90 °C (40 Pa)], was prepared according to the method reported by Lucas *et al.*^[11] and Edmundson^[12]. Infrared (IR) spectra were obtained with Nexus 870 FT-IR spectrometer. ^1H -NMR spectra were recorded on DPX0-300 (300 MHz) spectrometer. Proton chemical shifts were referenced to tetramethylsilane (TMS).

Synthesis of 2-(6-Hydroxy)hexanoxo-2-oxo-1,3,2-dioxaphospholane (HOP)

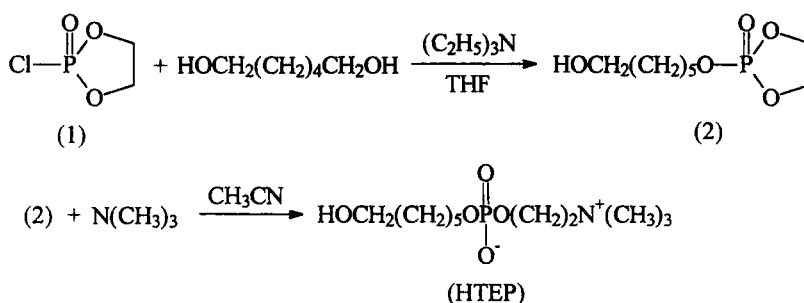
Into a thoroughly dried 250 mL three-necked round-bottomed flask equipped with a mechanical stirrer, a drying tube and a dropping funnel, 12.98 g (0.11 mol) of 1,6-hexanediol, 10.10 g (0.10 mol) of TEA and 180 mL of dry THF were added. After cooling with an ice/ CaCl_2 bath (−20 °C), 14.25 g (0.10 mol) of COP was slowly added dropwise to the stirred solution over a period of 2 h. During the reaction, $\text{TEA} \cdot \text{HCl}$ was precipitated. The reaction was maintained at −20 °C during the addition and then allowed to warm up naturally to 10–15 °C. After being kept at this temperature for 2 h the white precipitate was filtered off and washed with 10 mL THF. The filtrate was evaporated in vacuum to obtain a viscous liquid with a yield = 19.90 g (88.9%).

IR (KBr): 2937 and 2862 cm^{-1} (− CH_2 − CH_3), 3392 cm^{-1} (−OH), 1253 cm^{-1} (P=O), 1024 cm^{-1} (POCH₂). ^1H -NMR (CDCl_3 , δ): 3.95–4.30 (m, 6H, −OCH₂CH₂O− and −POCH₂−(CH₂)₅−), 3.62–3.81 (t, 2H, HO−CH₂−(CH₂)₅−), 1.20–1.80 (m, 8H, CH₂−(CH₂)₄−CH₂).

Synthesis of (6-Hydroxy)hexyl-2-(trimethylammonio)ethyl Phosphate (HTEP)

After 11.20 g (0.05 mol) of HOP in 30 mL dry acetonitrile was placed into a 300 mL pressure bottle, 3.55 g (0.06 mol) of trimethylamine dissolved in 30 mL dry acetonitrile was quickly added. The pressure bottle was closed and then shaken for 48 h in a thermostat maintained at 70 °C. After the reaction mixture was cooled to room temperature, the pressure bottle was opened and acetonitrile was removed under reduced pressure. The crude product was dissolved in methanol and then precipitated from acetone. This operation was repeated at least 3 times to give pure HTEP as a yellow viscous liquid with a yield = 9.10 g (64.1%).

IR (KBr): 3400 cm^{-1} (−OH), 1247 cm^{-1} (P=O), 1025 cm^{-1} (POCH₂). ^1H -NMR (D_2O , δ): 1.24–1.43 (m, 8H, CH₂−(CH₂)₄−CH₂), 3.07 (s, 9H, $\text{N}^+(\text{CH}_3)_3$), 3.42–4.12 (m, 8H, −CH₂OPOCH₂−CH₂− N^+ and HO−CH₂−).



Scheme 1 The reaction scheme for HTEP synthesis

Synthesis of SPU_s

Synthesis of HTEP-HDI monomer

Into a thoroughly dried four-necked round-bottomed flask, a solution of 1.68 g (0.01 mol) of HDI dissolved in 40 mL DMSO was added. Under a nitrogen atmosphere, 2.83 g (0.01 mol) of HTEP dissolved in 10 mL DMSO was added dropwise to the stirred solution over a period of 0.5 h. The reaction was maintained at 50 °C.

According to the titration, the content of NCO group was nearly constant after 2.5 h changing from 1.62 wt% to a constant value at 0.80 wt%, which showed that the monomer of HTEP-HDI was obtained completely. NCO%: 0.14% (mol%).

Synthesis of SPU_{HTEP} (PTMG-MDI -BD-HTEP-HDI)

The process of synthesizing SPUs was similar to those reported previously^[13, 14]. In the first step, a solution of 3.9 g (15.6 mmol) MDI dissolved in 4.5 mL DMSO and MIBK was added to 5.0 g (5.0 mmol) PTMG dissolved in 4.5 mL DMSO and MIBK with stirring under a nitrogen atmosphere. After 1 h at 70°C, in the second step, 0.9 g (10.0 mmol) BD dissolved in 4.5 mL DMSO and MIBK was added dropwise to the reaction mixture. For the third step, about 2.0 g (4.4 mmol) of HTEP-HDI, which was dissolved in 20 mL DMSO, was slowly added into the reaction solution. Stirring was continued at 70°C for 2 h. For the last step, 20 mL of DMF was added to the solution with stirring. After 1 h, the polymer was poured into a plate mould and dried in a vacuum oven at 50°C for at least 48 h. A pale yellow elastomer of SPU_{HTEP} was obtained.

Synthesis of SPU_{control} (PTMG-MDI -BD)

Using the same procedure but not adding monomer HTEP-HDI, SPU_{control} (PTMG-MDI -BD) was also prepared as a control.

Surface Property Characterization

The resulting SPUs were rinsed with freshly distilled water continuously for five days to remove the unreacted monomer of HTEP-HDI and then these SPUs were dried in a vacuum oven at 50°C for at least 48 h. ATR-FTIR was performed on the surface of the cast films. The spectrum was recorded using Nexus 870 FT-IR.

XPS spectra were obtained using Mg K_α radiation. In addition to survey scans (0–1000 eV) to determine the elemental composition of the various surfaces, elemental compositions were also determined on the basis of peak areas from the C_{1s}, N_{1s}, O_{1s} and P_{2p} orbitals. The binding energy was referenced by setting the C_{1s} hydrocarbon peak to 285 eV.

The contact values angle are for each sample the average of 8 measurements taken at 1 min contact of the water droplet on the air-exposed side.

Blood Compatibility Evaluation

The hemolytic test was the same as that described previously^[15]. The blood sample was prepared by mixing 8 mL anticoagulated whole blood with 10 mL 0.9% NaCl solution. The films were cut into pieces of 1 cm diameter each and leached in 0.9% NaCl solution for 24 h. Having been dried, they were placed in 10 mL 0.9% NaCl solution and 0.2 mL blood sample was added. Then the whole system was subjected to centrifugation at 2000 rpm for 10 min. The upper clear solution of the blood mixture was characterized at an absorption frequency of 545 nm with a UV-Vis spectrophotometer. The sample for positive control was prepared by mixing 10 mL distilled water with 0.2 mL blood sample and the sample for negative control was just a mixture of 10 mL 0.9% NaCl and 0.2 mL blood sample. Six parallel experiments were performed for each film sample.

Blood platelet adhesion studies were conducted as that described previously^[16] but with some modifications. After the films were equilibrated with pre-warmed (37°C) phosphate buffer saline (PBS; pH 7.4) for 2 h, they were incubated at 37°C for 1 h with freshly prepared platelet-rich plasma (PRP) obtained from Nanjing Red Cross Center. The films were washed three times with fresh PBS by mild shaking to remove nonadhesion platelets. After thorough washing with PBS, the platelets adhering to the film surfaces were dehydrated in an ethanol-graded series (50, 60, 70, 80, 90, and 100%) for 30 min each and allowed to dry on a clean bench at room temperature. The platelet-attached surfaces were examined by SEM (Model JSM6300, Japan) after gold-sputtering treatment.

RESULTS AND DISCUSSION

Characterization of Surface Properties

Surface properties of the polymer films were investigated by ATR-FTIR, XPS and contact angle measurements.

The air-facing surface of the films was blood-contacting, therefore, all surfaces and the blood compatibility properties were related to the air-facing surface.

Figure 1 shows the ATR-FTIR spectra of SPU_{HTEP} and SPU_{control}. The spectra of the polymers show the characteristic C-H and N-H stretching vibration at 2940 and 3322 cm⁻¹, respectively. Compared with the control sample without phosphatidylcholine analogue, SPU_{HTEP} displayed additional stretching vibration bands of P=O, P-O-CH₂ and N⁺(CH₃)₃ groups at 1226, 1019 and 966 cm⁻¹, respectively.

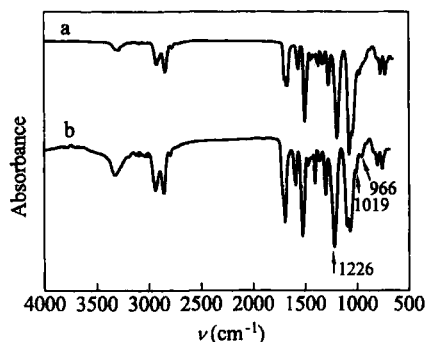


Fig. 1 ATR-FTIR spectra for SPU_{control} (a) and SPU_{HTEP} (b)

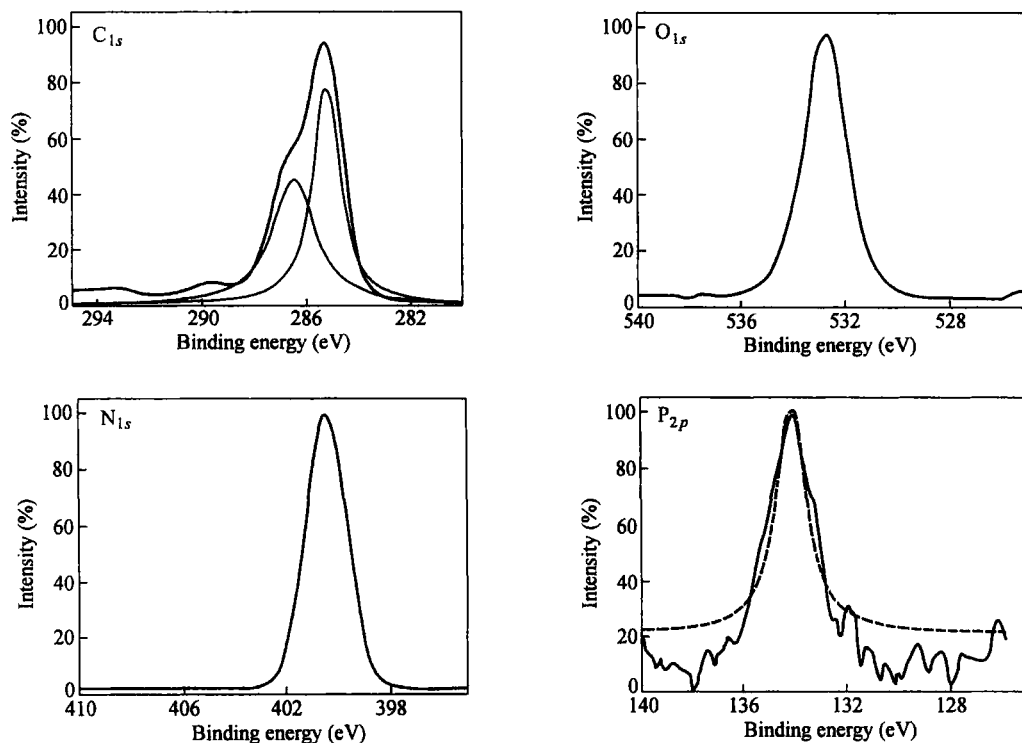


Fig. 2 XPS spectra of the cast film surface of SPU_{HTEP}

Figure 2 shows the typical C_{1s}, N_{1s}, O_{1s} and P_{2p} spectra for SPU_{HTEP}. The C_{1s} peak for SPU can be resolved into 2 component peaks including a hydrocarbon (C-C-C) peak at 285.1 eV, and an ether (O-C) peak at 286.5 eV. The urethane (NH-CO) peak, when compared to hydrocarbon (C-C-C) peak or ether (O-C) peak, is too small to allow accurate peak resolution. A peak at 532.9 eV was used to fit the SPU O_{1s}. The high-

energy and low-energy tailings on the N_{1s} peak reflect a combination of contributions from the urethane nitrogen (N – COO) and phospholipid ammonium nitrogen (N⁺(CH₃)₃). A Gaussian peak of N_{1s} is at 400.6 eV. A Lorentzian peak at 134.1 eV was used to fit P_{2p} peak (PO₄³⁻) in SPU_{HTEP}.

Table 1 lists the XPS elemental surface compositions of SPUs. The ratio of the peak area of P_{2p}/C_{1s} was also calculated and summarized in Table 1. The P_{2p} data suggests that phosphatidylcholine structure appears on the surface of SPU_{HTEP}. Some difference between theoretical and experimental data of P_{2p} may be attributed to the incompleteness of the reaction between SPU and HTEP-HDI other than the error in measurement.

Table 1. XPS elemental surface composition (%) and water contact angle (°) of SPUs*

Polymer	C _{1s}	N _{1s}	O _{1s}	P _{2p}	P _{2p} /C _{1s}	[θ] _(air)
SPU _{control}	73.96	3.13	22.76	0.15 (0.00)	0.0020	78.1 ± 3
SPU _{HTEP}	69.83	4.41	24.81	0.95 (1.16)	0.0136	62.3 ± 3

* Values in parenthesis are expected theoretical elemental compositions, which are based on blending.

To investigate the hydrophilicity/hydrophobicity of the surfaces of the polymers, water contact angle measurements were carried out (Table 1). The SPU_{HTEP} is relatively hydrophilic, and this result agrees with that of XPS. SPU_{HTEP} with phosphatidylcholine structure on the surface of SPU shows stronger hydrophilicity.

Blood Compatibility Evaluation

Hemolytic Test

The absorption band at 545nm of the samples was characterized with a spectrophotometer. The degree of hemolysis was calculated as follows:

$$D\ (\%) = [(A_{\text{sample}} - A_{\text{negative}})/(A_{\text{positive}} - A_{\text{negative}})] \times 100\%$$

where *D* is the degree of hemolysis, *A*_{sample} is the absorbance of the SPUs, *A*_{positive} is the absorbance of the positive control and *A*_{negative} is the absorbance of the negative control. The degree of hemolysis (%) of SPU_{HTEP} is 0.56 and that of SPU_{control} is 5.60. This result indicates that only little hemolysis take place on the SPU_{HTEP}.

Platelet Adhesion

The synthesized SPUs were assessed as biomaterials by their degree and nature of blood platelet adhesion resulting from exposure to PRP. Figure 3 shows the SEM photographs of the surface of SPU_{HTEP} and SPU_{control}. For synthesized SPU_{HTEP}, a relatively limited number of platelets adhered. In contrast with SPU_{HTEP}, the SPU_{control} not only exhibited substantial platelet adhesion, but also showed some degree of shape variation for the adhered platelets. These results indicate that the blood compatibility of SPU_{HTEP} is generally better than that of SPU_{control}. The polymers with sulfo-ammonium zwitterions on their surfaces have good antithrombogenicity^[17]. Similarly, SPUs with the phosphorylcholine structure on the surfaces also have good antithrombogenicity. It is considered that the improved antithrombogenicity can be attributed to the zwitterionic structure. In aqueous (blood) medium, molecules with zwitterionic structure not only can not diffuse into the interior of proteins' tertiary/quaternary structure, which is mainly maintained by hydrophobic bonds and hydrogen bonds, to effect the synergetic interaction between main chain and side portions, but also minimize the effect on exterior surface ions (cations/anions) of protein, thus contributing to the maintenance of normal conformation of protein and its assembly^[18].

As for the application of this kind of zwitterionic structure to improve the antithrombogenicity of biomaterials, it is easy to synthesize various SPUs with phosphorylcholine through hydroxyl-containing phosphorylcholine; furthermore, the hydroxyl phosphorylcholine can also be covalently bonded to surfaces of many biomaterials via the –NCO group by surface coating and it thus has unique advantages for surface coating cardiovascular devices to improve their blood compatibilities. The development of such blood-compatible phospholipid polyurethane is ongoing in our research group.

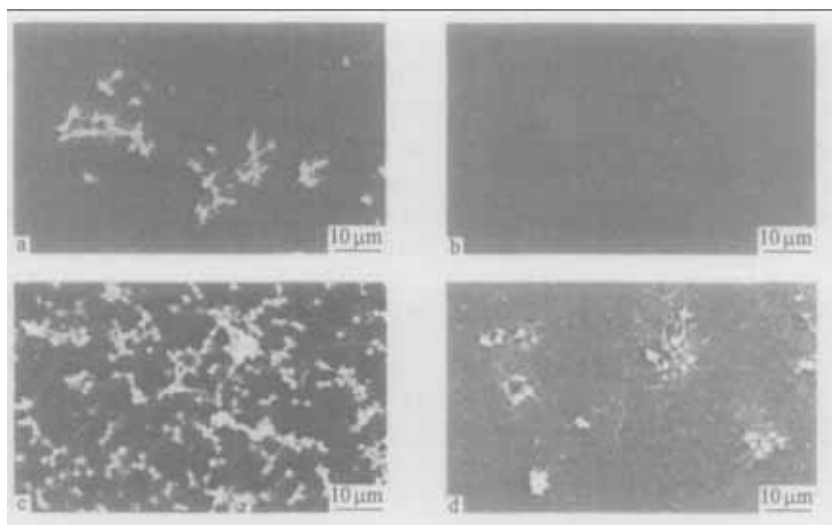


Fig. 3 SEM photographs of the surface of SPU films after 60 min and 120 min of PRP exposure
(a) SPU_{control} 60 min; (b) SPU_{HTEP} 60 min; (c) SPU_{control} 120 min; (d) SPU_{HTEP} 120 min

CONCLUSIONS

A novel phosphorylcholine, (6-hydroxy)hexyl-2-(trimethylammonio)ethyl phosphate (HTEP), was synthesized and characterized. It was demonstrated that the segmented polyurethane (SPU) containing such phosphorylcholine structure shows better blood compatibility.

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