Synthesis and hemocompatibility evaluation of segmented polyurethane end-capped with both a fluorine tail and phosphatidylcholine polar headgroups

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To improve the hemocompatibility of polyurethanes, an amine monomer containing a long fluorine tail and phosphatidylcholine polar headgroups, 2-amino-3-oxo-3-(2-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctano amido) ethyl amino) propyl phosphorylcholine (FASPC) was firstly synthesized and characterized. Then four kinds of fluorinated phosphatidylcholine end-capped polyurethanes with different chemical structures were prepared. The surface properties of these prepared polyurethanes were characterized using X-ray photoelectron spectroscopic analysis (XPS) and water contact angle measurements. The results indicated that the phosphatidylcholine (PC) polar headgroups along with the fluorine tail could be easily enriched on the top surfaces, and the PC groups could be highly oriented on the outmost surface when the polymer film was in contact with water for only 30 s at room temperature. The evaluation of hemocompatibility was carried out via fibrinogen adsorption and platelet adhesion. Fibrinogen adsorption (37°C for 90 min) decreased by 98% to 87% compared to that on ordinary polyurethane surfaces, and almost no platelet adhesion and activation was observed at 37°C for 2 h.

Keywords: synthesis; hemocompatibility; segmented polyurethane; phosphatidylcholine

Introduction

Surface-induced thrombosis remains a significant challenge for polyurethanes used as long-term implanted devices. In particular, protein adsorption and platelet adhesion on the foreign surface usually elicit the activation of the coagulation cascade and promote thrombus formation (Davie and Fujikawa 1975; Wootten and Ku 1999). Therefore, several strategies have been developed to improve polyurethane hemocompatibility, such as chemical modification or the introduction of surface-active additives. One way to efficiently suppress protein adsorption and platelet adhesion is to introduce phosphatidylcholine groups (PC) into the side chains of polyurethanes. Polymers containing phosphatidylcholine groups, such as 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers, have already been shown to suppress biological reactions and are now clinically used on intravascular stents, soft contact lenses, and artificial knee joints (Goda and Ishihara 2006; Chan et al. 2007; Moro et al. 2010). However, the PC group cannot easily migrate to the surface. Thus, >16% (molar ratio) of phosphatidylcholine chain extender or a PC content of 5 wt% in the bulk is necessary to sufficiently reduce the adhesion of platelets on the polyurethane surface. This high PC content causes high water adsorption in the bulk, and consequently results in poor mechanical properties (Li et al. 1998; Nagase et al. 2008).

Another way to achieve good hemocompatibility is to introduce fluorocarbon chains into polyurethane. It is well-known that fluorocarbon chains exhibit many useful surface characteristics, such as low surface energy, oxidative stability, and good water and oil repulsion (Kashimoto et al. 2008; Xie et al. 2009; Leitsmann et al. 2010). As a result, a large number of fluorinated polymers have been found to be relatively hemocompatible (Chen and Kuo 2002; Jahangir et al. 2002). Tang et al. (1996) and McCloskey et al. (2001) investigated the fluorocarbon chain end-capped poly(ether urethane)s used as surface-modifying macromolecules (SMMs) to the base polyurethanes and demonstrated that SMMs are able to migrate to the surface and establish a fluorine-rich surface. Jahangir et al. (2002) showed that introduction of SMMs into polyurethane could reduce platelet adhesion and protein absorption. More recently, the SMMs have been modified to include an additional bioactive element, and it was found that vitamin E or peptides could be co-delivered to the surface of a polymer with a fluorine component of the oligomeric molecule to improve biostability or stimulate cell adhesion (Ernsting et al. 2003, 2005, 2007).
Thus, the current authors developed the idea of introducing both fluorocarbon chains and phosphatidylcholine head groups into polyurethane for better hemocompatibility. On the one hand, fluorocarbon chains, alone, have good hemocompatibility and, on the other hand, fluorocarbon chains can help the migration of PC to the surface of polyurethane (Iwasaki and Akiyoshi 2006). In a previous study, a novel diol-containing fluorinated phosphatidylcholine head group was prepared and attached to the hard block of polyurethanes as a side chain (Tan et al. 2006). It was revealed that PC groups can be easily transferred to the surface with the help of a long fluorinated chain, and the synergistic effect of the PC group and fluorinated chain on polyurethanes provides better hemocompatibility than other phospholipid-containing polyurethanes reported in the literature (Li et al. 1998; Yung and Cooper 1998). However, the efficiency of the suppression of protein adsorption and platelet adhesion to the fluorinated phosphatidylcholine was only 75%. In addition, a pretreatment process in water at 80°C was needed to relocate the PC group to the outer surface to form the biomimetic structure on the surface of the polyurethanes (Tan et al. 2005b). This process would increase thromboresistance before clinical application of such devices.

In the study reported here, to prepare polyurethanes with good hemocompatibility, an amine monomer containing long fluorine tails (−(CF₂)₆CF₃) and phosphatidylcholine polar head group, and a series of polyurethanes end-capped with fluorinated phosphatidylcholine were designed and synthesized. Compared to the fluorinated phosphatidylcholine unit (−R₁−(CF₂)₆−R₂−PC) applied in the previous work (Tan et al. 2006, 2011), the design was optimized for the current monomer as shown in Scheme 1, CF₃(CF₂)₆−R−PC. Firstly, the two links (R₁, R₂) among the three parts, the phosphatidylcholine polar head group, the fluorocarbon chain, and the chain end of polyurethane, were simplified to one (R), which was designed to reduce the inhibition to the mobility of the fluorocarbon chain. Secondly, since the interfacial free energy of a CF₂ group is six times that of a CF₃ group, with the driving force from CF₃ group, it was expected that the fluorine tail (−(CF₂)₆CF₃) would be easier to enrich on the surface. In addition, the introduced site of fluorinated phosphatidylcholine moiety was no longer at a hard segment, but at a chain-end. Since spontaneous enrichment of PC groups at the sub-stratum surface by end-group synthesis has been demonstrated to be an effective approach for polymers (Farrar et al. 1988; Nederberg et al. 2004), a shorter time for relocation of the PC group to the outer surface for polyurethane end-capped with both a fluorine tail and phosphatidylcholine polar headgroups than those prepared in the previous work would be expected. In the present article, the synthesis, surface properties and hemocompatibility of these novel fluorinated phosphatidylcholine end-capped polyurethanes are reported.

### Materials and methods

#### Materials

Isobutyl chlorocarbonate (iBuOCOCl) and acetyl chloride were distilled under atmospheric pressure. N-methylmorpholine was distilled under atmospheric pressure in the presence of acetic anhydride. 1, 6-hexamethylene diisocyanate (HDI), methylenebis(phenylene isocyanates) (MDI) and 1,4-butanediol (BDO) were distilled under vacuum. Poly(tetramethylene glycol)s (PTMG, Mn = 1000, obtained from Dupont) was dehydrated at 100°C for 4 h under vacuum before use. Poly(tetramethylene glycol)s (PTMG, Mn = 1058) was synthesized in the laboratory and dehydrated under reduced pressure at 100°C for 4 h (Tan et al. 2004). Poly(ether urethane)s (PT-MPU) were synthesized with MDI, polytetramethyleneoxide (PTMO) and 1,4-butanediol (BDO) (molar ratio 3:2:1) using solution polymerization \((\text{Mn} = 5.56 \times 10^{-4}, \text{D} = 2.25)\) (Zhang 2010). The other reagents were used as received.
Synthesis of tert-butyl 3-hydroxy-1-oxo-1-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanamido)ethylamino)propan-2-ylcarbamate (BPFAOH)

A solution of BOC-\(\text{L-serine} \) (6.2 g, 30 mmol, synthetic process in (see Supplementary material) [Supplementary material is available via a multimedia link on the online article webpage]) and N-methylmorpholine (3.6 ml, 33 mmol) in anhydrous tetrahydrofuran (THF, 30 ml) was cooled to \(-15^\circ\text{C}\), and then isobutyl chlorocarbonate (4.1 ml, 31.5 mmol) was added. After the mixture was stirred for 10 min, N-(2-aminoethyl)-2,2,5,5,6,6,7,7,8,8,8-pentadecafluorooctanamido (APFDE C, 13.6 g, 30 mmol, synthetic process in (see Supplementary material) [Supplementary material is available via a multimedia link on the online article webpage]) in anhydrous tetrahydrofuran was added. The reaction mixture was stirred for 3 h at room temperature and then filtered under vacuum. The filtrate was concentrated under reduced pressure to give residue, and then the residue was dissolved in ethyl acetate. The solution was washed with distilled water, brine, dried over MgSO₄, filtered under vacuum, and evaporated in vacuum to give crude BPFAOH. The crude BPFAOH was purified by silica gel column chromatography using gradient petroleum ether/ethyl acetate to yield 9.5 g of BPFAOH (49.2%), \(R_f \) 0.52 in 60% petroleum ether/ethyl acetate. The synthesis route is shown in Scheme 1.

The Fourier Transform infrared spectrum (FTIR) of the polymer against KBr included the following absorptions: 3512 (\(\text{NH}–\)), 3326 (\(-\text{OH}\)), 2981, 2934 (\(-\text{CH}3–\)), 1699 (\(-\text{C}=\text{O}\)), 1647 (\(-\text{NH}–\)), 1202, 1143 (\(-\text{CF}2–, -\text{CF}_3\)). Atmospheric pressure chemical ionization (APCI) mass spectrometry results were (positive) \(m/z\) observed 644 g mol⁻¹, theoretical was 643 g mol⁻¹.

\(^1\text{H} \) NMR spectroscopy (CDCl₃, 400 MHz) provided these results (\(\delta \) ppm): 1.44 (s, 9H, \(-\text{C}(\text{CH}_3)3\)), 2.0 (s, 1H, \(-\text{OH}\)), 3.47–3.53 (m, 4H, \(-\text{CH}_2\text{CH}_2–\)), 3.66–3.68 (m, 1H, \(-\text{CH}_3\text{OH}\)), 4.05–4.08 (m, 1H, \(-\text{CH}_2\text{O}H\)), 4.13 (br, 1H, \(-\text{CH}–\)), 5.57 (s, 1H, \(-\text{NH}–\)), 7.13 (s, 1H, \(-\text{NH}–\)), 7.82 (s, 1H, \(-\text{NH}–\)).

Synthesis of 2-amino-3-oxo-3-(2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluorooctanamido)ethylamino)propyl phosphorycholine (FASPC)

BPFAOH (9.5 g, 14.8 mmol) and triethylamine (2.5 ml, 17.8 mmol) were dissolved in 40 ml anhydrous tetrahydrofuran. After cooling the solution to \(-15^\circ\text{C}\), 2-chloro-1,3,2-dioxaphospholane-2-oxide (COP, 2.3 g, 16.3 mmol) dissolved in anhydrous tetrahydrofuran (20 ml) was added slowly to the stirred solution under nitrogen over 30 min. The temperature of the reaction mixture was maintained at \(-15^\circ\text{C}\) for 1 h and allowed to slowly warm to room temperature. Triethylammonium chloride precipitate was filtered off and washed with tetrahydrofuran. The filtrate was evaporated under vacuum to give residue as yellowish oil. The residue then was dissolved in 60 ml dry acetonitrile and transferred to a 100 ml glass pressure bottle. After the pressure bottle was cooled to \(-18^\circ\text{C}\), excess trimethylamine was rapidly added to the solution. The pressure bottle was then sealed and maintained at \(60^\circ\text{C}\) for 24 h; subsequently, the solution was evaporated under vacuum to produce a viscous liquid. The viscous liquid was dissolved in methanol and 30 ml saturated HCl/ethyl acetate was added with stirring for 24 h at room temperature. Sodium bicarbonate solution then was added to adjust to pH 10. Finally, the crude FASPC was purified by C18 reverse silica gel column chromatography using water/methanol to yield 8.9 g of FASPC (85.1%, \(R_f \) 0.45 in developing agent of BuOH/H₂O/HAc = 3/2/0.5). The synthesis route is shown in Scheme 1. Characterization of the material included FTIR against KBr (3435 (\(\text{NH}–\)), 2973, 2918 (\(-\text{CH}2–\)), 1704 (\(-\text{C}=\text{O}\)), 1647 (\(-\text{NH}–\)), 1204 (\(-\text{CF}2–, -\text{CF}_3\)), 1241 (\(\text{P}=\text{O}\)), 1149 (\(\text{CF}_2–, -\text{CF}_3\), \(\text{C}–\text{O}–\text{C}\)), 1093 (\(\text{P}–\text{O}–\text{CH}_2\)); APCI ms (positive) \(m/z\) theoretical 708 g mol⁻¹, observed 709 g mol⁻¹; and \(^1\text{H} \) NMR (DMSO, 400 MHz) (\(\delta \) ppm: 3.12 (s, 9H, \(-\text{N}^+(\text{CH}_3)_3\)), 3.26–3.31 (m, 4H, \(-\text{CH}_2\text{CH}_2–\)), 3.49–3.51 (t, 2H, \(-\text{CH}_2\text{N}^+\)), 3.64–3.77 (m, 2H, \(-\text{CH}–, -\text{CHOP}–\)), 4.02 (br, 3H, \(-\text{CHOP}–, -\text{POCH}_2–\)).

Synthesis of FASPC end-capped polyurethanes

FASPC end-capped polyurethanes based on 3/2/0.2/1.6 molar ratio of disocyanate/polymeric diol/BDO/FASPC were prepared by solution polymerization in a suitable solvent with DBTDL catalyst. All polyurethanes were synthesized by similar methods. A representative synthesis, of PT-HFPC (PTMG-HDI-BDO-FASPC), is described here.

In the first reaction step: HDI and 1% DBTDL were added to the stirred toluene solution of PTMG under a dry nitrogen atmosphere at 40°C. The reaction was maintained within a range of 60°C for 2 h. In the second step, chain extender (BDO) was added to the reaction solution, while the temperature was kept at 65 ~ 70°C for 1 h. Following this, the FASPC dissolved in DMF was added to the products of the second step and the mixture stirred at 80°C for 3 h. The solution was then cooled to room temperature and stored for 1 week. The polymer was precipitated in methanol/distilled water mixed solvent (vol/vol = 2/1) to remove any residual FASPC and the low molecular weight fraction, then dried in an oven for 24 h, followed by drying at 60°C under vacuum for 48 h. The reaction scheme for synthesis of...
fluorinated phosphorycholine (FASPC) end-capped polyurethanes is shown in Scheme 2. Characteristics of the polymer are summarized here: FTIR against KBr: 3300(NH), 2937, 2856(CH2), 1718(carbonyl of NHCOO), 1690 (carbonyl of NHCONH), 1244(P=O), 1113(C=O–C); and 1H NMR (DMSO, 400 MHz) δ ppm: 1.21 (s, −CH2−), 1.35 (s, 2H, −CH2−), 1.50 (s, 2H, −CH2−), 2.92–2.94 (m, 2H, −CH2−), 3.12 (s, 9H, −N+(CH3)3), 3.28 (br, 4H, −CH2CH2−), 3.5 (br, 2H, −CH2N+), 3.64–3.77 (br, −CH−, −CHOP−), 3.89–3.91 (m, 2H, −CH2−), 4.04 (br, −CHOP−, −POCH2−).

Characterization of materials
To confirm intermediates and FASPC chemical structures, 1H NMR spectra were conducted with a Bulker-400 spectrometer (400 MHz). Mass spectra were obtained on a TSQ Quantum ultra-LC/MS with atmosphere pressure chemical ionization (positive mode). Infrared analysis was performed on a Nicolet560 spectrophotometer between 4000 and 400 cm−1 at a resolution of 4 cm−1.

Polymer film preparation
The polymer films for evaluating the surface properties (XPS, contact angle measurements, ELISA, platelet adhesion measurements) and mechanical testing were prepared by drop-casting the clear polymer solution onto clean hydrophilic glass coverslips from 3% (m/v) THF. The films then were put into an oven at 30°C for 24 h, followed by drying at 60°C under vacuum for 48 h. The air-facing side of the film was analyzed.

Molecular weight determination
Gel permeation chromatography was carried out on an HL-GPC220 using two PLgel columns (5 um MIXED-C, 300 × 7.5 mm; PLgel 5 um MIXED-D, 300 × 7.5 mm) with a detection range from 1.0 × 103 to 2.0 × 105. Polymers were dissolved as 2.5 mg ml−1 in N, N-dimethylformamide (DMF) mobile phase, and injected onto styragel columns at 80°C, with a mobile phase flow rate 1 ml min−1. Average molecular weights were calibrated based on narrow molecular weight polymethylmethacrylate standards.

Mechanical testing
Mechanical testing was carried out with an Instron 5567 Model Universal Testing machine at 23°C and 50% relative humidity. The crosshead speed was 500 mm min−1. Samples of 50 mm × 6 mm × 0.2 mm were cut from films that were prepared as described above. The results reported are the mean values of five replicates.

X-ray Photoelectron Spectroscopy (XPS)
XPS was carried out on an XSAM-800 electron spectrometer. The spectrometer was equipped with an Mg-Kα achromatic X-ray source (20 kV, 10 mA) and two take-off angles (15° and 90°) were used. The take-off angle represents the sampling depth with 15° near the surface (two nanometers) and 90° away from the surface (10 nm). The air-facing side of each membrane sample was analyzed (Briggs et al. 1979). Every film (10 mm × 10 mm) was scanned three times for each sample type. Deconvolution of the high resolution spectra was made by means of a least-squares peak analysis software, XPSPEAK version 4.1, using the Gaussian/Lorenzian sum function. Initial assumptions on the possible species were derived from the results of previous studies (Beamson and Briggs 1992; Tasker et al. 1994; Amaral et al. 2005).

Contact angle measurements
Time-based water contact angle measurements on the air-facing side of each sample were obtained with a digital optical contact angle meter (DSA100, KRUSS GmbH, Germany) using the sessile drop method at ambient temperature. A distilled water droplet (3 μl) was applied with a syringe needle on a polyurethane film at 25°C, and the image of it was recorded automatically every 15 s to measure its contact angle. A small transparent glass box (5 cm × 3 cm × 3 cm) was used during the measurement to reduce evaporation. The values quoted are the average of five measurements on each sample.

Scheme 2. Reaction scheme for synthesis of fluorinated phosphorycholine (FASPC) end-capped polyurethanes.
Evaluation of protein adsorption

The adsorption of human fibrinogen (Fg, purchased from Aldrich) onto the films was evaluated using enzyme-linked immunosorbent assay (ELISA) according to the standard protocol (Zhang et al. 2006; Chang et al. 2007). The detailed process can be found in the Supplementary material [Supplementary material is available via a multimedia link on the online article webpage]. Polymer films with 1.0 × 1.0 cm² of surface area were incubated with 500 µl of PBS solution at 37°C for 60 min at first, and were then in sequence incubated with 500 µl of 0.5 mg ml⁻¹ human fibrinogen (Fg, purchased from Aldrich) in PBS solution at 37°C for 90 min, 500 µl of bovine serum albumin (BSA, 2 mg ml⁻¹ in PBS) solution at 37°C for 90 min, and 500 µl of 5.5 µg ml⁻¹ horseradish peroxidase (HRP) conjugated goat anti-human fibrinogen in PBS solution at 37°C for 30 min. The polymer films then were rinsed five times with 500 µl of washing buffer and transferred into clean wells, after which the enzyme-induced color reaction was carried out by adding 500 µl of 0.1 M citrate-phosphate buffer (pH = 5.0) containing 1 mg ml⁻¹ chromogen of o-phenylenediamine (OPD) and 0.03% hydrogen peroxide. Absorbance of light intensity at 490 nm was determined by a microplate reader. The absorbance from an ordinary poly(ether urethane)s was taken to be equivalent to 100% for calculating relative adsorption values. Results were presented as mean values of five samples for each polymer with the standard deviation (SD), and a comparative analysis was done using the analysis of variance and the Student t-test.

Platelet adhesion

Platelet-rich plasma (PRP) was obtained by centrifuging healthy pig blood and used in measuring platelet adhesion on polyurethane films (Ishihara et al. 1999). The films were washed three times with distilled water, the films were then treated with 2.5% PBS, the films were then rinsed five times with 500 µl of washing buffer and transferred into clean wells, after which the enzyme-induced color reaction was carried out by adding 500 µl of 0.1 M citrate-phosphate buffer (pH = 5.0) containing 1 mg ml⁻¹ chromogen of o-phenylenediamine (OPD) and 0.03% hydrogen peroxide. Absorbance of light intensity at 490 nm was determined by a microplate reader. The absorbance from an ordinary poly(ether urethane)s was taken to be equivalent to 100% for calculating relative adsorption values. Results were presented as mean values of five samples for each polymer with the standard deviation (SD), and a comparative analysis was done using the analysis of variance and the Student t-test.

Results and discussion

Synthesis of a novel amine monomer with fluorinated alkyl phosphatidylcholine polar headgroups

In a previous study (Tan et al. 2011), an end-capping reagent containing a long fluorocarbon chain (-(CF₂)₈CF₃) inserted between hydroxyl group and phosphatidylcholine polar headgroups was synthesized and used to prepare a series of phospholipid polyurethanes. But, phosphorus was not detected (ie <0.05%) on the surface of those phospholipid polyurethanes by XPS. This is because the reactivity of hydroxyl groups was reduced by the adjacent long fluorocarbon chain, resulting in only a small amount of end-capping reagent involved in those polyurethanes. Based on that study, a new amine monomer (FASPC) with a long fluorinated tail (-(CF₂)₈CF₃) and PC unit was designed and synthesized in the present study. The whole synthesis route and chemical structure of FASPC are shown in Scheme 1. In order to eliminate the impact of strong electronegativity of fluorine atoms on the reactivity of the amine group, ethylene diamine was chosen to enlarge the distance of fluorine atom and amine group of the L-serine. It was expected that more FASPC would be incorporated into the polyurethanes at the chain termination step due to the high reactivity of amine group than that of hydroxyl group. In addition, as the phosphatidylcholine polar headgroup and long fluorine tail are linked to the L-serine and then attached to each end of the molecule chain, it provides potential for the PC unit and the fluorinated chain to migrate together to the outer surface of the polyurethanes. The method of FASPC synthesized with trimethylamine in dry acetonitrile is according to the Nakaya method (Umeda et al. 1982), and the final product was a white power. The structures of all the synthetic intermediates and the resulting phosphatidylcholine (FASPC) shown in Scheme 1 were demonstrated by FTIR, 1H NMR and MS. The mass spectrogram (positive) of this product showed a single peak at 709 g mol⁻¹ (M_FASPC = 708 g mol⁻¹, Figure S1). The 1H NMR spectrum of the FASPC showed characteristic peaks of −N⁺(CH₃)₃, −CH₂N⁺, and −CH− at 3.12, 3.49–3.51, and 3.64–3.77 ppm, respectively. Moreover, the FTIR spectrum showed the C−F (−CF₂−, −CF₃) signal at 1204 cm⁻¹, P=O signal at 1241 cm⁻¹, and P−O−CH₂ signal at 1093 cm⁻¹. These results suggested that the FASPC has been synthesized successfully.

Synthesis and characterization of segment polyurethanes end-capped with fluorinated phosphatidylcholine

Due to the segmented nature of polyurethanes, it is possible to achieve a broad range of properties by
varying the hard and soft segments of these polymers (Lee and Tsai 1999; Woo et al. 2000; Tang 2001; Tang et al. 2001). The validity of the approach in the current study was tested, at first, with four kinds of segmented oligourethanes with different chemical structures, which were prepared by polyaaddition of HDI or MDI with PTMG or PHPC in toluene, followed by the addition of BDO as a chain extender. In the polymerization vessels, FASPC was finally added to react with diisocyanate of the prepolymers to obtain fluorinated phosphatidylcholine end-capped polyurethanes (PT-HFPC, PH-HFPC, PT-MFPC and PH-MFPC, where the acronyms represent the composition of the polyurethanes, PT representing PTMG, PH representing PHPC, M representing MDI, H representing HDI, FPC representing FASPC), as shown in Scheme 2. In order to avoid any confusion caused by unreacted FASPC or low molecular weight fractions, the polymers were precipitated in methanol/distilled water mixed solvent; no micromolecule signals arose on the GPC spectra (Figure S2–S5), which indicated the purity of the polymers.

The chemical structures of these polyurethanes were then confirmed by $^1$H NMR, FTIR and XPS spectra, as described in the Experimental section. The single peak at 3.12 ppm in $^1$H NMR spectra observed in all phospholipid polyurethanes was assigned to the methyl groups of the quaternary ammonium proton in phosphatidylcholine (PC) unit. In FTIR spectra (Figure 1), the PC group was identified from the peak 1244 cm$^{-1}$ (P=O). In addition, the infrared carbonyl band envelope of polyurethanes was composed of separate absorptions attributed to ‘free’ and hydrogen-bonded (ordered and disordered) groups (Coleman et al. 1988; Tan et al. 2005a). The absorption peak of ‘free’ C=O always appears in higher wave-number regions than hydrogen-bonded C=O. As shown in Figure 1, the ‘free’ and hydrogen-bonded C=O absorption peak positions were 1721 cm$^{-1}$ and 1684 cm$^{-1}$, respectively, in PT-MPU. However, the hydrogen-bonded C=O absorption peak migrated to about 1700 cm$^{-1}$, and the ‘free’/hydrogen-bonded C=O peak-area ratio increased in PT-MFPC. This indicated that the ratio of ‘free’ C=O groups sharply increased after introducing the large terminal groups.

After leaching unreacted FASPC monomer, the XPS data can offer direct evidence of successful end-capping. As shown in Figure 2, the XPS spectra of carbon (C$_{1s}$) and nitrogen (N$_{1s}$) were analyzed. The sample of PT-HFPC representing fluorinated phosphatidylcholine end-capped polyurethanes was compared with original polyurethanes (PT-HPU). The C$_{1s}$ peak in PT-HFPC was fitted to seven component peaks, viz. 284.9 eV (C–H and C–C), 285.9 eV (C–N–C=O), 286.4 eV (C–O–P), 286.6 eV (C–O–C=O and C–O–C), 288.8 eV (N–C=O), 291.5 eV (CF$_2$) and 293.6 eV (CF$_3$). Among them, the C–O–P, CF$_2$ and CF$_3$ peaks belong to the end-capped FASPC moiety, which do not appear in the C$_{1s}$ peak in PT-HPU. The characteristic N$_{1s}$ (CH$_3$)$_3$ peak also appeared at 402.1 eV in N$_{1s}$ spectra of PT-HFPC. These results indicated that FASPC was successfully incorporated to the end of the polyurethane chains.

Table 1 lists the molecular weights and molecular weight distributions of these polyurethanes as determined by GPC. The molecular weights of the obtained phospholipids polyurethanes were $>1 \times 10^4$, and smooth films were prepared by a solvent casting
method for surface characterization and hemocompatibility evaluation. However, due to their relatively low molecular weights, the phospholipid polyurethanes had low tensile strengths (Table 1). The tensile strength of PH-MFPC could not be detected in this test condition, which indicated that it can hardly be used as a bulk material.

**Surface property characterization**

As potential biomaterials, the surface properties of the FASPC end-capped polyurethanes deserve careful investigation. Table 2 lists the atomic molar percentages of fluorine (F 1s) and phosphorus (P 2p) at depths of 2 nm (15° take-off angle in XPS) and 10 nm (90° take-off angle) from the upper surfaces. Their bulk contents were calculated from 1H NMR spectra (Figure S6 and S7) and are shown for comparison. It should be noted that the ‘surface’ zone of the polyurethanes refers to the range of several nanometers below the air-polyurethane interface ( 5–10 nm) in the XPS study, and the ‘bulk’ represents the entire film including the ‘surface’ zone in the NMR study. Over 0.4% P 2p and 20% F 1s on the surfaces of FASPC end-capped polyurethanes were detected by XPS, indicating that phosphatidylcholine polar groups and long fluorine tails had migrated to these polymeric surfaces. And the fluorine concentration at the top surface (obtained by 15° take-off angle) was higher than that at the near surface (obtained by 90° take-off angle) for all FASPC end-capped polyurethanes. Furthermore, it can be observed that the F 1s content in the top surfaces (2 nm depth) was >4 times higher than that in the bulk of these polyurethanes. For example, the atomic percentage of fluorine on the top surfaces was 26.72%, which was 4.27 times greater than the 6.26% in bulk PT-HFPC; this surface-to-bulk value was 14.34 times in PH-MFPC. These results suggest that the long fluorine tails easily enriched the top surfaces of these polyurethanes due to the low surface energy of fluorine atoms and a stronger migration activity of the terminal group. In addition, phosphorus was also detected in each of the FASPC end-capped polyurethane films, indicating that phosphorylcholine head groups were indeed enriched at the surfaces with the help of the long fluorine tails.

It should be noted that the ratio of F:P on the surface (>35:1) was much higher than that in FASPC (15:1), which indicates that the large terminal groups were not in a random distribution but in the form of fluoroalkyl chains partially orienting to the outer surface (Iwasaki and Akiyoshi 2006). Due to the low interfacial free energy of CF 2 and CF 3, they tended to migrate to the surface for a stable interface, and the appearance of other functional groups (including phosphorylcholine) on the surface is then hindered. The ratio of F:P had a depth-dependence, and the maximum value of this ratio was observed on the outmost surface. For example, the ratio of F:P was 56:1 at 2 nm depth for PT-MFPC, but decreased to

| Sample       | Take off angle (°) | F 1s (surface, %) | F (bulk, %) | P 2p (surface, %) | P (bulk, %) |
|--------------|-------------------|-------------------|-------------|-------------------|-------------|
| PT-HFPC      | 15                | 26.72             | 6.26        | 0.51              | 0.42        |
|              | 90                | 22.70             | 5.00        | 0.67              | 0.33        |
| PH-HFPC      | 15                | 30.68             | 5.00        | 0.67              | 0.33        |
|              | 90                | 29.01             | 6.02        | 0.52              | 0.40        |
| PT-MFPC      | 15                | 33.27             | 2.32        | 0.52              | 0.40        |
|              | 90                | 25.84             | 0.41        | 0.52              | 0.40        |
| PH-MFPC      | 15                | 0                 | 0           | 0.52              | 0.40        |
| PT-HPU       | 15                | 0                 | 0           | 0.52              | 0.40        |

Note: *Values detected by XPS; **values calculated from 1H NMR spectra.

Table 1. Stoichiometry, molecular weights, yield and mechanical properties of the FASPC end-capped polyurethanes.
38:1 at 10 nm depth. The other samples showed the same trends. Because of linking to the fluorocarbon chain, it is fair to assume that the phosphorus also tended to be at the top-surfaces but not near-surfaces; that indeed occurred in the polyurethanes with a PHPC soft segment.

The opposite tendency for phosphorus concentration was observed for polyurethanes containing fluorinated phosphatidylcholine group in the side chain (Tan et al. 2005b). This could be due to the lower interfacial free energy of the CF₃ group, compared to that of the CF₂ group. The end groups would migrate more easily to the top-surfaces with the help of the long fluorine tail (−(CF₂)nCF₃). While this driving force seemed not to be enough to draw the phosphatidylcholine group effectively to the top-surfaces for the PTMG segment-containing polyurethanes (such as PT-MFPC), that could be due to the lower glass transition temperature of the PTMG segment compared to the PHPC segment (Figure S8). The lower glass transition temperature of the PTMG soft segment gives its higher mobility and makes it prone to appear on the surface (Yoon and Ratner 1988). However, there is still an incongruence in that the P content is greater for the PT polymer vs the PH polymer in the MFPC series, which is different from the F content comparison between them. As noted earlier, the F and P contents in the bulk were then calculated from ¹H NMR spectra and are listed in Table 2. It can be seen that the P content in the bulk of PH-MFPC was less than half of that in the PT-MFPC bulk, which may be the reason why the P content on the surface was greater for PT-MFPC. When the P content increases in the bulk, even if it is still less than in the PH polymer, the PH-HFPC can have a higher P content than PT-HFPC on the surface due to the higher glass transition temperature of the PHPC soft segment. As for the F content, caused by its low surface energy, the influence of the soft segment seems insignificant. The XPS spectra of carbon (C₁s) and nitrogen (N 1s) obtained at different sample depths of PT-HFPC are shown in Figure 2. It can be seen that the areas of N⁺(CH₃)₃, C−O−P, CF₂ and CF₃ peaks were clearly depth-dependent. All these characteristic peaks, which belong to the end-capped FASPC moiety, had larger peak areas at the 2 nm depth than at the 10 nm depth, especially for the CF₃ group at the lowest surface energy. These results provide strong further evidence that the fluorinated phosphatidylcholine groups enriched on the top-surfaces of the fluorinated phosphatidylcholine end-capped polyurethanes.

To further investigate the surfaces of fluorinated phosphorylcholine end-capped polyurethanes, the changes in the water contact angles on the surfaces of the polymer films with time were determined, and the results are shown in Figure 3. The ‘surface’ here refers to the air-facing interface of polyurethane films that were cast on glass. Although a small transparent glass box was used, the evaporation of the water droplets could not be completely avoided in the time-based contact angle measurement processing, but there was little variation in the water contact angles on the control polymer samples in the relatively short times of this study. If longer times had been used, a receding type of contact angle measurement would occur. In Figure 3, it can be seen that the water contact angles varied little with time on the surfaces of ordinary (control) polyurethanes, while a sharp decrease in the water contact angles on the surfaces of the FASPC end-capped polyurethanes occurred except for PH-HFPC. For example, the momentary water contact angle of PT-HFPC was 119°, but the contact angle quickly dropped to 83° after contacting the surface for 30 s. After only 1 min, except for PH-HFPC, the water contact angles on all the surfaces of fluorinated phosphorylcholine polyurethanes dropped to less than that of ordinary polyurethanes. It is not likely that this phenomenon was caused by the residual FASPC monomers, since they had been extracted by the methanol/water mixed solvent. So the result indicates that the top-surface structures of these FASPC end-capped polyurethanes rearranged rapidly from hydrophobic to hydrophilic with environmental change from air to water at room temperature. As for PH-HFPC, this rearrangement not only takes place, but apparently occurs as soon as the water contacts the film surface, so quickly that it could not be observed within the manipulation time for the measurement.

A detailed investigation of XPS, above, combined with the water contact angle measurement results
suggests that the phosphorycholine groups may orient on the top-surface due to the long fluorine tails bending over to the sub-surface when the samples are in contact with water; the model is shown in Scheme 3. Thus time-consuming pretreatment may not be required for medical devices prepared by these FASPC end-capped polyurethanes, since a biomimetic surface can be formed in the physiological environment within a short time.

**Hemocompatibility evaluation**

Thrombus formation occurring soon after blood perfusion is a major concern that will limit application of many of biomaterials for blood vessels. Fibrinogen is one of the most abundant proteins present in blood and plays a vital role in thrombosis on material surface. In addition, platelet adhesion and activation on the surface will trigger the coagulation of blood, leading to thrombus formation (Mackman 2008). Thus, protein adsorption and platelet adhesion were used to investigate the hemocompatibility of these FASPC end-capped polyurethanes.

The direct ELISA, using a polyclonal anti-fibrinogen conjugated to HRP, was used to detect the fibrinogen adsorption to those fluorinated phosphatidylcholine polyurethanes (Zhang et al. 2006). Fibrinogen adsorption to the control poly(ether urethane)s (PT-MPU) was used as a reference. Relative protein adsorption for various samples with respect to that on PT-MPU is shown in Figure 4, where it can be seen that the amount of protein adsorption on the FASPC end-capped polyurethanes films was significantly reduced as compared with that on PT-MPU. The amount of adsorbed human Fg on the PH-HFPC, PT-FPC, PT-MFPC and PH-MFPC was 12.9%, 11.3%, 7.9% and 2.2% of that on PT-MPU, respectively. It should be noted that the direct ELISA method is not the best method for assaying the amount of fibrinogen adsorbed to polymer surfaces because of its controversial degree of accuracy, but it is a convenient and swift approach for detecting protein adsorption on polymer membranes. More precise means, such as radiolabeling, to assay for the amount of fibrinogen adsorbed to polymer surfaces will be applied in future.

Figure 5 shows typical SEM photographs of platelet adhesion to the PT-MPU and FASPC end-capped polyurethanes. A substantial number of adhered platelets can be observed on the surfaces of PT-MPU and also some deformed platelets, indicating activation on adherent platelets (Suggs et al. 1999). Only a minor number of platelets were observed on the surfaces of PT-HFPC and PH-MFPC, and almost no platelets were observed on the surfaces of PH-HFPC and PT-MFPC. Close morphological examination

![Scheme 3. Schematic illustration of surface structure changes of FASPC end-capped polyurethanes in different circumstance. (a) Air or vacuum; (b) water contact.](image)

![Figure 4. Relative human fibrinogen adsorption on various material surfaces determined from ELISA with ordinary poly(ether urethane) (PT-MPU) as a reference.](image)

![Figure 5. SEM photographs of the surface of FASPC end-capped polyurethanes films after PRP exposure for 2 h. (A) PT-MPU; (B) PT-HFPC; (C) PH-HFPC; (D) PT-MFPC; (E) PH-MFPC. Actual magnification: left 1000 ×; right 5000 ×.](image)
further revealed that platelets on the PH-HFPC and PH-MFPC kept their original shapes and smooth surfaces, and looked like they were standing up on the surfaces of the polymer films, suggesting a strong suppression of platelet adhesion by these phospholipids polyurethanes.

Besides typical SEM photographs, counting the platelets will be needed in future for quantitative analysis. While other results from the FASPC end-capped polyurethanes indicated hemocompatibility, most of the films showed pristine surfaces after soaking in PRP; hardly a platelet was found with SEM, so analysis by counting was not completed. It should be noted that the promising qualitative data were not likely caused by platelet lysis due to leaching materials, since the unreacted FASPC and low molecular weight fractions were removed by precipitating the polymers in methanol/distilled water mixed solvent, and no micromolecule signal arose on the GPC spectra (Figure S2–S5). The surface holes seen in the SEM images, giving rise to possible confusion, were probably caused by solvent evaporation in the oven during film preparation.

The trend of the relative amounts of protein adsorption and platelet adhesion matches neither the changes in the fluorine content or the phosphorus content on the surfaces, which indicates that the inhibition of protein and platelet adhesion was not determined by a single factor, but was related to both the PC group and fluorinated chain. This synergistic effect is not clear-cut in this work. In addition, polyurethanes in different structures (various soft and hard segments) would exhibit different surface properties (Tan et al. 2005a), and the surfaces of polyurethanes cannot be completely covered with FASPC moieties. A concentration threshold effect of fluorinated phosphorylcholine on the polyurethane surfaces could be another important cause and needs to be studied in future. In spite of the variation in the polymeric backbones used in this study, the preliminary results suggest good hemocompatibility of all the FASPC end-capped polyurethanes. It is believed that the FASPC end-capped polyurethanes demonstrate good potential for the development of surfaces with minimal thrombogenic character in in vivo applications.

Conclusions

In this study, an amine monomer containing a fluorinated PC unit was successfully synthesized and used as the end-capper for the preparation of a series of new fluorinated phosphorylcholine polyurethanes. The enrichment of fluorinated phosphorylcholine on the surface of polyurethanes was detected by XPS. Furthermore, the surface structures of such polyurethanes could be rearranged rapidly in a water environment at room temperature. Protein adsorption and platelet adhesion results indicated that these novel fluorinated phosphorylcholine end-capped polyurethanes had good hemocompatibility. More importantly, it was found that the positive hemocompatibility did not greatly depend on the chemical structure of the polyurethanes, which suggests that this is a general method for the development of polyurethanes with potential bio-applications. Due to the relatively weak mechanical properties of the polyurethanes described here, the polymers are still unsuitable for use as bulk materials, and are being explored as polymeric additives in the authors’ laboratory.

Supplementary material

The synthetic process for N-(2-aminoethyl)-2,2,3,3,4,4,5,6,7,7,8,8,8-pentadecafluorocatanamide (APFD EC) and BOC-t-serine, evaluation of protein adsorption using the enzyme-linked immunosorbent assay (ELISA), and platelet adhesion on the polymer films. Refer to the Web version on PubMed Central for Supplementary material [Supplementary material is available via a multimedia link on the online article webpage].

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References

Amaral IF, Granja PL, Barbosa MA. 2005. Chemical modification of chitosan by phosphorylation: an XPS, FT-IR and SEM study. J Biomater Sci Polym Ed 16:1575–1593.

Beamson G, Briggs D. 1992. High resolution XPS of organic polymers. The Scienta ESCA300 Database. New York (NY): John Wiley & Sons Inc.

Briggs D, Brewis DM, Konieczko MB. 1979. X-ray photoelectron spectroscopy studies of polymer surfaces. J Mater Sci 14:1344–1348.

Chan KH, Armstrong J, Withers S, Malik N, Cumberland DC, Gunn J, Holt CM. 2007. Vascular delivery of e-myc antisense from cationically modified phosphorylcholine coated stents. Biomaterials 28:1218–1224.

Chang Y, Chen SF, Yu QM, Zhang Z, Bernards M, Jiang SY. 2007. Development of biocompatible interpenetrating polymer networks containing a sulfobetaine-based polymer and a segmented polyurethane for protein resistance. Biomacromolecules 8:122–127.
Chen KY, Kuo JF. 2002. Surface characterization and platelet adhesion studies of aliphatic polyurethanes grafted by fluorocarbon oligomers: effect of fluorocarbon chain length and carboxylic acid group. J Mater Sci Mater Med 13:37–45.

Coleman MM, Skrovnek DJ, Hu J, Painter PC. 1988. Hydrogen bonding in polymer blends. 1. FTIR studies of urethane-ether blends. Macromolecules 21:59–65.

Davie EW, Fujikawa K. 1975. Basic mechanisms in blood coagulation. Annu Rev Biochem 44:799–829.

Ernsting MJ, Labow RS, Santerre JP. 2003. Surface modification of a polycarbonate-urethane using a vitamin-E-derivatized fluoroalkyl surface modifier. J Biomat Sci Polym Ed 14:1411–1426.

Ernsting MJ, Labow RS, Santerre JP. 2007. Human monocyte adhesion onto RGD and PHSRN peptides delivered to the surface of a polycarbonate polyurethane using bioactive fluorinated surface modifiers. J Biomed Mater Res 83A:759–769.

Ernsting MJ, Bonin GC, Yang ML, Labow RS, Santerre JP. 2005. Generation of cell adhesive substrates using peptide fluoroalkyl surface modifiers. Biomaterials 26:6536–6546.

Farrar DJ, Litwak P, Lawson JH, Ward RS, White KA, Robinson AJ, Rodvien R, Hill JD. 1988. In vivo evaluations of a new thromboreistant polyurethane for artificial heart blood pumps. J Thorac Cardiovasc Surg 95:191–200.

Gods T, Ishihara K. 2006. Soft contact lens biomaterials from biospired phospholipid polymers. Expert Rev Med Devices 3:167–174.

Ishihara K, Fukumoto K, Iwasaki Y, Nakabayashi N. 1999. Modification of polysulfone with phospholipid polymer for improvement of the blood compatibility. Part 2. Protein adsorption and platelet adhesion. Biomaterials 20:1553–1559.

Iwasaki Y, Akiyoshi K. 2006. Highly wettable polyethylene films generated by spontaneous surface enrichment of perfluoroalkylated phosphorylcholines. J Appl Polym Sci 102:2868–2874.

Jahangir AR, McClung WG, Cornelius RM, McCloskey CB, Brash JL, Santerre JP. 2002. Fluorurated surface-modifying macromolecules: modulating adhesive protein and platelet interactions on a polycarbonate-urethane. J Biomed Mater Res 60:135–147.

Kashimoto K, Yoon J, Hou BY, Chen CH, Lin BH, Aratono M, Takie T, Schlossman ML. 2008. Structure and depletion at fluorocarbon and hydrocarbon water liquid/liquid interfaces. Phys Rev Lett 101:076102.

Lee DK, Tsai HB. 1999. Properties of segmented polyurethanes derived from different disocyanates. J Appl Polym Sci 75:167–174.

Leitmann R, Bohm O, Planitz P, Radehaus C, Schaller M, Schreiber M. 2010. Adsorption mechanisms of fluoro-carbon polymers at ultra low-k surfaces. Surf Sci 604:1808–1812.

Li YJ, Tomita T, Tanda K, Nakaya T. 1998. Synthesis and hemocompatibility evaluation of novel segmented polyurethanes with phosphorylchololine polar headgroups. Chem Mater 10:1596–1603.

Mackn N. 2008. Triggers, targets and treatments for thrombosis. Nature 451:914–918.

McCloskey CB, Yip CM, Santerre JP. 2001. Effect of fluorinated surface-modifying macromolecules on the molecular surface structure of a polyether poly(urethane urea). Macromolecules 35:924–933.

Moro T, Takatori Y, Kyomoto M, Ishihara K, Saiga K, Nakamura K, Kawaguchi H. 2010. Surface grafting of biocompatible phospholipid polymer MPC provides wear resistance of tibal polyethylene insert in artificial knee joints. Osteoarthr Cartilage 18:1174–1182.

Nagase Y, Nakajima S, Oku M, Iwasaki Y, Ishihara K. 2008. Synthesis and properties of segmented poly(urethane-urea)s containing phosphorylcholine moiety in the side-chain. Polym J 40:1149–1156.

Nederberg F, Bowden T, Nilsson B, Hong J, Hilborn J. 2004. Phosphoryl choline introduces dual activity in biomimetic ionomers. J Am Chem Soc 126:15350–15351.

Suggs LJ, West JL, Mikos AG. 1999. Platelet adhesion on a bioreasorbable poly(propylene fumurate-co-ethylene glycol) copolymer. Biomaterials 20:683–690.

Tang YW, Labow RS, Santerre JP. 2001. Enzyme-induced biodegradation of polycarbonate-urethanes: dependence on hard-segment chemistry. J Biomed Mater Res 56:516–528.

Tang YW, Santerre JP, Labow RS, Taylor DG. 1996. Synthesis of surface-modifying macromolecules for use in segmented polyurethanes. J Appl Polym Sci 62:1133–1145.

Tasker S, Chamber RD, Badyal JPS. 1994. Surface defluorination of PTFE by sodium atoms. J Phys Chem 98:12442–12446.

Umeda T, Nakaya T, Imoto M. 1982. Polymeric phospholipid analogues, 14. The convenient preparation of a vinyl monomer containing a phospholipid analogue. Macromol Rapid Commun 3:457–459.

Wu GLY, Mittelman MW, Santerre JP. 2000. Synthesis and characterization of a novel biodegradable antimicrobial polymer. Biomaterials 21:1235–1246.

Wootton DM, Ku DN. 1999. Fluid mechanics of vascular systems, diseases, and thrombosis. Annu Rev Biomed Eng 1:299–329.

Xie XY, Wang RF, Li JH, Luo L, Wen D, Zhong YP, Zhao CS. 2009. Fluorocarbon chain end-capped poly(carbonate urethane)s as biomaterials: blood compatibility and chemical stability assessments. J Biomed Mater Res B 89B:223–241.
Yoon SC, Ratner BD. 1988. Surface and bulk structure of segmented poly(ether urethanes) with perfluoro chain extenders. 3. Effects of annealing, casting solvent, and casting conditions. Macromolecules 21:2401–2404.

Yung LYL, Cooper SL. 1998. Neutrophil adhesion on phosphorylcholine-containing polyurethanes. Biomaterials 19:31–40.

Zhang XQ. 2010. Synthesis and characterization of fluorinated phosphorylcholine end-capped polyurethanes [Dissertation]. Sichuan University. 62 pp.

Zhang Z, Chao T, Chen SF, Jiang SY. 2006. Superlow fouling sulfobetaine and carboxybetaine polymers on glass slides. Langmuir 22:10072–10077.