

LARGELY IMPROVED BLOOD COMPATIBILITY OF POLYURETHANE BY BLENDING WITH FLUORINATED PHOSPHATIDYLCHOLINE POLYURETHANE*

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Abstract The improvement of biocompatibility of polyurethanes was investigated. The results demonstrate that the blood compatibility of polyurethanes can be further improved by just simply mixing with the fluorinated phosphatidylcholine poly(carbonate urethane)s (FPCPCUs). The solution blending was done by mixing poly(ether urethane) (PEU) with FPCPCU in different compositions. An increased blood compatibility of the blend films was observed with the increase of FPCPCU content, and when FPCPCU content reached to 40 wt% (40FPCPCU film), 6 times and 23 times decrease of platelet adhesion number have been achieved, compared with that of FPCPCU and PEU, respectively. More interestingly, the blend films showed even better blood compatibility than that of FPCPCU. DSC, AFM and XPS were used to characterize the miscibility and surface structure of the blends film. The largely improved blood compatibility was rationalized based on the combined effect of phase separation between PEU and FPCPCU and the formation of surface nanostructures induced by segregation of FPCPCU at the surface.

Keywords: Fluorinated phosphatidylcholine poly(carbonate urethane)s; Blood compatibility; Migration and segregation.

INTRODUCTION

Polyurethanes (PU) are widely used for manufacturing medical devices because of their excellent mechanical properties, and good blood compatibility^[1]. However, the blood compatibility of polyurethanes is not satisfactory for long-term implantation as a small-diameter vascular graft (< 6 mm). New concepts for making blood compatible polymer surfaces have been proposed with considerably attention to the outer surface of the cell membrane, the biomembrane, which is mainly constructed of natural phospholipid molecules^[2]. Chapman and his group examined this point in the middle of the 1980s and proposed to make a non-thrombogenic surface by direct modification of the material surface with a phospholipid group. Although their preliminary data suggested the wisdom of the concept, they could not supply new materials and medical devices using their modification method^[3–6]. On the other hand, the molecular design and synthesis of a new methacrylate monomer with a phospholipid polar group, 2-methacryloyloxyethyl phosphorylcholine (MPC) and its polymer was reported by one of the authors in 1978 for development of new biocompatible and blood-compatible materials^[7]. The MPC could be polymerized with various other vinyl compounds, and hemocompatibility of the MPC polymer was carefully evaluated^[8–14]. The platelet adhesion and activation were suppressed on the surface of the MPC polymers when the MPC composition was above 30 mol%^[9, 10, 15, 16]. These non-thrombogenic properties appeared when the MPC polymers came in contact with whole human blood even in the absence of

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anticoagulants^[11]. One step further, a series of 2-methacryloyloxyethyl phosphorylcholine (MPC) were blended with polyurethanes^[17–21], and it was found that the blood compatibility of the PU/MPC polymer blends was superior compared with that of the original PEU, in terms of platelet adhesion and protein adsorption.

Nevertheless, these phosphatidylcholine polar headgroups cannot easily migrate to the surface. Thus, in order to achieve phosphatidylcholine polar head groups on the surface, a large amount of phospholipid chain extender has to be used, which leading to a high water adsorption and deteriorated mechanical property^[22, 23]. In order to obtain biomedical polyurethane with excellent biocompatibility and biostability as well as good mechanical properties, a series of poly(carbonate urethane)s containing fluorinated phosphatidylcholine side groups (FPCPCUs) were designed and synthesized recently in our group^[24]. In addition, our previous studies also revealed that the fluorinated side chains of fluorinated polyurethanes were prone to migrate to the uppermost surfaces of the polyurethanes blends^[25]. In this way, the phosphatidylcholine polar head groups can be easily brought out onto the surface or sub-surfaces with the help of migration of fluorinated side chain that is directly connected with phosphatidylcholine polar head group. Thus only a small amount of phosphatidylcholine has been proved enough to improve the hemocompatibility of polyurethanes. In this paper, we will report our recent efforts to further improve the blood compatibility of PEU by blending with (FPCPCUs). It was found that the blood compatibility of the PEU/FPCPCU blends with attention to platelet adhesion was superior compared with that of the original PEU, and could be much better than that of the pure FPCPCU.

EXPERIMENTAL

Materials

A novel chain extender with a long side chain fluorinated alkyl phosphatidylcholine polar head group 2-[2-[2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluoro-10-ethoxy-decyloxy]-*N*-(2-hydroxy-1-hydroxymethyl-1-methyl-ethyl)-acetamide] phosphatidylcholine (HFDAPC) was synthesized from 2,2,3,3,4,4,5,5,6,6,7,7,8,8,9,9-hexadecafluoro-decane-1,10-diol by multi-step reaction. Fluorinated phosphatidylcholine poly(carbonate urethane)s (FPCPCU) were synthesized with methylenebis(phenylene isocyanate)(MDI), poly(1,6-hexyl-1,5-pentylcarbonate) diol (PHPCD, $M_w = 1058$) or polytetramethyleneoxide(PTMO, $M_n = 1000$), HFDEAPC and 1,4-butandiol (BDO) (molar ratio 4:2:1:1) using two-step solution polymerization in *N,N*-dimethylacetamide (DMAc). The schematic structure of FPCPCU is shown in Fig. 1. And the FPCPCU used in this work had 50%

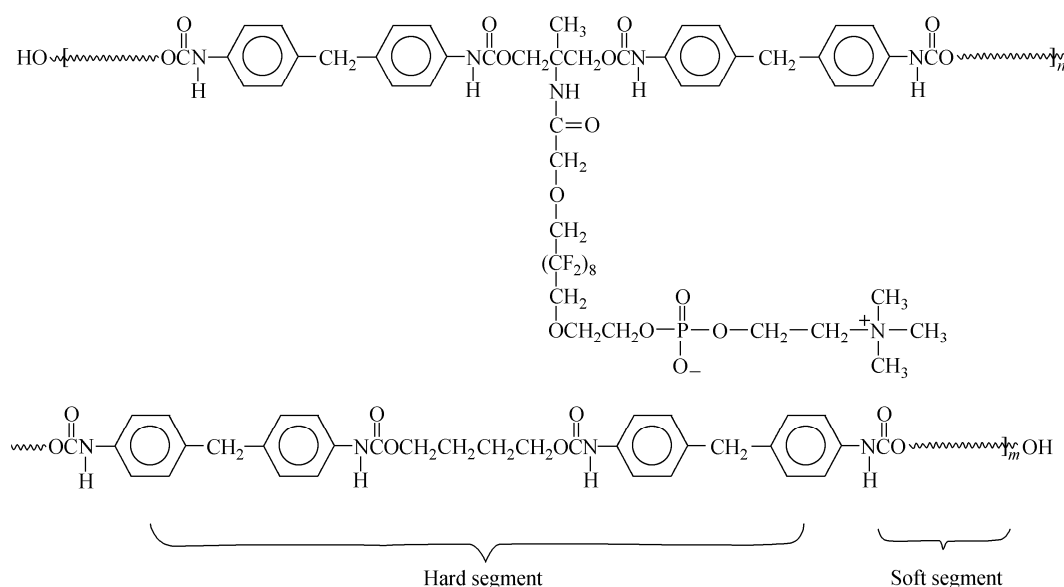


Fig. 1 The schematic structure of fluorinated phosphatidylcholine poly(carbonate urethane)s (FPCPCU). Soft segment is poly(1,6-hexyl-1,5-pentylcarbonate) diol (PHPCD)

long fluorinated phosphatidylcholine in side chain as the chain extender. The molecular weights and molecular weights distribution of the FPCPCU, determined by GPC using polystyrene standards and THF as the mobile phase, were 3.7×10^4 and 2.06, respectively. Pure poly(ether urethane) (MDI:BDO:PTMO = 2:1:1) with a molecular weight of $M_n = 5.8 \times 10^4$, and hard segment percentage of 37.1 wt% was used.

Sample Preparation

The series of PEU/FPCPCU blends were prepared in 50 g/L via solution mixing in DMAc. The content of FPCPCU in the blends are 10, 25, 40 and 50 wt%, and labeled as 10FPCPCU, 25FPCPCU, 40FPCPCU and 50FPCPCU, respectively. The sample films were prepared by casting the solution onto clean hydrophilic glass plates, and then put the plates into an oven at 50°C for 24 h, then under vacuum at 50°C for 48 h, 80°C for 2 h. These specimens were used for evaluation of blood compatibility and measurement of the surface and bulk structure.

Evaluation of Platelet Adhesion

Fresh whole blood was extracted from healthy white rabbit, and the platelet-rich plasma (PRP) was obtained with the citrated blood centrifuged at 190 g for 10 min. The blend films were washed three times with distilled water and physiological saline, respectively, and then soaked in PRP, which platelet density was adjusted to 3.85×10^5 cells/ μ L, for 1 h at 37°C. After the PRP solution was removed, samples were rinsed with PBS (Phosphate buffered saline, pH = 7.4), and treated with 2.5% glutaraldehyde at 4°C overnight. Then, the samples were dehydrated by systematic immersion in a series of ethanol/water solution (60, 70, 80, 90, 95, and 100 vol%) and later dehydrated by critical point drying using carbon dioxide as transitional fluid. Finally, the samples were gold coated for the analysis of scanning electron microscope (SEM) with JEOL Superprobe 733 scanning electron microscope. The number of platelets that adhered to the polymer films was counted in three regions with SEM pictures ($\times 1000$). Statistical analysis was performed using a student's t-test ($p < 0.05$).

X-ray Photoelectron Spectroscopy (XPS)

XPS was carried out on an XSAM-800 electron spectrometer. The spectrometer was equipped with a Mg-K α achromatic X-ray source (20 kV, 10 mA) and two take-off angles of 30° and 90° were used with X-ray source. The take-off angle represents the depth having been detected 30° is near the surface (fifty of angstroms) and 90° is ten nanometers away from the surface. Air-facing side of the membranes was analyzed.

Atomic Force Microscopy (AFM)

The AFM measurements were performed on SPA400 with an SPI3800N controller, Seiko Instruments Industry, Co. Ltd. at room temperature, using the micro-fabrication cantilevers with force constant of approximately 13 N/m. In tapping mode, the topographic and phase data were recorded simultaneously. In our study, the ratio of amplitude was adjusted to 0.4–0.7 of the free air amplitude for all the images. Under these moderate tapping condition, phase data are sensitive to the difference of surface stiffness between hard and soft segment of polyurethane^[26–28].

DSC Measurement

The polymer samples (17–20 mg) were cut off from the films prepared as described above. The DSC heating data was collected at 10 K/min from –100 to 100°C by TA instrument 2910 thermal analyzer. During the measurement, dried N₂ gas was purged at a constant flow rate. The temperature reading and calorific measurement were calibrated by using standard indium.

RESULTS AND DISCUSSION

Figure 2 shows SEM pictures of the materials surface after contact with the platelets for 60 min. On the PEU, 10FPCPCU and 25FPCPCU surface, the platelets are formed with large aggregates and the extension of pseudopodia is observed, indicating activation on adherent platelets^[29, 30]. However, much decreased number of adhered platelet is observed on the 50FPCPCU and FPCPCU films. On 40FPCPCU film almost no platelet can

be adhered and the adhered ones keep their original shape with smooth surface. Moreover, the platelets look like stand-up on the surfaces of the polymer blends, compared with aggregates and pseudopodia on the surfaces of PEU, 10FPCPCU and 25FPCPCU, suggesting a strong suppression of 40FPCPCU to platelet adhesion. To quantitatively estimate the blood compatibility, the number of adhered platelets on the films was calculated. They are $(4.59 \pm 0.54) \times 10^4$, $(4.43 \pm 1.63) \times 10^4$, $(3.28 \pm 0.04) \times 10^4$, $(0.17 \pm 0.05) \times 10^4$, $(2.65 \pm 0.53) \times 10^4$ and $(1.22 \pm 0.03) \times 10^4$ cells/mm² on PEU, 10FPCPCU, 25 FPCPCU, 40FPCPCU, 50FPCPCU, and FPCPCU films, respectively. The 40FPCPCU film surface shows remarkably less adhered cells than other polymer surfaces, even much better blood compatibility than pure FPCPCU film. Actually, it has been reported in our previous paper that FPCPCUs with only 5–12.5 mol% feed ratios of phosphatidylcholine extender already had very good hemocompatibility, as indicated by protein adsorption and platelet adhesion experiments^[24]. Here we found that the blood compatibility of PEU could be further largely improved just by simply blending with FPCPCU. 40FPCPCU film shows 6 times decrease of platelet adhesion number compared with FPCPCU, and 23 times decrease of platelet adhesion number compared with PEU. The excellent anti-adhesion to platelets and the good blood compatibility of these materials (particularly for 40FPCPCU) may bring great advantages for PEU to find various medical applications.

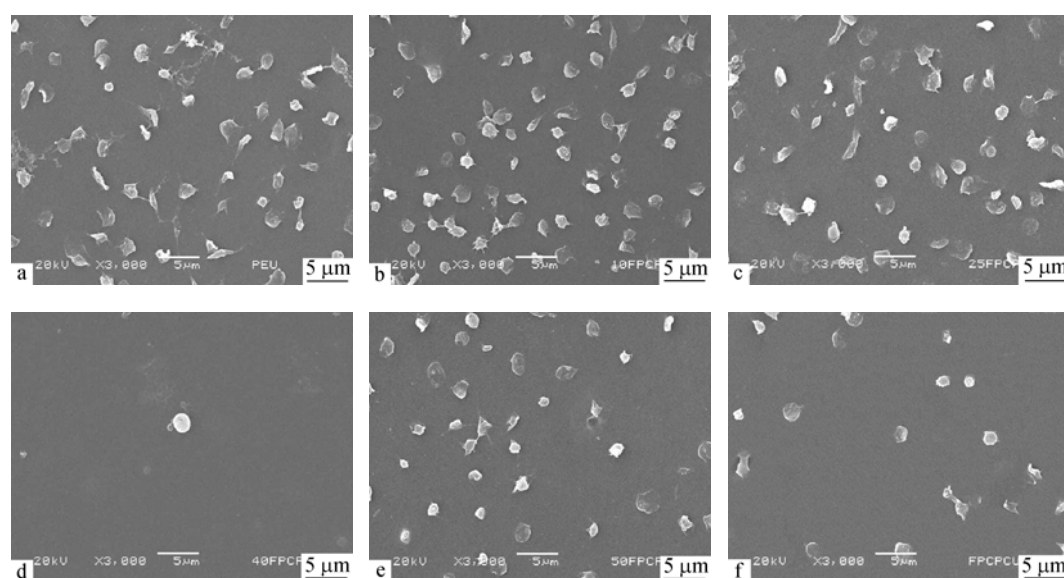


Fig. 2 Platelet adhesion on the blends, FPCPCU and PEU surfaces
a) PEU; b) 10FPCPCU; c) 25FPCPCU; d) 40FPCPCU; e) 50FPCPCU; f) FPCPCU

To understand the improved blood compatibility of the blends films, it is necessary to know the miscibility between the components. For this reason, DSC experiment was carried out to measure the change of glass transition temperature (T_g) of FPCPCU and PEU in the blends. This is shown in Fig. 3. Only the T_g of soft segment could be observed for both FPCPCU and PEU in the experimental temperature range. The T_g of FPCPCU and PEU is around -10°C and -40°C , respectively. One observes a single T_g located around -40°C for 10FPCPCU and 25FPCPCU. In this case, only T_g of PEU is observed and almost no T_g for FPCPCU is detected. This may be caused by (1) a phase miscible structure between FPCPCU and PEU, or (2) the less content of FPCPCU and its T_g cannot be easily observed by DSC. However, two obvious T_g s (the one is located around -40°C and the other is located around -10°C) for 40FPCPCU and 50FPCPCU can be observed. So it can be safely concluded that PEU could be phase miscible with FPCPCU at low content of FPCPCU, but phase separated at higher FPCPCU content.

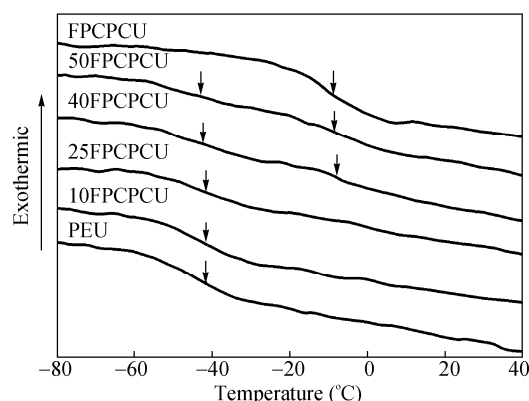


Fig. 3 DSC curves of fluorinated phosphatidylcholine poly(carbonate urethane)s and poly(ether urethane)s

Having known the phase behavior of the blend films, now let's check the surface change by using XPS. The atomic percentage of phosphorus (P_{2p}), fluorine (F_{1s}), carbon (C_{1s}), oxygen (O_{1s}) and nitrogen (N_{1s}) at the depth of 5 nm (Take-off angle 30°) and 10 nm (Take-off angle 90°) away from surface of the PEU/FPCPCU blends are listed in Table 1. It can be seen that the atomic percentage of fluorine element on the blends surface is increased with increasing content of FPCPCU in the blends, and the fluorine content on the surface of 40FPCPCU is the highest. It is interesting that the atomic concentration of fluorine at the depth of 5 nm and 10 nm away from the surface for 40FPCPCU is even higher than that of pure FPCPCU. And that the fluorine concentration at the uppermost surface (acquired by take-off angle 30°) is higher than that at the near surface (obtained by take-off angle 90°) for all the PEU/FPCPCU blends prepared. This result suggests that the low surface energy of fluorinate alkyl chains are very easy to migrate to the uppermost surface in the PEU/FPCPCU blends system. In addition, the phosphorus has also been detected for each sample of PEU/FPCPCU blends on the surface, except for 10FPCPCU, probably due to the low content of FPCPCU in the blend. This indicates that the phosphatidylcholine polar group is indeed brought out onto the surface with the help of migration of fluorinated side chain that is directly connected with it. Furthermore, the content of nitrogen (which general represents the hard segments of polyurethanes) on the uppermost surface of polymer blends is increased with the increasing depth away from the surface. The XPS spectrum of nitrogen (N_{1s}) is shown in Fig. 4 obtained at 90° . The N_{1s} peak for 40FPCPCU consist of two symmetric and Lorentzian peaks for urethane nitrogen ($-\text{NH}-(\text{C}=\text{O})-\text{O}$) at 399.88 eV and phospholipids ammonium nitrogen ($-\text{N}^+(\text{CH}_3)_3$) at 402.23 eV, as shown in Fig. 4(b). However, the PEU polymer surface shows only urethane nitrogen ($-\text{NH}-(\text{C}=\text{O})-\text{O}$)

Table 1. Atomic percentage of phosphorus, fluorine, and nitrogen at the depth of about 5 nm and 10 nm on the PEU/FPCPCU blend film surfaces

Sample	Take-off angle	P_{2p} (%)	F_{1s} (%)	N_{1s} (%)
PEU	30°	0.0	0.0	0.0
	90°	0.0	0.0	1.0
10FPCPCU	30°	0	7.9	2.9
	90°	0	4.4	3.0
25FPCPCU	30°	0.4	17.8	2.9
	90°	0.7	13.2	3.8
40FPCPCU	30°	1.3	22.7	2.6
	90°	0.7	16.0	3.1
50FPCPCU	30°	0.8	19.0	2.2
	90°	1.0	15.4	3.2
FPCPCU	30°	1.2	16.1	2.2
	90°	0.9	14.5	2.4

peak at 399.50 eV (Fig. 4a). These results provide strongly further evidence that the phosphatidylcholine polar group is also present on the surfaces of these blend films. The largely increased intensity of the peak at 399.50 eV for 40FPCPCU compared with that for PEU also suggests that a large amount of hard segments of polyurethanes has been brought onto the surface for 40FPCPCU.

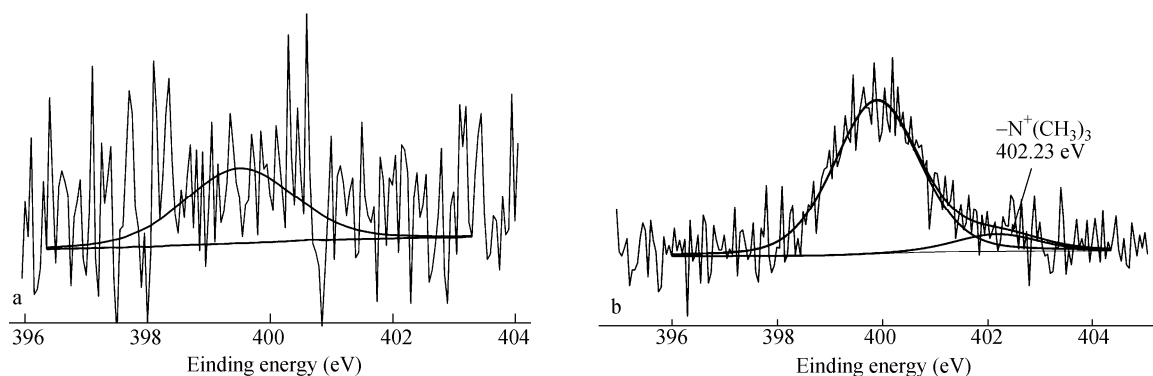


Fig. 4 XPS spectrum in the nitrogen (N1s) of PEU (a) and 40FPCPCU (b)
The take-off angle of photoelectron relative to the polymers surfaces was 90°.

Since the largely enhanced migration is observed only for PEU/FPCPCU blends with higher FPCPCU content, one may expect that phase separation between PEU and FPCPCU may play certain role for the enhanced migration. To prove this, PEU was also blended with fluorinated phosphatidylcholine poly(ether urethane) (FPCPEU), which should have much better miscibility with PEU, due to the similar ether structure. Only one glass transition temperature was observed for all the PEU/FPCPEU blends in DSC curves (not shown here), which suggesting indeed a good miscibility between PEU and FPCPEU. The XPS result is listed in Table 2. One observes that all the blend film surfaces have a lower atomic percentage of phosphorus and fluorine than that of pure FPCPEU. This result suggests that the surface migration for 40FPCPCU and 50FPCPCU could be indeed due to the phase separation between the PEU and FPCPCU since two T_g s are observed in these two blends as shown in Fig. 3.

Table 2. Atomic percentage of phosphorus, fluorine, and nitrogen at the depth of about 5 nm and 10 nm on the PEU/FPCPEU blend film surfaces

Sample	Take-off angle	P _{2p} (%)	F _{1s} (%)	N _{1s} (%)
PEU	30°	0.0	0.0	0.0
	90°	0.0	0.0	1.0
10FPCPEU	30°	0.0	3.7	0.0
	90°	0.0	2.7	2.6
25FPCPEU	30°	0.0	7.9	1.2
	90°	0.0	5.7	2.7
40FPCPEU	30°	0.0	9.9	2.0
	90°	0.2	9.0	2.6
50FPCPEU	30°	0.0	6.9	2.0
	90°	0.0	5.9	3.7
FPCPEU	30°	1.2	10.3	2.3
	90°	0.5	9.3	3.0

Anderson *et al.* carried out the blending of polymeric materials having a ternary amino group into polyurethanes and evaluated protein adsorption and cell adhesion on the modified materials^[31–33]. Both protein adsorption and cell adhesion on the polyurethane surface could be reduced by the addition of polymeric materials having a ternary amino group. Furthermore, a series of 2-methacryloyloxyethyl phosphorylcholine

(MPC) and polyurethanes (PUs) blends were reported by Ishihara group^[17–20]. The blood compatibility of the PU/MPC polymer blends with attention to platelet adhesion and protein adsorption was superior compared with that of the original PEU. However, neither Anderson nor Ishihara has investigated the change of surface structure of PEU films after blending with other materials and proposed a reasonable explanation for the improved blood compatibility observed in their studies. Following we will try to understand the improved blood compatibility from the change of surface structure by using AFM.

AFM was used to study the microdomains of polyurethanes lying underneath the upper most surface layer by 0.4–0.7 ratio of the free air amplitude tapping and the phase imaging at the tapping of the ratio provides information about surface stiffness variation related to changes in Young's modulus^[26, 27, 34]. The measured tensile modulus of the FPCPCU is 35 MPa, which is 2 times of that for PEU (17 MPa). So the surface morphology can be easily studied by the difference of surface stiffness between PEU and FPCPCU *via* AFM. Figure 5 shows the topographic and phase images of PEU, FPCPCU, 10FPCPCU, 25FPCPCU, 40FPCPCU and 50FPCPCU blends samples in region of 5 μm scan. The phase data for the hard area is bright and the soft area is dark, and yet, topography data for the hard area is low spot and the soft area is high spot. Topography and phase images of the blends are changed visibly compared with that of PEU and FPCPCU, where nearly only one phase is observed on their surfaces (Figs. 5a and 5b). Actually, polyurethanes alone possess a two-phase structure composed by the hard and soft segments. However, the phase separation between the hard and soft segments is so small and could not be detected *via* AFM in our experiment. For the PEU/FPCPCU blends, two phases

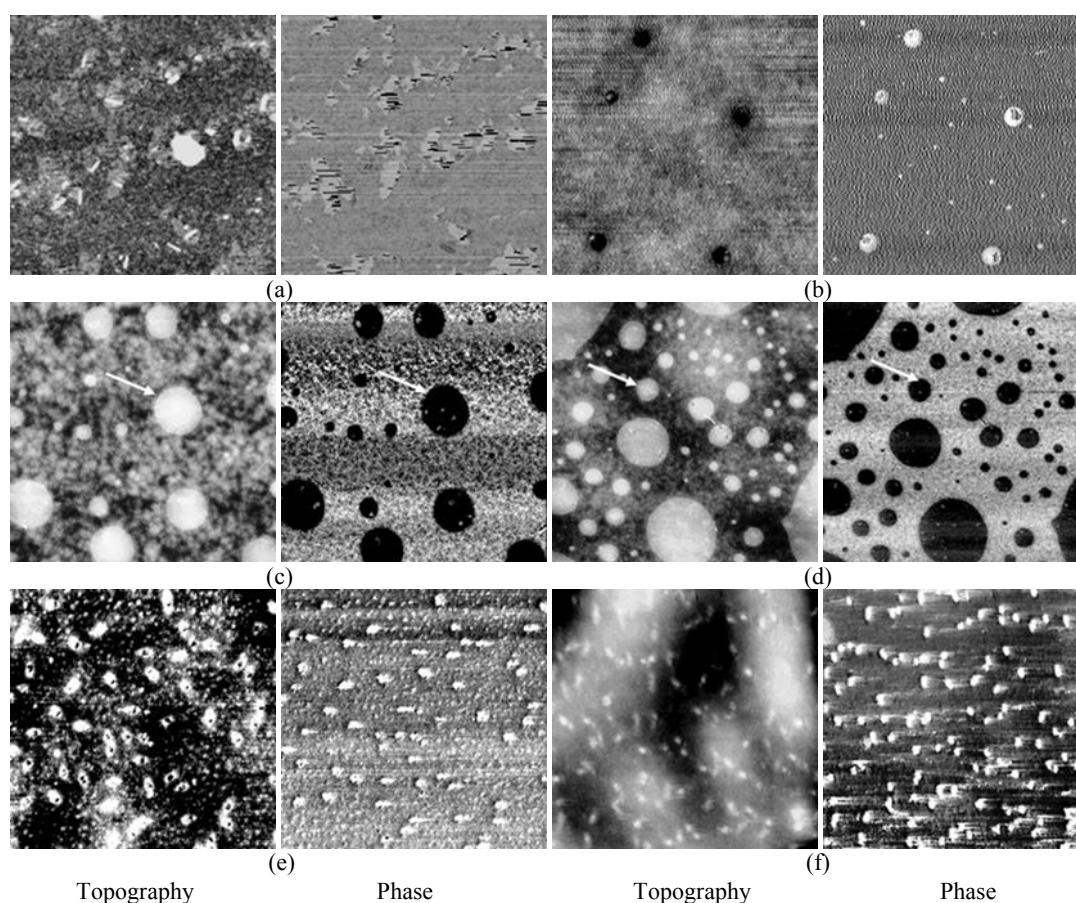


Fig. 5 Topography and phase images of PEU (a), FPCPCU (b), 10FPCPCU (c), 25FPCPCU (d), 40FPCPCU (e) and 50FPCPCU (f) at 5 μm scan sizes

surface structure is obviously evident, where the PEU phase appears as high spots in topographies and dark in phase images. For 10FPCPCU and 25FPCPCU films, it is interesting to see that the minor component FPCPCU forms the continuous phase and the major component PEU forms droplets with a size range from 0.3 μm to 1.0 μm . This is certainly due to the migration of FPCPCU onto the surface. With increasing of FPCPCU content to 40 wt%, a peculiar surface structure that a combination of two FPCPCU domain sizes is observed. One is in the range of 150–200 nm and another is in the range of 50–80 nm. When the content of FPCPCU increases to 50 wt%, the large droplets still remain there, however, the small ones disappear with some dark areas exposed on the surface. The change of surface structure of PEU induced by adding FPCPCU could be understood as due to the migration and phase separation of FPCPCU at the surface, particularly for 40FPCPCU and 50FPCPCU.

AFM observation is in very good agreement with XPS result and DSC measurement. And the DSC, AFM and XPS results together can be well used to understand the large improved blood compatibility of PEU/FPCPCU blends, particularly for 40FPCPCU. With increasing of FPCPCU content, an increased content of PC group is achieved due to the migration of FPCPCU onto the surface, resulting in increased blood compatibility. For 40FPCPCU and 50FPCPCU, the migration of FPCPCU onto the surface is not only driven by low surface energy of fluorine group, but more importantly, could be also driven by the phase separation between the PEU and FPCPCU. For 40FPCPCU sample, a peculiar surface structure with a combination of micro and nano domain sizes is formed. Since the formation of nanostructure was found also an effective approach for excellent blood compatibility^[35], the large improved blood compatibility of 40FPCPCU could be understood as due to the peculiar surface structure and the highest content of PC group on the surface. As further increasing of FPCPCU to 50 wt%, the phase separation takes place seriously on the surface and no more the nano-domain exists, resulting in a decreased atomic percentage of fluorine and phosphorus, thus decreased blood compatibility.

CONCLUSIONS

The blood compatibility of polyurethanes could be largely improved by just simply mixing with these fluorinated phosphatidylcholine poly(carbonate urethane)s (FPCPCUs). The surface structure and miscibility of the blend film have been confirmed by XPS, AFM and DSC. With increasing of FPCPCU content, an increased content of PC group was achieved due to the migration of FPCPCU onto the surface. Results in platelet adhesion experiment imply strongly that a considerable enhancement of blood compatibility can be achieved. Since the PEU/FPCPCU blends have shown also good mechanical properties and much cheaper price compared with FPCPCU, together with the excellent blood compatibility, it can be expected these materials may play important roles in future medical applications.

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