Preparation of a stable phospholipid monolayer grafted onto a methacryloyl-terminated substrate as blood compatible materials

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Abstract—We have prepared a surface-grafted phospholipid monolayer by in situ polymerization carried out at the interface between a pre-assembled phospholipid monolayer and a methacryloyl-terminated substrate. The phospholipid containing an acryloyl moiety, 1-stearoyl-2-[12-(acryloyloxy)-dodecanoyl]sn-glycero-3-phosphocholine (acryloyl-PC), was pre-assembled by vesicle fusion onto methacryloyl-terminated substrates which were silanized with 3-(trimethoxysilyl)propyl methacrylate (TSM). The acryloyl-PC monolayer and methacryloyl-terminated substrates were then polymerized in situ by adding a water-soluble initiator, 2,2-azobis(2-methylpropionamide) dihydrochloride (AAPD), at 60°C for 15 min. Atomic force microscopy (AFM) and X-ray photoelectron spectroscopy (XPS) measurements indicated that the polymerized phospholipid surface on the TSM-silanized substrates formed a lipid monolayer structure with some defects. The polymerized phospholipid surfaces also showed good stability in methanol due to chemical bonding to solid surfaces. The grafting efficiency of acryloyl-PC monolayer on the TSM substrate, which was calculated by the relative carbon ratio of the polymerized acryloyl-PC monolayer on TSM substrate before and after methanol washing, was 94.5%. For comparative analysis, the acryloyl-PC monolayer was also polymerized onto dimethyl-terminated substrates silanized with dichlorodimethylsilane (DCM). In the absence of surface grafting moieties on solid substrates, the laterally polymerized acryloyl-PC monolayer physically adsorbed on substrates was easily removed in an organic solvent. The surface-grafted phospholipid monolayer was also greatly effective in the prevention of platelet adhesion.

Key words: Phospholipid monolayer; in situ polymerization; surface grafting; blood compatibility.

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INTRODUCTION

Neutral phosphorylcholine (PC) surfaces have been recognized as one of the most biologically inert surfaces for developing blood compatible materials, since outer cell membranes of red blood cells and endothelial cells are mainly composed of PC lipids [1, 2]. As such, there have been many reports regarding the development of cytomimetic biomaterials containing a PC group. Ishihara et al. synthesized 2-methacryloyloxyethyl phosphorylcholine (MPC) copolymers with enhanced blood compatibility [3–5]. The MPC copolymers were designed to mimic the surface structure of the cell membranes and their PC group played an important role of creating excellent blood compatibility.

Supported phospholipid monolayers and bilayers containing highly-packed PC lipids on substrates have been also extensively studied using vesicle fusion and Langmuir–Blodgett (LB) techniques [6, 7]. Both vesicle fusion and LB techniques are used to engineer the surface in which the constituent members can be controlled, modified and easily assembled with a high level of control order [8]. Although these supported phospholipid surfaces could prevent non-specific protein absorption and platelet adhesion, they showed limited stability in clinical applications [9, 10], i.e. physically-adsorbed phospholipid monolayers could be dissociated from the surface by shear stress in high blood flow and hydrophobic interactions with plasma proteins. Furthermore, the physically-adsorbed phospholipid monolayer would be removed from the surface during sterilization processes, such as autoclave and ethylene oxide gas sterilization.

Polymerization of phospholipid monolayers containing reactive moieties, such as mono- or bis-acetylene, acryloyl and sorbyl groups, have been proposed for increasing the stability of phospholipid monolayers on solid substrates [11–14]. The laterally-polymerized phospholipid assemblies with mono reactive moieties exhibited acceptable stability under static conditions in water and air, as well as in the presence of high shear flow environment [13]. On the other hand, the polymerization of a bis-substitute phospholipid led to a more complex cross-linked polymer network with corresponding stability in organic solvents and surfactants [14]. However, the stability of polymerized phospholipid monolayers still needs to be increased in order to successfully apply these monolayers in clinical applications, because the polymerized phospholipids are associated with non-covalent interaction on solid substrates.

In this study, the surface of a phospholipid monolayer, which was directly grafted to a solid substrate, was engineered so as to increase its stability in the physiological conditions. We have developed a new in situ polymerization system that was applied at the interface of an acryloyl-PC monolayer and a methacryloyl-terminated substrate using a water-soluble initiator. And, the surface properties of and platelet adhesion on the surface-grafted phospholipid monolayer were also characterized.
EXPERIMENTAL

Materials

1,12-dodecanediol, 2,6-di-tert-butyl-p-cresol, pyridine, 4-(N,N-dimethylamino)pyridine (DMAP), dicyclohexylcarbodiimide (DCC) and pyridinium dichromate (PDC) were obtained from Sigma (St. Louis, MO, USA). 1-Stearoly-2-hydroxy-sn-glycero-3-phosphocholine was obtained from Avanti Polar Lipids (Alabaster, AL, USA) and was used without further purification. 3-(Trimethoxysilyl)propyl methacrylate (TSM) was purchased from Gelest (Tyllytown, PA, USA). Dichlorodimethylsilane (DCM), acryloyl chloride, 2,2'-azobis(2-methylpropionamide) dihydrochloride (AAPD), chloroform, toluene and THF were purchased from Aldrich (Milwaukee, WI, USA). These solvents were HPLC grade and were used without further purification.

Synthesis of 1-stearoyl-2-[12-(acryloyloxy)-dodecanoyl]-sn-glycero-3-phosphocholine (acyrloyl-PC)

The phospholipid containing mono-acryloyl moiety (acyrloyl-PC) was synthesized as described by Marra et al. and Sells et al. [13, 15]. 1-Stearoly-2-hydroxy-sn-glycero-3-phosphocholine (0.5 g, 0.89 mmol), 12-(acryloyloxy)-1-dodecanoic acid (0.46 g, 1.70 mmol), DMAP (100 mg, 0.82 mmol) and one crystal of 2,6-di-tert-butyl-p-cresol were dissolved in 5 ml chloroform. DCC (0.20 g, 0.98 mmol) was added to the solution and then stirred at room temperature in the dark under an argon atmosphere for 48 h. The precipitated dicyclohexylurea was removed by filtering and the filtrate was evaporated. The residue was dissolved in 20 ml methanol and an ion-exchange resin (5.0 g, AG-501-8X, Bio-Rad, Hercules, CA, USA) was added to the solution. After stirring for 1 h, the resin was filtered and washed with methanol. The filtrate was dried with Na₂SO₄ and evaporated. The residue was dissolved in chloroform and purified by flash chromatography on silica gel (chloroform/methanol/water, 65:25:1). The synthesized phospholipid was clear oil. Yield: 0.3 g (48%). TLC: Rf = 0.27 (chloroform/methanol/water, 65:25:4). ¹H-NMR (CDCl₃): δ 6.35–6.41 (d, 1 H, vinyl), 6.12–6.22 (q, 1 H, vinyl), 5.80–5.83 (d, 1 H, vinyl), 5.16–5.20 (s, 1 H, POCH₂CH), 4.20–4.44 (m, 3 H, CHCH₂OP, CHOCO), 4.12 (m, 3 H, CHOCO, CH₂OOC), 3.83–3.95 (m, 4 H, NCH₂CH₂O), 3.32 (s, 9 H, (CH₃)₃N⁺), 2.22–2.38 (m, 4 H, COCCH₂), 1.61 (br, 6 H, CH₂), 1.28 (s, 42 H, CH₂), 0.85 (t, 3 H, CH₂CH₃).

Preparation of TSM and DCM monolayers on solid substrates

Silicon wafers to be used as solid substrates were cut into 1 cm × 2 cm pieces. They were sonicated in soap water, rinsed with large amount of distilled water and further sonicated in dichloromethane. The substrates were then placed in a freshly prepared ‘piranha’ solution (a mixture of 70% concentrated sulfuric acid and 30% hydrogen peroxide) for 10 min at 80°C. Subsequently, the substrates were rinsed
with distilled water and then dried under an argon atmosphere. The cleaned surfaces had a water contact angle of nearly zero, indicating that the surfaces were covered by silanol moieties.

The cleaned silicon wafers were silanized with DCM according to the established procedure [8]. The produced dimethyl-terminated DCM monolayer had no grafting moieties with the acryloyl-PC. For making grafting moieties on solid substrates, we prepared methacryloyl-terminated substrates by immersing the cleaned silicon substrate into the solution of 5 ml anhydrous toluene and 2% TSM. After the solution was stirred at 80°C for 12 h, the substrate was withdrawn from the solution and immersed in toluene to remove unreacted TSM molecules. After further rinsing with toluene, the substrate was rinsed with methanol and distilled water. Finally, the TSM-silanized substrate was dried under an argon atmosphere, followed by baking at 100°C for 12 h.

Preparation of acryloyl-PC monolayers on DCM and TSM-silanized substrates

A solution of acryloyl-PC in a small amount of chloroform/methanol (1:1, v/v) was placed in glass test tubes. The solvent was removed under a stream of argon and any remaining solvent was evaporated in a vacuum. The acryloyl-PC film was rehydrated in distilled water (1 mg/ml) and vortexed. The dispersed lipid solution was sonicated at 10 watt for 10 min in an ice bath, thereby producing vesicles with the average diameter of 120 nm, as confirmed by dynamic light scattering (Series 4700, Malvern Instruments, Malvern, UK). Assembled acryloyl-PC monolayers were then prepared by immersing the DCM and TSM silanized substrates into the vesicle solutions with gentle stirring for 3 h at room temperature.

In situ polymerization of pre-assembled phospholipid monolayers on DCM and TSM-silanized substrates

After vesicle incubation for 3 h, the appropriate amount of AAPD was directly added into glass test tubes containing acryloyl-PC vesicles and the silanized substrate. The glass test tubes were purged with argon before sealing with a rubber septum and then placed in an oil bath at 60°C for 15 min. The concentration of the initiator was varied from 0.01% to 1% in the vesicle solution. After the in situ polymerization, the substrates were rinsed 3 times with distilled water to remove AAPD molecules and dried under an argon atmosphere. In order to evaluate the stability of phospholipid monolayers on solid substrates, we performed a ‘wash off’ test in methanol. The polymerized phospholipid monolayers on DCM and TSM-silanized substrates were immersed in methanol for 10 min and then washed 10 times with methanol and distilled water, respectively. After the ‘wash off’ test, the polymerized phospholipid monolayers were dried under a stream of argon and further characterized.
Instruments

The chemical structure of an acryloyl-PC was analyzed by $^1$H-NMR (JEOL JNM-LA 300 WB FT-NMR, Jeol, Tokyo, Japan). The average thickness of all the samples was measured using an automatic ellipsometer (S2000, Rudolph Research, Flanders, NJ, USA) with a He-Ne laser light source ($\lambda = 632.8$ nm) at an angle of incidence of 70°. The water contact angles of each sample were measured using DCA 322 (Cahn Instrument, Madison, WI, USA). The measurements for each of the four samples were reported as the average advancing/receding degree ± standard deviation. The AFM images were obtained in air at room temperature using an Autoprobe CP system (Park Scientific, Sunnyvale, CA, USA). The contact mode images were made using a contact silicon ultralevers with a spring constant of 0.06 N/m. The AFM images were acquired in a constant-force mode at a scan rate of 1 Hz using a piezoscanner (100 μm) and analyzed by using PSI Proscan Software. We integrated the histogram of 10 images per sample using bearing analysis and the surface coverage of acryloyl-PC molecules on a TSM-silanized surface was calculated as an average surface coverage ± standard deviation. The XPS (EscaLab 210, UK) measurements were performed using monochromatic Mg $K_\alpha$ radiation of 1253.6 eV. Each sample was measured with an experimental resolution of 1.8 eV and at a take-off angle of 45°. The XPS results for each of the three samples were reported as average atomic percentages ± standard deviation.

Platelet adhesion

Whole blood from male rabbits (New Zealand white rabbit, 2.5 kg, Fine Exp. Lab., Naju, South Korea) was carefully collected in a polypropylene syringe containing 3.8% sodium citrate solution. Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 2500 rpm for 3 min at 4°C. After collecting the supernatant, the remainder was centrifuged further at 4500 rpm for 5 min to prepare platelet-poor plasma (PPP). PRP and PPP were mixed to a concentration of $3.8 \times 10^5$ cells/μl and the number of platelets was counted using a cell counter (Coulter Z1, Coulter Electronics, UK). Each sample (20 mm × 8 mm) was hydrated for 1 h in PBS, immersed in 5 ml PRP for 2 h at 37°C and then rinsed with PBS with gentle agitation to remove the weakly adhered platelets. The adhered platelets were fixed with 2.5% glutaraldehyde in PBS for 2 h and then dehydrated with a series of ethanol/water mixtures. The dehydrated samples were freeze-dried with liquid nitrogen and analyzed with scanning electron microscopy (SEM, JSM-5800, Jeol, Tokyo, Japan). Quantitative measurements were also made for 15 representative areas of three different specimens.
RESULTS AND DISCUSSION

Surface properties of polymerized phospholipid monolayers on TSM-silanized substrates

The *in situ* polymerization of phospholipid monolayers on solid substrate was proceeded by three processes, as shown in Fig. 1: (a) preparation of the polymerizable surface with methacryloyl moieties on solid substrates (TSM monolayer), (b) preparation of an acryloyl-PC monolayer on the methacryloyl-terminated surface (PC-TSM) and (c) *in situ* polymerization of the pre-assembled acryloyl-PC molecules and the methacryloyl molecules on TSM-terminated surfaces (poly-PC-TSM).

The methacryloyl-terminated surface was prepared by the silanization of a silicon wafer with TSM. TSM was used to functionalize silicon oxide surfaces with methacryloyl groups and it gave rise to uniform monolayers on the surfaces [16, 17]. The TSM-silanized surfaces had hydrophobic properties due to the methacryloyl groups of TSM. The advancing and receding contact angles of TSM-silanized surface were 81.7 ± 3.2° and 64.2 ± 2.5°, respectively, while the contact angle of the cleaned silicon wafer surface was nearly zero. The AFM image showed that the TSM-silanized surface had a monolayer structure with a root-mean-square (rms) roughness of 1.6 Å (Fig. 2b), whereas the rms roughness of the cleaned silicon wafer surface was 0.15 Å.

![Diagram](image.png)

**Figure 1.** Schematic illustration of the procedure for preparing a surface-grafted phospholipid monolayer on a methacrylated-terminated substrate by using an *in situ* polymerization.
was 1.2 Å (Fig. 2a). The thickness of the TSM monolayer measured by ellipsometry was 15 ± 2 Å, which was very similar to the length of a TSM molecule. This confirmed that a TSM monolayer had formed on the silicon surface over its oxide layer.

After vesicle fusion for 3 h, AAPD, a water-soluble initiator was added directly into the glass test tubes and the polymerization was initiated by heating to produce free radicals. It was reported that AAPD could penetrate the hydrocarbon chains of acryloyl-PC monolayers or bilayers above the lipid phase transition temperature [13, 15]. Thus, it was expected that AAPD could initiate the polymerization carried out at the interface between an acryloyl-PC monolayer and a TSM monolayer above the lipid phase-transition temperature (32.0°C). The polymerization condition was optimized by varying the AAPD concentration from 0.01% to 1% and the reaction was performed at 60°C for 15 min. When the AAPD concentration was more than 1%, nitrogen gas was produced vigorously and damaged the monolayer structure. Therefore, the maximum AAPD concentration was determined to be 1%.

The effect of initiator concentration on the in situ polymerization was determined using water contact-angle measurements, as shown in Table 1. The hydrophilicity of poly-PC-TSM was slightly increased with the increase of AAPD concentration, indicating that the surface coverage of hydrophilic acryloyl-PC head groups had increased on the substrate. This was because the polymerized phospholipid monolayer had formed a covalent bond with the substrate and that it could now be stable, in contrast to the physically adsorbed acryloyl-PC molecules which could be partially dissociated during the copious washing step. In the case of 1% AAPD concentration, the minimum advancing and receding contact angles of poly-PC-TSM were observed to be 58.5° and 32.3°, respectively, and they indicated the maximum surface coverage of acryloyl-PC molecules. This poly-PC-TSM polymerized with 1% AAPD showed a very smooth and flat surface with the rms roughness of 2.1 Å, and the surface coverage of phospholipid molecules, measured by bearing analysis, was above 97 ± 2.4% (Fig. 2c). The average thickness of the film measured by ellipsometry was 31 ± 1.2 Å which was an apparent thickness of a supported phospholipid monolayer.

<table>
<thead>
<tr>
<th>Initiator concentration (%)</th>
<th>Contact angle (deg) (advancing/receding)</th>
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<tbody>
<tr>
<td></td>
<td>Before methanol washing</td>
</tr>
<tr>
<td>0.01</td>
<td>62.2 ± 2.5/42.7 ± 1.9</td>
</tr>
<tr>
<td>0.1</td>
<td>60.3 ± 3.2/42.5 ± 3.1</td>
</tr>
<tr>
<td>1</td>
<td>58.5 ± 2.2/32.3 ± 1.8</td>
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The data represent mean ± SD, n = 4.
Figure 2. AFM images (5 $\mu$m $\times$ 5 $\mu$m) of (a) cleaned silicon wafer, (b) TSM-monolayer and (c) poly-PC-TSM. Inset: height profile along the dashed line in the AFM image.
It was evidently confirmed that the degree of surface grafting was closely related to the AAPD concentration by methanol washing test, as shown in Table 1. For the AAPD concentration below 0.1%, the poly-PC-TSM surface showed hydrophobic property after methanol washing, indicating the dissociation of hydrophilic PC groups from the substrate. However, the poly-PC-TSM polymerized with 1% AAPD still maintained its hydrophilicity after methanol washing, as shown by its advancing and receding contact angles of 60.1° and 38.1°, respectively. From the ‘wash off’ test, we finally optimized the in situ polymerization of acryloyl-PC monolayers by using 1% initiator at 60°C for 15 min.

During the in situ polymerization, three different polymerizations could occur: between (a) TSM and TSM, (b) acryloyl-PC and acryloyl-PC and (c) TSM and acryloyl-PC. Polymerization between TSM and acryloyl-PC could graft a polymerized phospholipid monolayer onto the substrate. The stability of the poly-PC-TSM was compared with physically-adsorbed phospholipid monolayers, such as a PC-TSM and a polymerized phospholipid monolayer on the DCM-silanized substrate (poly-PC-DCM). All different phospholipid monolayers on substrates were washed with methanol and the amount of remaining phospholipid molecules on substrates were quantitatively analyzed by using AFM and XPS measurements.
Figure 3. AFM images (5 μm × 5 μm) of phospholipid surfaces on solid substrates which were washed with methanol; (a) PC-TSM, (b) poly-PC-DCM and (c) poly-PC-TSM. Inset: height profile along the dashed line in the respective AFM images.
After methanol washing, most of the assembled phospholipid molecules or phospholipid polymers on PC-TSM and poly-PC-DCM surfaces were removed from the substrate (Fig. 3a, b). However, the poly-PC-TSM surface showed an uneven morphology with many holes, compared with the poly-PC-TSM surface in Fig. 2c (Fig. 3c). We assumed that these holes indicate defects of \textit{in situ} polymerization of acryloyl-PC molecules to methacryloyl moieties on a TSM-silanized surface. The surface coverage of the poly-PC-TSM, measured using bearing analysis, was $85 \pm 5.7\%$ after methanol wash. Therefore, the grafting efficiency by \textit{in situ} polymerization of an acryloyl-PC on the TSM substrate was calculated as $85\%$. The surface of a methanol washed poly-PC-TSM had an rms roughness of 3.2 Å and an average thickness of 24 Å. From this methanol-washed phospholipid-modified surface, we concluded that the physically-adsorbed acryloyl-PC molecules or acryloyl-PC polymer are completely removed from surface by methanol washing. Only the surface-grafted acryloyl-PC polymers are stable to methanol washing, due to covalent bonding to a solid surface.

The element compositions of TSM-silanized substrates and phospholipid surfaces were determined by XPS analysis, as shown in Table 2. The major components of each surface were C(1s), O(1s), S(2p) and N(1s). O and Si atoms of the silicon oxide substrate were observed in all samples, which indicated that supported phospholipid monolayers on silanized substrates were thinner than the scan depth of the XPS at a take-off angle of 45° [13]. The presence of a N(1s) peak at 403.3 eV for the
Table 2.
XPS measurements for phospholipid monolayers on substrates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>XPS analysis (atomic %)</th>
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<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>SiO₂</td>
<td>4.7 ± 0.2</td>
</tr>
<tr>
<td>TSM</td>
<td>15.2 ± 0.9</td>
</tr>
<tr>
<td>DCM</td>
<td>14.6 ± 1.1</td>
</tr>
<tr>
<td>PC-TSM</td>
<td>62.5 ± 2.7</td>
</tr>
<tr>
<td>PC-DCM</td>
<td>63.2 ± 3.3</td>
</tr>
<tr>
<td>poly-PC-TSM</td>
<td>65.2 ± 2.1</td>
</tr>
<tr>
<td>poly-PC-DCM</td>
<td>63.4 ± 3.7</td>
</tr>
<tr>
<td>Methanol-washed PC-TSM</td>
<td>16.4 ± 1.6</td>
</tr>
<tr>
<td>Methanol-washed PC-DCM</td>
<td>15.9 ± 1.2</td>
</tr>
<tr>
<td>Methanol-washed poly-PC-TSM</td>
<td>61.6 ± 2.1</td>
</tr>
<tr>
<td>Methanol-washed poly-PC-DCM</td>
<td>15.5 ± 1.1</td>
</tr>
</tbody>
</table>

The data represent mean ± SD, n = 3.

Figure 4. XPS spectra in the N(1s) region of (a) TSM monolayer, (b) PC-TSM, (c) methanol-washed PC-TSM, (d) poly-PC-TSM and (e) methanol-washed poly-PC-TSM.

The quaternary nitrogen of the phospholipid head group on the XPS spectra provided a clear evidence that phospholipid monolayers were grafted to the solid substrate (Fig. 4). However, the P(2p) peak could not be observed because of a low intensity and an overlap with the silicon atom peak in the XPS spectra. The nitrogen peak was apparently observed on the PC-TSM and poly-PC-TSM surfaces, whereas both
TSM and the methanol washed PC-TSM did not present any nitrogen peaks. Only poly-PC-TSM showed a nitrogen peak after methanol washing, indicating that the polymerized acryloyl-PC monolayer had formed a covalent bond with the solid substrate.

When the phospholipid monolayer was formed on the DCM- or TSM-silanized substrate, the carbon composition increased and oxygen and silicon compositions decreased. After being washed with methanol, PC-DCM and PC-TSM gave the same quantitative results as the TSM-silanized substrate, because the physically adsorbed phospholipid monolayer was completely removed from the substrates. After polymerization, poly-PC-DCM and poly-PC-TSM were found to the same compositions as PC-DCM and PC-TSM, respectively. It means that the phospholipid monolayers could not be damaged during the polymerization. However, poly-PC-DCM presented a decreased carbon atom composition and increased oxygen and silicon atom compositions after the methanol washing, indicating that acryloyl-PC molecules had been removed. Poly-PC-TSM, in particular, presented the similar atom compositions before and after the methanol washing. The grafting efficiency of an acryloyl-PC monolayer on the TSM substrate, which was calculated by the relative carbon ratio of the poly-PC-TSM surface before and after the methanol washing, was 94.5%.

Platelet adhesion

Figure 5 shows SEM pictures of TSM-silanized substrates and phospholipid surfaces after they had been in contact with PRP. Platelets were severely adhered on the TSM surface. Furthermore, morphological changes of the adhered platelets, such as extended pseudopods and flattering, were observed. On the other hand, platelet adhesion was reduced effectively on the PC-TSM surface. The number of adhered platelets on the PC-TSM surface was much less than that on the TSM surface (Fig. 6). The difference was statistically significant at the 95% confidence level. After the methanol washing, the number of adhered platelets on the PC-TSM surface was similar to that on the TSM surface without any statistically significant difference. Only the poly-PC-TSM surface showed few platelets, even after methanol washing, which was different at the 95% confidence level from TSM surfaces. It was confirmed that the poly-PC-TSM surface was very stable, and that it also greatly suppressed platelet adhesion with a platelet density of 5500 platelets/cm², which was only 8.9% of that for TSM surface.

CONCLUSIONS

In this study, we proposed a new stable phospholipid monolayer produced by a new in situ polymerization method using a pre-assembled phospholipid monolayer to a TSM-silanized solid surface. Under the optimized polymerization conditions with a 1% AAPD initiator, the polymerized phospholipid surface showed a very flat
Figure 5. Scanning electron micrographs of platelet adhesion on (a) TSM monolayer, (b) PC-TSM, (c) poly-PC-TSM, (d) PC-TSM after methanol washing and (e) poly-PC-TSM after methanol washing. The magnification of each image is ×2000.
A stable phospholipid monolayer grafted onto a methacryloyl-terminated substrate

Figure 6. Number of adhered platelets on TSM monolayer, PC-TSM and poly-PC-TSM; before methanol washing (■) and after methanol washing (□). The error bar is for standard deviation (n = 10) and asterisk indicates a statistically significant (* p < 0.05) difference when compared to TSM monolayer.

and smooth monolayer structure of PC lipids. The XPS study further confirmed that the polymerized phospholipid monolayer was successfully grafted to a solid surface and that it was very stable. This surface-grafted phospholipid monolayer significantly prevented platelet adhesion. Therefore, the stabilized phospholipid monolayer could be proposed as a new stabilized cytomimetic biomembrane to be used as implantable biomaterials.

Acknowledgements

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