In Situ Photopolymerization of a Polymerizable Poly(ethylene glycol)-Covered Phospholipid Monolayer on a Methacryloyl-Terminated Substrate

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We have prepared a chemically anchored monolayer of PEG (poly(ethylene glycol)) and phospholipid mixture (PEG/phospholipid) on a methacryloyl-terminated substrate by in situ photopolymerization. Both monoacryloyl phospholipid (acryloyl-PC, 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine) and monoacryloyl PEG (acryloyl-PEG, 12-(acryloyloxy)dodecanoyl-PEG) were synthesized by modifying phospholipid and PEG with 12-(acryloyloxy)-1-dodecanoic acid and 12-(acryloyloxy)-1-dodecanol, respectively. The surface pressure−area (Π−*A*) isotherm showed that acryloyl-PEG molecules were stable in the phospholipid monolayer and that they could be evenly inserted into a phospholipid monolayer at the air/water interface. By adding 10 mol % acryloyl-PEG into phosholipid vesicles, we could produce a PEG/phosholipid monolayer on methacryloyl-terminated substrates using vesicle fusion for 3 h. Then, this polymerizable PEG/phospholipid monolayer was in situ photopolymerized onto a methacryloyl-terminated substrate with eosin Y/triethanolamine as co-initiators. Optimal vesicle fusion and irradiation condition were determined with respect to the vesicle fusion time and duration of irradiation. As confirmed by atomic force microscopy and X-ray reflectivity studies, the polymerized PEG/phosholipid surface formed a PEG-covered phospholipid monolayer with thicknesses of 3 and 6 nm for the base phospholipid monolayer and the covering PEG layer, respectively. The chemical anchoring efficiency of polymerized PEG and phospholipid molecules, which was calculated by the relative carbon ratio of each surface before and after methanol washing using X-ray photoelectron spectroscopy, was 98%. This polymerized PEG/phosholipid monolayer showed good stability in organic solution due to firm chemical anchoring to a solid surface.

Introduction

Considerable research effort has been devoted to the development of bioinert surfaces for blood-contacting devices such as artificial organs, artificial blood vessels, and biosensors. Many research groups have utilized zwitterionic phosphorylcholine-modified surfaces in order to mimic the endothelial cell membrane since it is inert, nonfouling (i.e., resistant to protein adsorption and cell adhesion), and nontoxic (i.e., resistant to immune reaction) under physiological conditions.[1−5]

Supported phospholipid assemblies on solids have been proven to be useful for mimicking biomembrane structures as a bioinert surface. Phospholipid monolayers on solids can be commonly prepared using the Langmuir−Blodgett (LB) or vesicle fusion.[6] When observed by infrared spectroscopy, glancing incident angle X-ray technique, and atomic force microscopy (AFM), these phospholipid assemblies form a relatively well oriented and highly packed phospholipid surface,[7−9] and the adsorption of proteins and blood cells to zwitterionic phospholipid assemblies is much lower than the control surface.[10−13] However, supported phospholipid membranes have an obvious drawback of limited stability since the physically adsorbed phospholipids are easily solubilized and washed out in surfactants and organic solvents.

To increase the stability of phospholipid assemblies, Chaikof et al. have introduced in situ polymerization of phospholipid monolayers containing monoacryloyl groups on solids.[4,14] The laterally polymerized phospholipid monolayers resulted in acceptable stability under static conditions in water and air, as well as in the presence of a high-shear flow environment, but phospholipids were significantly desorbed by being exposing to surfactant and organic solvents.[15] Recently, we have reported a method to prepare a chemically anchored phospholipid monolayer using in situ polymerization at the interface between a monoacrylated phospholipid monolayer and a methacryloyl-terminated surface.[15] The phospholipid mono-

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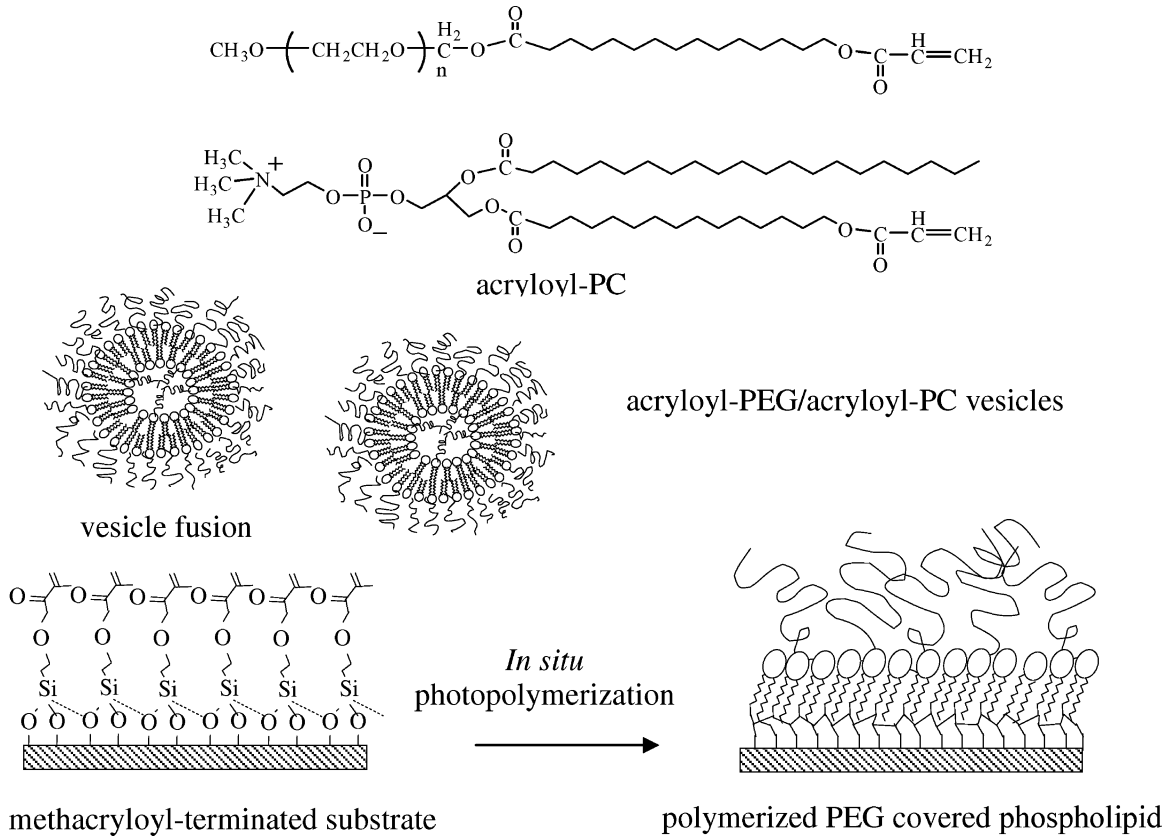

Figure 1. Schematic illustration of the procedure for preparing a PEG-covered phospholipid monolayer, which is chemically anchored to a methacryloyl-terminated substrate.

layer, chemically anchored to solid substrate, formed an oriented and packed monolayer structure with a high anchored phosholipid coverage above 87%. The chemically anchored phospholipid monolayer on a solid substrate was very stable in surfactant and organic solvent. Moreover, this polymerized phospholipid surface prevented protein adsorption and platelet adhesion, in vitro, and it suppressed immune reaction, in vivo.[16] But the defects of unpolymerized phosholipid molecules, which are unstable in water, surfactant, and organic solvents, still activated protein adsorption and blood cell adhesion.

The PEG covered phospholipid surface is highly conducive to ordered PEG coating for drug delivery systems and to producing biocompatible materials.[17−21] The major protective effect of a PEG layer on a phospholipid surface is attributed to the steric repulsion and bound water on PEG layers.[22,23] In particular, a dense PEG layer could be formed on phospholipid surfaces by controlling PEG−lipid concentration during transfer of phospholipid monolayers at the air/water interface or during vesicle fusion onto solids.[20,21] It is also well-known that the adsorption of protein and blood cells is much lower on PEG-covered phospholipid surfaces than on pure phospholipid monolayers.[20,21,24] Thus, a PEG layer on phospholipid surfaces can act an additional bioinert barrier to protein adsorption and platelet adhesion.

In this study, we designed a stable PEG-covered phospholipid monolayer as new bioinert surfaces for blood-contacting devices. As shown in Figure 1, PEG and phospholipid were monoacrylated with a hydrophobic moiety of dodecane to form assembled PEG and phospholipid mixed (PEG/phospholipid) monolayers or vesicles. The acrylated PEG/phospholipid monolayers were produced using LB techniques at the air/water interface or vesicle fusion onto methacryloyl-terminated substrates, respectively. Then, 10 mol % acryloyl-PEG mixed phospholipid monolayers, produced by vesicle fusion, were chemically anchored onto methacryloyl-terminated substrates using in situ photopolymerization. The surface property and stability of the polymerized PEG/phospholipid monolayer anchored on a solid were characterized using water contact angle measurements, AFM, X-ray reflectivity (XR), and X-ray photoelectron spectroscopy (XPS) measurements.

Experimental Section

Materials. mPEG (monomethoxyl poly(ethylene glycol), M_w 2000), DCC (dicyclohexylcarbodiimide), DMAP (4-(*N*,*N*-dimethylamino)pyridine), 2,6-*di*-*tert*-butyl-*p*-cresol, EY (eosin Y, 5% in water), TEA (triethanolamine), and VP (1-vinyl-2-pyrrolidinone) were obtained from Sigma Chemical Co. (St. Louis, MO). TSM (3-(trimethoxysilyl)propylmethacrylate) (Gelest Inc., Tyllytown, PA) was used without further purification. Chloroform, toluene, and ethanol of analytical grade were purchased from Aldrich Chemical Co. (Milwaukee, WI). A monoacrylated phospholipid (acryloyl-PC, 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine) and 12-(acryloyloxy)-1-dodecanol were prepared as described by Marra et al.[4] and Kim et al.[15]

Synthesis of Acryloyl-PEG (12-(Acryloyloxy)dodecanoyl-PEG). To prepare an acrylated PEG, mPEG was first carbox-

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ylated, as described by Zalipsky et al.[25] Then, mPEG-COOH (1.0 g, 0.5 mmol), 12-(acryloyloxy)-1-dodecanol (256 mg, 1.0 mmol), DMAP (61 mg, 0.5 mmol), and one crystal of DTBC were dissolved in chloroform (5 mL). DCC (120 mg, 0.59 mmol) was added to the solution, and then the mixture was stirred for 24 h at room temperature in the dark under an argon atmosphere. The dicyclohexylurea was removed by filtering, the filtrate was slowly added into the cold diethyl ether with vigorous stirring, and the precipitate was filtered. Finally, the obtained acryloyl-PEG was purified by using ion exchange chromatography (DEAE-sephadex A-25, Sigma). The product is a white powder (54% yield). ^{1}H NMR ($CDCl_3$): 1.27 ppm (s, 16 H, CH_2) 1.62 ppm, (br, 4 H, CH_2), 2.2 ppm (m, 2 H, $-O-CO-CH_2-$), 3.4 ppm, (s, 3 H, $CH_3O-CH_2-CH_2-O-$), 3.6 ppm (183.1 H, PEG), 4.3 ppm (t, 4 H, $-CH_2-CH_2-O-CH_2-O-CO-$, $-CH_2-CH_2-O-CO-C=CH_2$), 5.8 ppm (d, 1 H, $CH_2=CH-COO-$), 6.1 ppm, 6.4 ppm (d, 2 H, $CH_2=CH-COO-$).

Acryloyl-PEG/Acryloyl-PC Monolayers at the Air/Water Interface. A commercial NIMA trough (type 611, Warwick Science Park Inc., Conventry, U.K.) was used for the preparation of LB monolayers and the measurement of surface pressure. Both acryloyl-PEG and acryloyl-PC were mixed in chloroform/methanol (1/1 v/v) at 0−30 mol % of acryloyl-PEG, and the solution was spread on the ultrapure water surface at 20 °C. After time was allowed for solvent evaporation, the monolayer was compressed and the surface pressure was measured by compressing the barrier at the rate of 30 cm^2/min.

Vesicle Fusion. Each pure acryloyl-PC and acryloyl-PEG (10 mol %)/acryloyl-PC mixture in chloroform/methanol (1/1, v/v) was placed in a glass tube. The solvent was removed under a stream of argon, followed by evaporating in a vacuum. The dried film was rehydrated in distilled water at 1 mg/mL for acryloyl-PC and 1.24 mg/mL for acryloyl-PEG (10 mol %)/acryloyl-PC mixture, respectively. After vortexing, each dispersed vesicle solution was sonicated at 10 W for 10 min in an ice bath, thereby producing acryloyl-PC vesicles and 10 mol % acryloyl-PEG mixed phospholipid vesicles with the average diameters about 120 and 100 nm, respectively, as confirmed by dynamic light scattering (Marvern Instruments Ltd., series 4700, Malvern, U.K.). Finally, supported acryloyl-PC and acryloyl-PEG/acryloyl-PC monolayers were prepared by immersing the TSM-silanized substrate into each vesicle solution, respectively, while gently stirring it for 3 h at room temperature. The methacryloyl-terminated substrate was prepared by the silanization of silicon wafers with TSM in toluene at 80 °C for 12 h, as described in a previous study.[15]

In Situ Photopolymerization. Visible-light-mediated in situ photopolymerizaiton was carried out using EY/TEA co-initiators.[14] A 10 μL portion of stock solution of co-initiators prepared as 10 mM EY, 225 mM TEA, and 37 mM VP in water was directly added to 1 mL of each vesicle solution containing a TSM-silanized substrate. Then, the glass test tube was purged with argon before sealing with a rubber septum and irradiated with a filtered lamp (ILC Technology, Sunnyvale, CA) producing 100 mV/cm^2 of light between 480 and 520 nm for 5−30 min at 25 °C. After in situ photopolymerization, the substrates were removed from the glass test tubes, washed with water to remove physically adsorbed phospholipid, PEG, and initiator molecules, followed by drying under an argon atmosphere.

Instruments. The static water contact angles for each sample were measured by a contact angle goniometer (100-00, Rame-Hart, Inc., Mountain Lakes, NJ). One water drop (10 μL) was deposited on the sample, and the static contact angle was measured as soon as possible using the goniometer. All measurements were done at room temperature and at 40% relative humidity. AFM images were obtained in air at room temperature using an Autoprobe CP system (Park Scientific, Inc., Sunnyvale, CA). Contact mode images were made using contact silicon ultralevers with a spring constant of 0.06 N/m in air. The constant force between the tip and the sample was kept as low as possible, and images were acquired in the constant-force mode at a scan rate of 1 Hz using a piezoscanner (100 μm). AFM images were analyzed by using PSI Proscan Soft ware (Park). Images presented were obtained after several repetitions of the samples, which always revealed similar features with regard to the depth of holes and the root-mean-square (rms) roughness. X-ray reflectivity (XR) was performed in the standard symmetric reflection geometry using a horizontal reflectometer (D8 Advance, Bruker) with a Cu Kα source. In the θ/θ geometry, the scattering wave-vector transfer is given by $q_z = 4\pi/\lambda \sin(\theta)$ and is varied by changing the angles θ of the incident radiation and the detector simultaneously to meet the specular condition. The horizontal detector slits were wide open to achieve integration over q_y. Specular reflectivity is sensitive to the variation of the electron density $\rho(z)$ in the direction of the surface normal, averaged in the (x,y)-plane region being studied, making these results sensitive to the layer thickness, density contrast, and interfacial roughness. Parallel beam geometry was employed using a Göbel mirror. The goodness of fit was evaluated by a least-squares algorithm. We systematically varied and then optimized fitting parameters, electron density, thickness (d), and roughness (σ) until the goodness of fit, χ^2, was minimized. The X-ray photoelectron spectroscopy (XPS) (EscaLab 210, Thermo Electron Corp.) measurements were performed using monochromatic Mg Kα radiation of 1253.6 eV. Each sample was measured with an experimental resolution of 1.8 eV at a takeoff angle of 45°. The XPS results for each of the three samples were reported as average atomic percentages ± standard deviation.

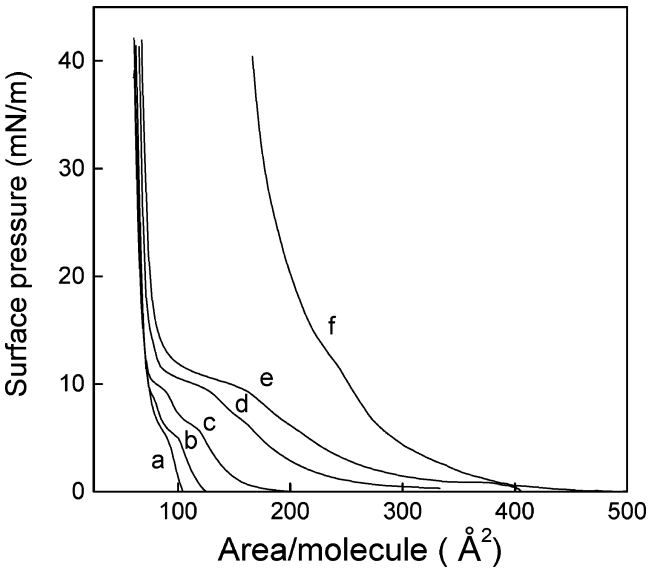

Figure 2. Surface−pressure area ($\Pi-A$) isotherms of acryloyl-PC monolayers containing acryloyl-PEG at 20 °C: (a) pure acryloyl-PC; (b) 1 mol % acryloyl-PEG; (c) 3 mol % acryloyl-PEG; (d) 5 mol % acryloyl-PEG; (e) 10 mol % acryloyl-PEG; (f) 30 mol % acryloyl-PEG.

Results and Discussion

Figure 2 shows the surface pressure−area ($\Pi-A$) curves of acryloyl-PC monolayers containing various concentrations of acryloyl-PEG (0, 1, 3, 5, 10, and 30 mol %) at 20 °C. The pure acryloyl-PC monolayer exhibited a distinctive liquid condensed/liquid expanded (LC/LE) coexistent phase transition at the surface pressure of 5.2 mN/m. As the acryloyl-PEG concentration increased, the LC/LE coexistent phase transition of mixed monolayers becomes more broaden with increasing amount of acryloyl-PEG. In the case of 3 mol % of acryloyl-PEG, there were two prominent transitions at 5.6 mN/m that appeared as the acryloyl-PC chains rearranged and at 8.7 mN/m as the acryloyl-PEG chains rearranged. But, the transition range by acryloyl-PEG chains was very narrow due to the lower acryloyl-PEG concentration. When the acrylated PEG concentration was above 10 mol %, only the transition of acryloyl-PEG chains was observed at 9.5 mN/m. It is known that the structure of PEG−lipid chains in PEG−lipid/phospholipid monolayer converts from mushroom to

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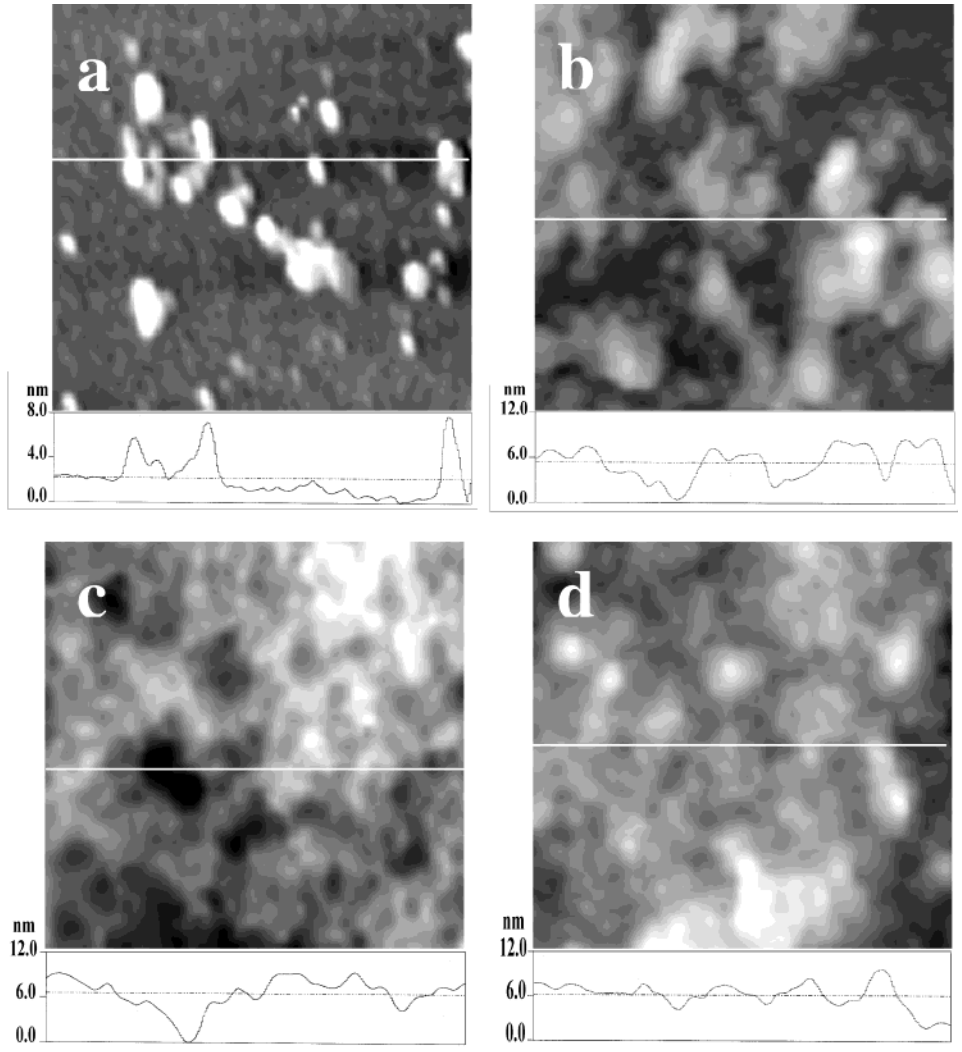

Figure 3. AFM images (2 μm × 2 μm) of growth mechanism of an acryloyl-PEG (10 mol %)/acryloyl-PC monolayer on a TSM-silanized substrate with incubation times of (a) 1 min, (b) 10 min, (c) 1 h, and (d) 3 h. The inset image presents a height profile along the line in the AFM image.

brush regime in the plateau transition region.[26−28] However, the 30 mol % acryloyl-PEG mixed monolayer showed a significant repulsive interaction of PEG chains and the mean molecular area at solid phase showed a shift to the right at 220 Å^2, which is much higher than that of pure acryloyl-PC monolayer (60 Å^2). Therefore, the critical concentration of acryloyl-PEG to form a dense PEG layer on the surface of a phospholipid monolayer, as shown in Figure 1, was determined to 10 mol % in the phospholipid monolayer at the air/water interface.

By use of vesicle fusion, pure acryloyl-PC and acryloyl-PEG (10 mol %)/acryloyl-PC monolayers were prepared by immersing the TSM-silanized substrate in the vesicle solution while gently stirring it at room temperature. In a previous study, we had already confirmed that a monoacrylated phospholipid monolayer could be successfully prepared on a TSM-silanized substrate using vesicle fusion and that it presented a phospholipid monolayer structure.[15,16] The surface coverage of the acryloyl-PEG/acryloyl-PC monolayer with respect to different incubation times was evaluated by performing a bearing analysis of AFM images (Figure 3). Since the TSM-silanized substrate had a very flat surface, bearing analysis can be used to evaluate the surface coverage of assembled acryloyl-PEG/acryloyl-PC monolayers. The AFM images showed that acryloyl-PEG/acryloyl-PC vesicles initially aggregated to form large domains with surface coverage of 34%, and the maximum roughness was about 7.9 nm within 1 min (see the inset image of Figure 3a). After 10 min of incubation, the large domains increased to include the partially covered monolayer with the surface coverage of 63%. Over 90% of the TSM-silanized substrate was covered with an acryloyl-PEG/acryloyl-PC monolayer of the maximum roughness of 7−8.5 nm after 1 h of incubation. After 3 h of incubation, the acryloyl-PEG/acryloyl-PC monolayer can be completely formed with the surface coverage of 98% and the maximum roughness decreased to 6 nm. Therefore, the results indicate that the vesicle incubation time should be longer than 3 h to form a PEG-covered phospholipid monolayer on the TSM-silanized substrate.

After pure acryloyl-PC and acryloyl-PEG/acryloyl-PC monolayers were prepared on TSM-silanized substrates, the monolayers were in situ photopolymerized using EY/TEA co-initiators. The effect of irradiation time on the chemical anchoring efficiency of the monolayers was determined by water contact angle measurements, as shown in Figure 4a. Contact angles decreased with the increasing irradiation time. After 10 min of irradiation

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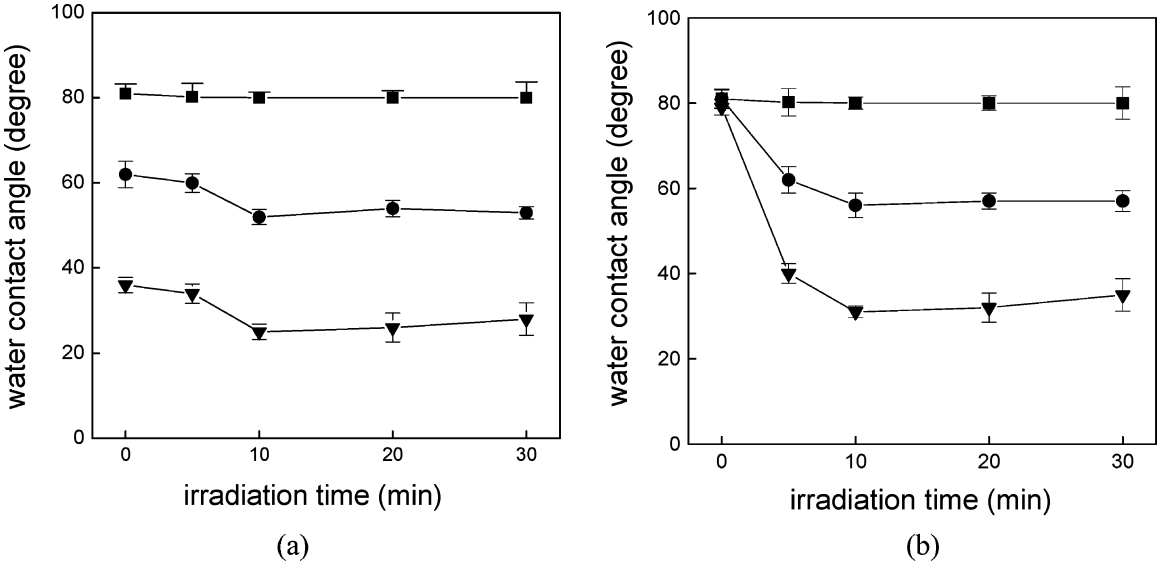

Figure 4. Water contact angles of TSM-silanized substrates (■), polymerized acryloyl-PC monolayers (●), and acryloyl-PEG(10 mol %)/acryloyl-PC monolayers (▼) according to photopolymerization times (a) before methanol wash and (b) after methanol wash.

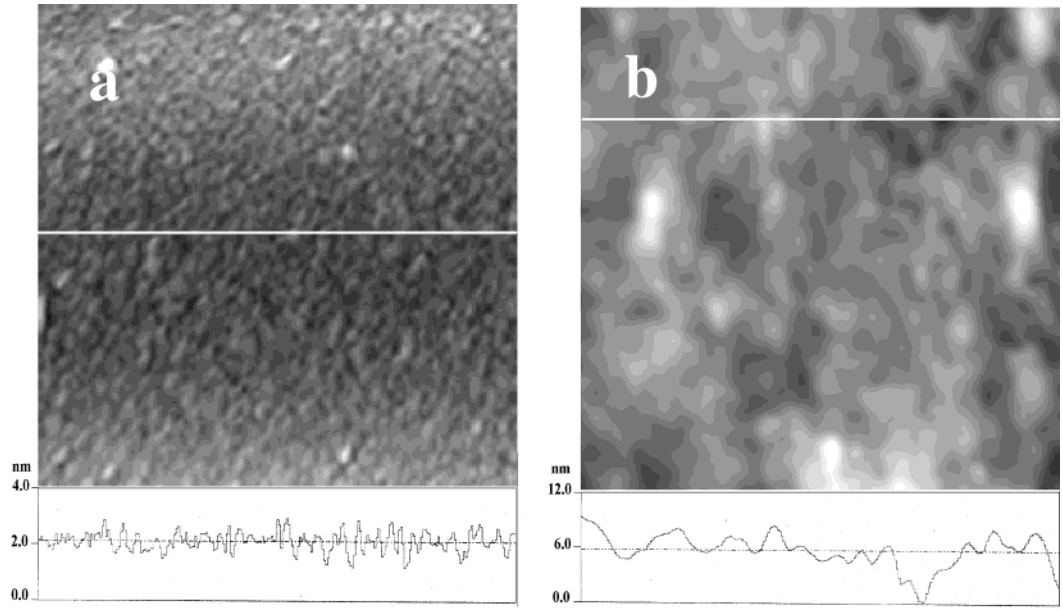

Figure 5. AFM images (2 μm × 2 μm) of (a) a polymerized acryloyl-PC monolayer and (b) a polymerized acryloyl-PEG (10 mol %)/acryloyl-PC monolayer. The inset image presents a height profile along the line in the AFM image.

time, the minimum contact angles of polymerized phospholipid and PEG/phospholipid monolayers were measured to be 52° and 25°, respectively. These contact angle data have been reported for highly dense phospholipid and PEG surfaces, respectively.[14,23] To clarify the grafting efficiency and stability of the polymerized monolayers, the polymerized monolayers were washed with methanol. Without in situ photopolymerization, the physically assembled phospholipid and PEG/phospholipid monolayers showed the same hydrophobicity of the TSM-silanized substrate, indicating complete removal of phospholipid and PEG molecules from the TSM-silanized substrate. However, the polymerized phospholipid and PEG/phospholipid monolayer, which were irradiated for 10 min, maintained their hydrophilicity before methanol washing. Therefore, it was confirmed that the polymerized phospholipid and PEG/phospholipid monolayers had been substantially grafted onto the TSM-silanized substrate by the in situ photopolymerization. The in situ photopolymerization was set up with 3 h of vesicle fusion, followed by 10 min of irradiation.

Figure 5 shows the AFM images of polymerized phospholipid and PEG/phospholipid monolayers (2 μm × 2 μm) in air. The surface of polymerized phospholipid monolayer was smooth with a root-mean-square (rms) roughness less than 0.2 nm with an average height of 2.5 nm from bottom to top surface. On the other hand, the polymerized PEG/phospholipid monolayer showed an uneven PEG morphology with a high rms roughness of 1.2 nm and an average height of 6.0 nm.

XR curves were obtained from the TSM-silanized substrate and the polymerized PEG/phospholipid monolayer on the TSM-silanized substrate washed with methanol in order to reveal their structures in detail (Figure 6). The solid lines in Figure 6 were calculated by fitting the experimental data (symbols) to the corresponding dispersion (δ) profiles, which were shown in the inset to the figure. Each layer is modeled using three adjustable fitting parameters: thickness (d), dispersion (δ) at an incident energy of 8.05 keV, and roughness (σ) for the interface between that layer and the next layer away from the Si substrate. Each data set shows unique interference fringes,

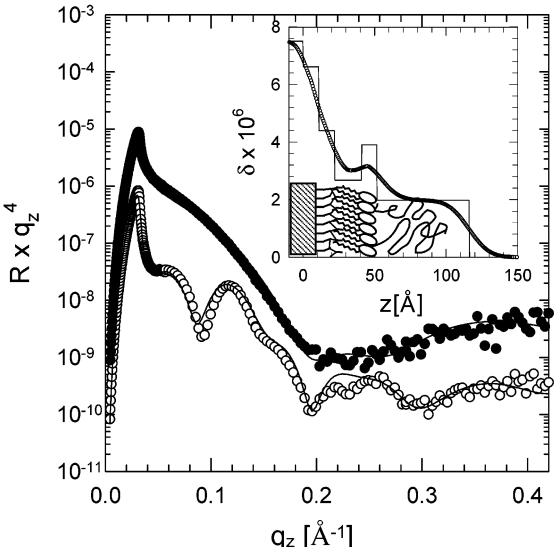

Figure 6. X-ray reflectivity for a TSM-silanized substrate (●) and a polymerized acryloyl-PEG (10 mol %)/acryloyl-PC monolayer (○) and their fits (line). The samples were washed with methanol and characterized with XR studies. Inset: Dispersion profile of the polymerized acryloyl-PEG (10 mol %)/acryloyl-PC monolayer (○) and unsmeared dispersion profile (line) together with schematic configuration of the PEG/phospholipid monolayer on the TSM-silanized substrate.

which contained damping factors and oscillating terms due to surface roughnesses and the film thicknesses, respectively, without considering q^{-4} decay due to the Fresnel law.[29] The upper (closed circles) and bottom curves (open circles) were obtained from the TSM-silanized substrate and the polymerized PEG/phospholipid monolayer, respectively.

Note that the silanization of TSM was processed on the oxide-covered silicon substrates, and the best fit was obtained when the silicon substrate had a 1.3 nm thick oxide layer. A pronounced low-frequency oscillation, first minimum at $q_z \sim 0.21$ Å^{-1}, indicates that the average thickness of the TSM-silanized monolayer prior to lipid fusion process is $d_{TSM} \sim 1.3$ nm on the native oxide layer. Multiple dips with higher frequencies arose from the thickness of the polymerized PEG/phospholipid monolayer, and the beating in the reflectivity profile of polymerized PEG/phospholipid monolayer was visible in the signal because several interfaces from PEG, phospholipid, and TSM layers were produced. All values were obtained through the fitting, the open circles in the inset to the figure giving the corresponding dispersion profile. Note that the steplike profile (line) in the inset is the unsmeared dispersion profile, and the effective-density model was employed to generate continuous model profiles.[30] The corresponding unsmeared dispersion profile shows that the maximum area fraction of phospholipid, occurring at the midplane of the head region in the phospholipid layer, is $\delta \approx 0.40$. The dispersion δ can be expressed in terms of the electron density ρ_{el} using $\delta = \lambda^2 \rho_{el} r_o / 2\pi$, where r_o is the classical electron radius (2.82×10^{-13} cm^{-13}), the wavelength λ is 1.54 Å, and ρ_{el} is the electron density of the material. Therefore, the equivalent electron density of the head region was about 79% of the reported phospholipid Langmuir layer.[31] This indicates

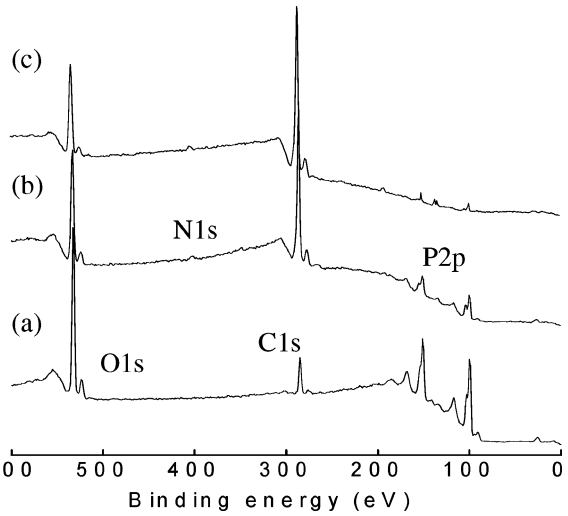

Figure 7. XPS spectra of (a) a TSM-silanized substrate, (b) a polymerized acryloyl-PC monolayer, and (c) a polymerized acryloyl-PEG (10 mol %)/acryloyl-PC monolayer. The samples were washed with methanol and characterized with XPS studies.

the formation of a relatively well packed polymerized phospholipid layer on the TSM substrate. The distribution obtained from fitting the data in the inset showed that the thicknesses of PEG and phospholipid layers were 6.4 and 2.9 nm, respectively, which were in agreement with the AFM results. The packing density of PEG layer was ca. 56%, which is given by $\delta(z)/\delta_{PEG}$, where δ_{PEO} is the dispersion of bulk PEG at an incident energy of 8.05 keV (3.958×10^{-6}) along the surface normal.

We infer from the results of AFM and XR studies that the PEG layer has the brush-type conformation with a thickness of 6.4 nm and a higher packing density of 56% in the dried state. Since the thickness of the PEG layer far exceeds the Flory radius of PEG lipid ($R_F = 3.5$ nm),[32] the thick PEG layer could be greatly expelled by hydration, thereby acquiring a hydrated brush-type conformation.[21,33] Our findings agree well with the experimental and theoretical studies on the brush-type conformation of the PEG layer.[32−35] According to the results, relatively high surface adsorption of acryloyl-PEG molecules can be anchored to a solid surface by adding 10 mol % of acryloyl-PEG into the lipid monolayer. As yet we do not have a clear answer to directly explain this result. But, it may be possible that some of acryloyl-PEG molecules could adsorb faster due to kinetically favorable interaction during vesicle fusion. Further experiments are in progress to study this effect in greater detail by imaging ellipsometry for in situ dynamics.

Figure 7 shows a XPS survey spectrum of polymerized phospholipid and PEG mixed monolayers on TSM-silanized substrates for an emission angle of 45° with respect to the surface plane. The main peaks are carbon (C 1s), nitrogen (N 1s), and phosphorus (P 2p) arising from the polymerized phospholipid molecules as well as silicon (Si

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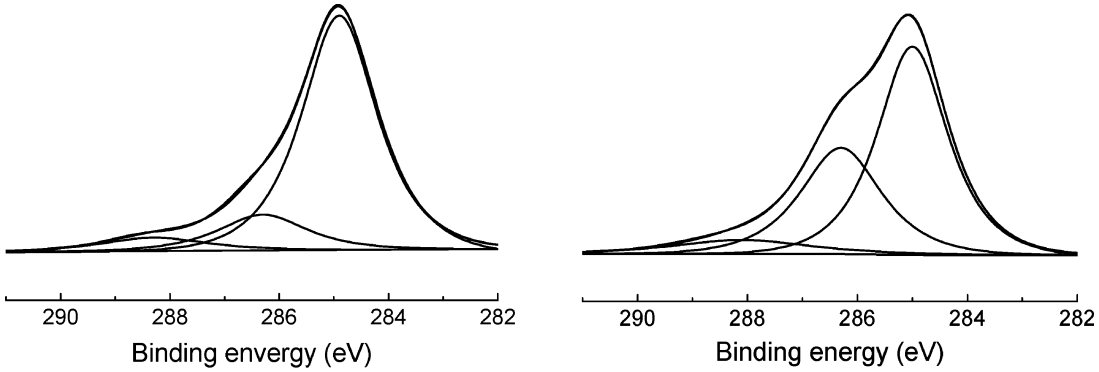

Figure 8. Typical deconvolution of carbon C 1s narrow scans of (a) a polymerized acryloyl-PC monolayer and (b) a polymerized acryloyl-PEG (10 mol %)/acryloyl-PC monolayer. Binding energies reported are in agreement with the literature values and usually vary within 0.2 eV for all monolayers. The samples were washed with methanol and characterized with XPS studies.

Table 1. XPS Results for TSM-Silanized Substrates, Polymerized Phospholipid Monolayers, and Polymerized PEG (10 mol %)/Phospholipid Monolayers

sample	C (%)	O (%)	Si (%)	N (%)	P (%)
TSM-silanized substrate	15.6 ± 0.5	42.9 ± 2.0	40.4 ± 0.3		
polymerized lipid	52.5 ± 1.7	21.9 ± 1.6	22.4 ± 1.0	1.9 ± 0.3	1.3 ± 0.3
polymerized PEG/lipid	65.8 ± 1.8	28.2 ± 1.3	5.0 ± 1.2	0.4 ± 0.1	0.6 ± 0.3
polymerized lipid[a]	50.2 ± 1.8	21.3 ± 1.3	25.9 ± 1.2	1.0 ± 0.3	1.3 ± 0.4
polymerized PEG/lipid[a]	61.1 ± 1.7	28.6 ± 2.1	10.3 ± 1.8	0.3 ± 0.1	0.5 ± 0.2

[a] The samples were washed with methanol and characterized with XPS studies.

2p) and oxygen (O 1s) from the PEG layers and silicon oxide substrates. Figure 8 gives a peak fitting for the narrow carbon (C 1s) scans of polymerized phospholipid and PEG/phospholipid monolayers on TSM-silanized substrates. In the case of a polymerized phospholipid monolayer, the component with the highest intensity had a binding energy of 285.0 eV due to the aliphatic carbons mainly present in the tail of the phospholipid molecule, and the lower binding energies of 286.4 eV (C−O) and 288.3 eV (C═O) were observed from acryloyl-PC molecules. However, the polymerized PEG/phospholipid monolayer presented a higher binding energy of 286.4 eV of the ether carbon from the PEG chains compared to the polymerized phospholipid monolayer.[36] This narrow carbon scan confirmed that the PEG layer was produced substantially on the phospholipid monolayer.

Presence of nitrogen (N 1s) at 403.3 eV and phosphorus (P 2p) at 134.6 eV on the polymerized phospholipid and PEG/phospholipid monolayers, respectively, provides evidence that acryloyl-PC molecules are indeed polymerized on TSM-silanized substrates. Moreover, the polymerized PEG/phospholipid monolayer increased carbon and oxygen composition but significantly decreased the silicon composition owing to the presence of a thick PEG layer on the phospholipid monolayer (Table 1). We also compared the atomic compositions of all samples after methanol wash. Only physically adsorbed phospholipid and PEG/phospholipid monolayers were completely washed out and the atomic compositions were very consistent with TSM-silanized substrates (not shown here). But, polymerized phospholipid and PEG/phospholipid monolayers still indicated similar atomic compositions after a "wash off" test. The decrease of carbon atom of polymerized phospholipid and PEG/phospholipid monolayers was only 2%, and the intensity of carbon ether of PEG layers was not changed after methanol wash (Table 2) because

Table 2. XPS Binding Energies/Peak Area for Polymerized Phospholipid Monolayers and Polymerized PEG (10 mol %)/Phospholipid Mixed Monolayers

sample	C−C (%) at 285 eV	C−O (%) at 286.4 eV	C═O (%) at 288.3 eV
polymerized lipid	80.6 ± 1.2	11.1 ± 0.8	8.3 ± 0.3
polymerized PEG/lipid	60.7 ± 2.1	32.1 ± 1.8	7.2 ± 0.9
polymerized lipid[a]	79.5 ± 1.3	11.6 ± 0.6	8.9 ± 0.7
polymerized PEG/lipid[a]	59.3 ± 2.1	33.4 ± 2.2	7.3 ± 0.5

[a] The samples were washed with methanol and characterized with XPS studies. The atomic compositions of all samples were determined by XPS analysis as shown in Table 1. The atomic composition of TSM-silanized substrates showed the same composition of a TSM monolayer.[15]

polymerized PEG and phospholipid molecules were chemically anchored to TSM-silanized substrates.

Conclusion

In this study, we proposed a stable PEG-covered phospholipid monolayer produced by in situ photopolymerization. Under the optimized photopolymerization condition with EY/TEA for 10 min, the PEG/phospholipid mixed monolayer was polymerized and anchored to a solid surface. The AFM and XR studies confirmed that the polymerized PEG/phospholipid mixed monolayer was successfully anchored to a solid surface and that it formed a stable PEG-covered phospholipid monolayer structure. The XPS measurements indicated that most of physically adsorbed lipid and PEG molecules on the TSM-silanized substrate were substantially anchored to a solid surface with high anchoring efficiency of 98%. Therefore, this stable PEG-covered phospholipid monolayer could be proposed as a new cytomimetic bioinert surface for implantable biomaterials or biosensors.

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