Cytomimetic Biomaterials. 1. *In-Situ* Polymerization of Phospholipids on an Alkylated Surface

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ABSTRACT: A stabilized, phosphatidylcholine-containing polymeric surface was produced by *in-situ* polymerization of a self-assembled lipid monolayer on an alkylated substrate. The phospholipid monomer 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphorylcholine was synthesized, prepared as unilamellar vesicles, and fused onto alkylated glass. Free-radical polymerization was carried out in aqueous solution at 70 °C and characterized using either the water-soluble initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPD) or an oil-soluble initiator 2,2′-azobis(isobutyronitrile) (AIBN). Under optimized conditions, the supported monolayer displayed advancing and receding water contact angles of 64 and 44°, respectively. Angle-dependent ESCA results confirmed the presence of phosphorus and nitrogen and were consistent with theoretical predictions for close-packed monolayer formation with near-normal alignment of lipid chains. In the absence of network formation, polymeric films demonstrated acceptable stability under static conditions in water and air, as well as in the presence of a high shear flow environment. Blood compatibility was assessed in a baboon arteriovenous shunt model, which revealed miminal platelet deposition over a 2 h observation period.

Introduction

The ability to repair, reconstruct, and replace components of the human cardiovascular system is dependent upon the the availability of blood compatible biomaterials. In this regard, the most intriguing development in the past decade has been the recognition that membrane-mimetic systems based on the phosphorylcholine head group limit the induction of surface-associated blood clot formation. Although not well understood, it has been speculated that this biological property may be related to the zwitterionic structure of phosphorylcholine and the large amount of bound water, or perhaps, the selective adsorption to this head group of specific plasma proteins that inhibit the blood-clotting process.[1] A more thorough review of blood−material interactions can be found elsewhere.[2,3]

In addition to the potential biological activity of biomembranes, they also offer a model for molecular engineering based upon their ability to self-organize as noncovalent aggregates. In this regard, phospholipids differing in chemical composition, saturation, and size have been utilized as building blocks in the design of structures of complex geometry, including lipid-based cylinders, cubes, and spheres. Most recently, surface-coupled bilayers have been produced by assembling a layer of closely packed hydrocarbon chains followed by exposure to either a dilute solution of emulsified lipids or unilamellar lipid vesicles.[4−6] Langmuir−Blodgett techniques have also been used to construct supported bilayers via a process of controlled dipping of a substrate through an organic amphiphilic monolayer.[7] The overall significance of these design strategies lies in the ability to engineer surfaces in which the constituent members can be controlled, modified, and easily assembled with a high level of control over both order and chemistry. Of particular importance is the dialkyl moiety, which facilitates the assembly of lipids with dissimilar head groups into surface structures of diverse biomolecular functionality and activity. Nonetheless, limited stability remains the major practical limitation of substrate-supported membranes in which the constituent members are associated solely by noncovalent interactions.

In order to create robust surface structures, most membrane-mimetic systems for blood-contacting applications have been designed as copolymers containing the phosphatidylcholine functional group in either side chains or, less frequently, the polymer backbone.[8−13] While these materials have improved stability and promising blood-contacting properties have been reported, a number of limitations exist. Notably, one loses the ability to engineer surface properties on a molecular level by taking advantage of the principal of self-organization intrinsic to amphiphilic molecules. Extensive literature exists on the two-dimensional polymerization of lipid monomers self-assembled in the form of vesicles.[14−22] However, far fewer studies have evaluated *in-situ* polymerization of dialkyl amphiphiles at a solid−liquid interface.[23−25] We report the synthesis of stabilized, phosphatidylcholine-containing polymeric surfaces by *in-situ* polymerization of a self-assembled lipid monolayer on an alkylated substrate. Both physiochemical surface properties and *in-vivo* blood compatibility in a primate animal model have been characterized.

Experimental Section

Materials. AAPD (2,2′-azobis(2-methylpropionamidine) dihydrochloride), DTBC (2,6-di-*tert*-butyl-*p*-cresol), 1,12-dodecanediol, pyridine, DMAP (4-(*N*,*N*-dimethylamino)pyridine), DCC (dicyclohexylcarbodiimide), and PDC (pyridinium dichromate) were obtained from Aldrich and used as received. 1-Palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine was obtained from Avanti Polar Lipids and used as received. THF and pyridine were obtained from Fisher and dried over 4 Å molecular sieves. Acryloyl chloride was obtained from Aldrich and vacuum distilled prior to use. Chloroform (Aldrich) was washed with water, dried over $CaCl_2$, distilled, and stored over 3 Å molecular sieves. AIBN (Aldrich) was recrystallized from methanol. The resin AG 501-X8 was obtained from Bio-Rad

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and used as received. Glass coverslips were purchased from Baxter.

Synthesis. 12-(Acryloyloxy)-1-dodecanol (1).[26] 1,12-Dodecanediol (50.0 g, 0.247 mol) was dissolved in 500 mL of THF with gentle heating. Pyridine (8.0 mL, 0.100 mol) was added, and the solution was cooled to room temperature. One crystal of 2,6-di-*tert*-butyl-*p*-cresol was added. Acryloyl chloride (6.0 mL, 0.074 mol) was dissolved in 40 mL of THF, and the solution was slowly added dropwise. After 24 h, the mixture was filtered to remove pyridine hydrochloride. The filtrate was rotoevaporated to give a white solid, which was taken up in 200 mL of $CHCl_3$, the resulting mixture being placed in an ice bath for 1 h. The mixture was filtered to remove unreacted diol, and the filtrate was dried over Na_2SO_4. The solvent was removed *in vacuo* to give a residue that was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 95/5). The product was a clear oil **(1)** [yield 14.90 g (79%); ^{1}H NMR ($CDCl_3$) δ 6.35−6.41 (d, vinyl, 1H), 6.10−6.20 (q, vinyl, 1H), 5.74−5.78 (d, vinyl, 1H), 4.10−4.18 (t, OCOCH_2, 2H), 3.61−3.65 (t, HOCH_2, 2H), 1.62−1.66 (br, CH_2, 4H), 1.26 (s, CH_2, 16H); HRMS calculated (FAB) 263.2198, observed 263.2203 (+Li)].

12-(Acryloyloxy)-1-dodecanoic Acid (2).[26] 12-(Acryloyloxy)-1-dodecanol (2.24 g, 8.75 mmol) was dissolved in 5.0 mL of DMF, and the solution was slowly added to a mixture of PDC (10.60 g, 28.00 mmol), one crystal of 2,6-di-*tert*-butyl-*p*-cresol, and 15.0 mL of DMF at 0 43 °C. The reaction slowly reached room temperature. After 23 h, the mixture was poured into 100 mL of H_2O and extracted five times into ether. The combined organic layers were washed with water and dried over Na_2SO_4. The solvent was removed *in vacuo* to give a residue that was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 97/3). The product was a white solid **(2)** [yield 1.07 g (46%); mp 30.0−31.0 °C; ^{1}H NMR ($CDCl_3$) δ 6.36−6.42 (d, vinyl, 1H), 6.12−6.22 (q, vinyl, 1H), 5.75−5.82 (d, vinyl, 1H), 4.10−4.20 (t, OCOCH_2, 2H), 2.25−2.43 (t, HOOCCH_2, 2H), 1.61−1.63 (br, CH_2, 4H), 1.27 (s, CH_2, 14H); HRMS calculated (FAB) 277.1991, observed 277.1997 (+Li)].

1-Palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphocholine (3).[26] To a mixture of 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (0.40 g, 0.82 mmol), 12-(acryloyloxy)-1-dodecanoic acid (0.46 g, 1.70 mmol), DMAP (0.10 g, 0.82 mmol), and one crystal of 2,6-di-*tert*-butyl-*p*-cresol, was added 6.0 mL of dry $CHCl_3$. DCC (0.20 g, 0.98 mmol) was added, and the reaction was stirred in the dark under argon. After 66 h, dicyclohexylurea was filtered off and washed with $CHCl_3$. The filtrate was evaporated, and the residue was dissolved in 20.0 mL of MeOH. Bio-Rad AG 501-8X (5.0 g) was added and the reaction stirred at room temperature for 1 h. The resin was filtered and washed with MeOH. The filtrate was dried over Na_2SO_4. The solvent was removed *in vacuo* to give a residue that was purified by flash chromatography on silica gel ($CHCl_3$/MeOH, 9/1, then $CHCl_3$/MeOH/H_2O, 65/25/4). The product was a clear oil obtained from the second fraction **(3)** [yield 0.32 g (53%); ^{1}H NMR ($CDCl_3$) δ 6.22−6.28 (d, vinyl, 1H), 5.88−6.12 (q, vinyl, 1H), 5.59−5.68 (d, vinyl, 1H), 5.07 (s, POCH$_2$C*H*, 1H), 4.24−4.44 (m, POCH_2CH, HCCH_2OCO, 4H), 4.10 (m, br, CH_2OCOCH=CH$_2$, 2H), 3.85−3.97 (m, NCH_2CH_2O, 4H), 3.07 (s, (CH_3)$_3$N$^+$, 9H), 2.16−2.20 (m, OOCCH_2, 4H), 1.46 (br, CH_2, 6H), 1.12 (s, CH_2, 38H), 0.73 (t, CH_2CH_3, 3H); HRMS calculated (FAB) 748.5129, observed 748.5099 (+H)].

Preparation of Silanized Glass. Materials. Microscope borosilicate glass coverslips (S/P Cover Glass, 24 × 40 × 0.25 mm, no. 2 thickness) were purchased from Baxter Scientific, Inc. Multi-Terge, a powerful high-pH chelating detergent, was purchased from EM Diagnostic Systems, Inc. (Gibbstown, NJ). All other chemicals and solvents (HPLC grade) were purchased from Aldrich. Hydrated $CHCl_3$ was prepared and stored in a clean screw-cap amber bottle by vigorously shaking 25 mL of $CHCl_3$ (1% ethanol-stabilized) with 10 μL of deionized water. A 63 mM octadecyltrichlorosilane (OTS) stock solution in dry CCl_4 was prepared under nitrogen by injecting 1.7 mL of OTS (95%, stored in dessicator at room temperature) with a 3-mL polypropylene syringe through a 0.1 μm PTFE syringe filter, directly into the commercial Sureseal container of 100 mL of anhydrous CCl_4 (99$^+$%, <0.005% water). The bottle was capped with a virgin rubber liner (Aldrich), closed with a bakelite screw-cap, and sealed with parafilm for long-term storage at room temperature. Liquid volumes below 30 mL were measured using nonlubricated polypropylene syringes (Aldrich). Dust-Off XL puff-duster cans (compressed gas filtered to 0.1 μm) were purchased from Falcon Safety Products, Inc. (Branchburg, NJ).

Method. Monolayers of OTS on atomically smooth glass coverslips were prepared according to a protocol derived from previously published methods[27] and adapted for large scale sample production. A typical single batch yielded 54 homogeneously coated hydrophobic slides. Specifically, commercial coverslips were cut longitudinally into two slabs of 12 × 40 × 0.25 mm with a diamond pen, puff dusted, and transferred to a Class 10 cleanroom. Two 400-mL glass beakers and one 200-mL amber glass bottle were then simultaneously degreased and deionized by application of a solution of Multi-Terge/H_2O 1/8 with a camel hair brush, followed by copious rinsing with running deionized water, and allowed to dry in open air, occasionally blowing with a nitrogen gun. The precleaned containers were subsequently etched under argon in a Harrick barrel plasma etcher (9 min, 100 W, 500 mTorr Ar). All 54 coverslips were precleaned (both sides) and etched similarly and mounted in a Teflon dip basket that we specifically designed for the purpose of (1) allowing quick handling of a large batch of slides, (2) enabling full exposition of the front and back sides of the coverslips to the reaction medium, and (3) minimizing the coverslip surface-to-solvent ratio in the reaction vessel. A mixture of 24.8 mL of hydrated $CHCl_3$, 248 mL of bicyclohexyl, and 27.0 mL of a 62.5 mM OTS stock solution in dry CCl_4 (filtered through a 0.1 μm PTFE syringe filter, Whatman) was well shaken in the above 200-mL screw-capped amber bottle and poured into the 400-mL clean beaker. The basket was fully immersed into this reaction mixture within 5 min of mixing. OTS deposition was allowed to proceed at room temperature in open air without agitation. After 1 h, the basket was lifted out of solution, rinsed by dipping in 2 × 300 mL of HPLC-grade $CHCl_3$, and sonicated (~47 kHz, ~130 W) in 2 × 300 mL of $CHCl_3$ for 10 min. The basket was then copiously rinsed with running deionized water and blown dry with a nitrogen gun. Finally, the slides were taken out, one by one with stainless steel forceps, and stored in wafer shippers (Fluoroware, Inc., Chaska, MN) interlayered with lint-free cleanroom tissue. For use in subsequent experiments, the OTS-coated slides were further cut to the appropriate size and puff-dusted.

Preparation of Vesicles and Fusion. A stock solution of the phospholipid in MeOH/$CHCl_3$ (1/1), was stored in the freezer and used throughout the experiments. One milliliter of the solution was added to a preweighed vial, and the solvent was removed by gentle blowing with an argon stream. The film (4−10 mg) was dried in the SpeedVac at room temperature for 1 h and then used immediately for vesicle preparation. Sodium phosphate buffer solution (20 mM) was added to the film in the vial. The vial was stoppered and sonicated for 1 min. Vesicles were then prepared by a freeze−thaw method. The cloudy solution was heated to 50 °C in a hot water bath, vortexed for 1 min, and then frozen in liquid nitrogen. This procedure was repeated three times, and the multilamellar vesicles were then extruded through 2000, 600, and 200 nm polycarbonate filters successively.[28] Fusion onto an alkylated glass coverslip consisted of diluting the unilamellar vesicles with buffer to the appropriate concentration and pouring the vesicle solution onto an alkylated slide. Fusion was initiated by addition of NaCl (750 mM buffered saline, pH 6.2) solution. The system was then kept in the dark, static, under argon, at either room temperature or 40 °C, for specified amounts of time.

Polymerization [General Procedure]. After vesicle fusion was complete, 0.1−4.0 mg of initiator was added to the system. This amount was calculated such that the [M]:[I] ratio was 10:1. The test tube containing the slide and buffer solution was stoppered and purged thoroughly with argon. The system was sealed and placed in an oil bath at 70 °C or subjected to UV light for varying amounts of time. After

polymerization was complete, the solution was pipeted from the tube, and the cover slip was rinsed 20−30 times with deionized water. The film was stored in water until further surface characterization.

Instrumentation. Contact angles were measured on a Rame-Hart goniometer, Model 100-00. The values reported are an average of at least five readings. Proton NMR data were obtained on a QE300 instrument. Angle-dependent ESCA data were obtained using a Physical Electronics (PHI) Model 5100 spectrometer equipped with a Mg/Ti dual-anode source and an Al/Be window. The system uses a hemispherical analyzer with a single-channel detector. Mg Kα X-rays (1253.6 eV) were used as an achromatic source, operated at 300 W (15 kV and 20 mA). The base pressure of the system was lower than 5×10^{-9} Torr, with an operating pressure no higher than 1×10^{-7} Torr. A pass energy of 89.45 eV was used when obtaining the survery spectra, and a pass energy of 35.75 eV was used for the high-resolution spectra of elemental regions. Spectra were obtained at the following takeoff angles: 15, 45, and 90°. The instrument was calibrated using Mg Kα X-radiation: the distance between Au $4f_{7/2}$ and Cu $2p_{3/2}$ was set at 848.67 eV, and the work function was set using Au $4f_{7/2}$ and Cu $2p_{3/2}$ and checked using Au $3d_{5/2}$. All metals were sputter cleaned to remove oxides. Full width at half-maximum for Ag $3d_{3/2}$ was measured to be 0.8 eV at a count rate of 30 000 counts.

Shear Flow Studies. Adherence assays were performed at a continuous shear stress of 200 dyn/cm^2 in a parallel plate flow chamber, as previously described.[29] The shear stress was held constant using a syringe pump (Harvard Apparatus, Southnatick, MA) and the temperature was maintained at 37 °C with the aid of a water bath (Nicholson Precision Instruments, Bethesda, MD).

Baboon Model. Arteriovenous shunt. *In-vivo* studies were performed as previously described.[30] Briefly, glass tubes (4 mm i.d. × 3 cm) were alkylated and *in-situ* lipid polymerization was performed, as noted above. Glass tubes were interposed into a permanent Silastic arteriovenous shunt that had been surgically implanted between the femoral artery and vein in male Baboons (*Papio anubis*). Circulating platelet concentrations averaged 391 000 platelets/μL. Ketamine hydrochloride (10 mg/kg intramuscularly) was given as a preanesthetic agent, and the operation was performed under general 1% halothane anesthesia. All procedures were in accordance with institutional guidelines. Mean blood flow rate through the shunt was measured continuously using a Doppler ultrasonic flow meter and held constant by an external screw clamp at 100 mL/min.

Platelet Radiolabeling. Autologous baboon platelets were radiolabeled on the day prior to the shunt study. Forty-five milliliters of whole blood were initially withdrawn into syringes containing 9 mL of acid citrate dextrose anticoagulant. The blood was centrifuged at 160*g* for 15 min and the platelet-rich plasma removed and centrifuged at 1500*g* for 15 min. The platelet pellet was then removed, washed in normal saline solution with 0.1% (w/v) dextrose, and 600 μCi of indium-111 oxine (Amersham Co.) was added to the platelet suspension. Following a 10-min incubation at room temperature, 3 mL of platelet-poor plasma was added and the platelets were incubated for an additional 2 min. The mix was centrifuged at 1500*g* for 5 min to form a platelet pellet, the supernatant and excess ^{111}In oxine removed, and the platelets resuspended in 5 mL of reserved plasma. Approximately 0.5 mCi of indium-111 oxine labeled platelets were reinjected into the baboon. Platelet function is not altered by this technique, when studied by either thrombin stimulated platelet release of ^{14}C serotonin or by morphological studies of dense body distribution.

Platelet Deposition Measurement. Platelet uptake on test surfaces was monitored over a 120 min period using scintillation camera imaging of the 172 keV ^{111}In γ photon peak. A high-sensitivity ^{99}Tc collimator was utilized, and images were aquired with a GE 400T scintillation camera (General Electric, Milwaukee, WI) interfaced with a Medical Data Systems A^3 image processing system (Ann Arbor, MI). Immediately before imaging, 2 min images were acquired of the 200 μl sample of platelet concentrate (injection standard)

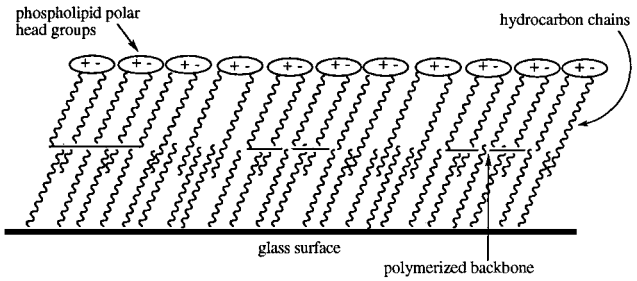


Figure 1. Polymerized phospholipid surface.

and of a segment of 4.0 mm i.d. Silastic tubing filled with autologous blood and having the same luminal volume as the glass tube segment (blood standard). Images were obtained continuously with data storage at 2-min intervals. Deposited ^{111}In-platelet activity was calculated by subtracting the blood standard activity from all dynamic study images. Data were converted, at each time point, to total platelet deposition per unit test surface, as follows:

$$\text{platelets/unit surface area} = \frac{\{[\text{test surface activity (cpm)} - \text{backd activity (cpm)}]}{\text{blood specific activity (cpm/mL)}} \times \text{platelets/mL} \quad (1)$$

where

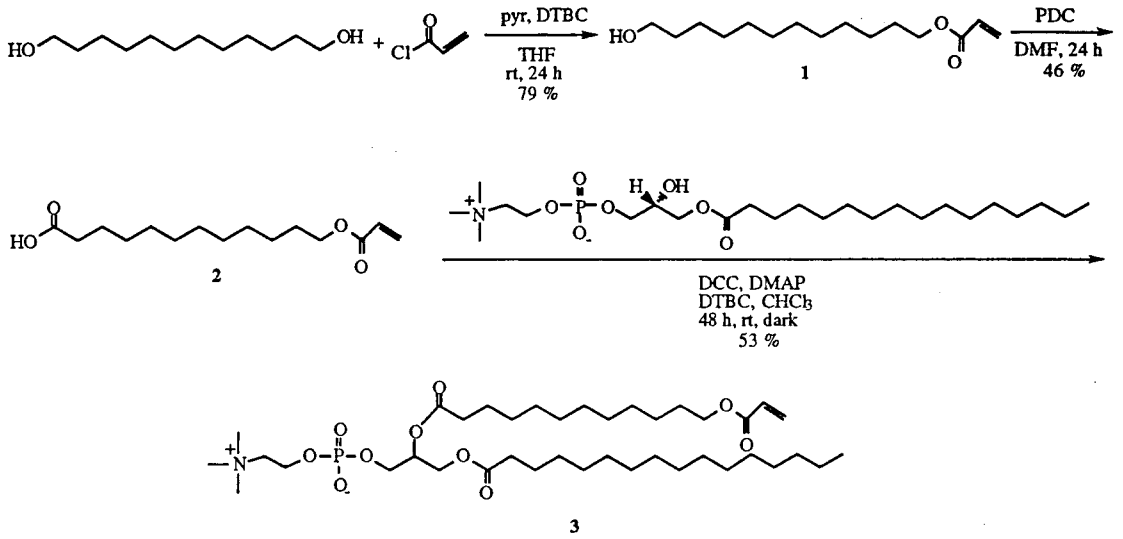
$$\text{blood specific activity} = \frac{\{[\text{blood std (cpm)} - \text{backgd (cpm)}]\,(^{111}\text{In fraction in platelets})\}}{\text{vol of the blood std (mL)}} \quad (2)$$

Results and Discussion

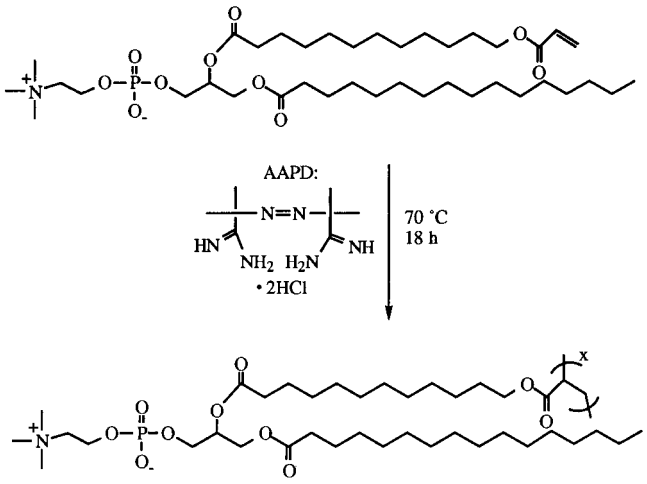
Stabilized phospholipid monolayer surfaces were prepared using a strategy based upon the fusion of unilamellar vesicles with an alkylated substrate followed by *in-situ* polymerization (Figure 1). Briefly, established general methodologies were utilized for the synthesis of the phospholipid monomer (Scheme 1),[26] preparation of liposomes, and fusion of the native lipid vesicles onto alkylated surfaces.[31] Optimized OTS-coated substrates, characterized by goniometric measurements and AFM, displayed advancing water contact angles of 110−113° and were topographically uniform with an average roughness of less than 2 Å over 1 μm^2. In order to facilitate vesicle/surface fusion, experiments were performed at 40 °C, above the known T_m for the acrylate-functionalized lipid monomer.[32] After fusion, either a water-soluble free radical initiator, AAPD, or an oil soluble initiator, AIBN, was added directly to the film in the buffer solution (Scheme 2) and polymerization was initiated by heat (65−70 °C). The polymerized film was rinsed copiously with water, and surface characterization was performed.

Several parameters were investigated in optimizing vesicle fusion and polymerization schemes, including vesicle size and concentration, fusion time, monomer/initiator concentration ratio, and type of initiator. In the process, it was postulated that a uniform lipid monolayer would be associated with a lower water contact angle than that associated with surface defects. While three different vesicle sizes were initially utilized, 200, 600, and 2000 nm, the effectiveness of surface fusion was unaffected by this parameter and all subsequent studies were performed with 600 nm vesicles. Likewise, monomer fusion to the alkylated substrate was examined as a function of vesicle concentration (750−1500 μM) and fusion time (24−48 h). Contact angles were unaffected by these parameters, and all

Scheme 1. Preparation of Phospholipid Monomer



Scheme 2. Polymerization of Phosholipid



ensuing studies were performed at a concentration of 1200 μM and a fusion time of 24 h. As we anticipated, larger ratios of monomer to initiator led to lower contact angles, presumably related to a higher degree of polymerization, and a monomer:initiator ratio of 10:1 was selected on the basis of these investigations. Finally, the effectiveness of two free radical initiators was studied: AAPD and AIBN, which are soluble in water and organic solvents, respectively. It was expected that AAPD, as a water-soluble initiator, would be more efficient since polymerization was carried out at a solid−aqueous interface. In addition, AAPD has also been shown to penetrate the hydrophobic layer above the T_m of polymerizable phospholipids.[26,33] Table 1 displays the contact angles for the two different initiator systems. Although successful polymerization was obtained by presonication of AIBN into vesicles, as reported by others,[34] lower contact angles were observed in the AAPD-initiated system.

Initial surface characterization of optimized surfaces, all polymerized with AAPD, was performed using contact angle goniometry (Table 2). Average advancing and receding water contact angles of 64 and 44°, respectively, were observed. As such, the hydrophilicity of the film was confirmed, particularly when compared to the base OTS substrate, which exhibited an average contact angle of 111°. In addition, an average hysteresis of 18° was noted. Hysteresis, defined as the difference between advancing and receding contact angles, is indicative of kinetic and thermodynamic film properties. For example, surface roughness and chemical heterogeneity, either intrinsically or due to molecular reorientation after penetration of water into the polymer film, may lead to large hysteresis values. In this regard, it bears reemphasis that advancing and receding angles measure the contact of water on dry and prewetted surfaces, respectively. Thus, hysteresis values in our polymer system likely represent, at least in part, the reorientation of hydrophilic phosphorylcholine moieties in order to minimize the free energy at the solid−water interface.

Angle-dependent ESCA measurements were carried out to further define atomic level surface properties (Tables 3 and 4). Assuming 2.5 OTS chains per phospholipid unit, theoretical atomic percent surface concentrations were calculated. In our system, P and N were identified, providing additional confirmation of a polymerized lipid film. Moreover, observed atomic percent concentrations, particularly C and O, were consistent with predictions for close-packed monolayer formation with near-normal alignment of lipid chains. Overall, ESCA has been used infrequently in the

Table 1. Final Polymer: Polymerization Conditions and Contact Angles

polymer run[a]	advancing contact angle (deg)	receding contact angle (deg)	hysteresis (deg)
1	63.6 ± 5.3	45.4 ± 2.7	18.2
2	63.3 ± 6.3	47.3 ± 6.9	16.0
3	64.8 ± 1.5	45.0 ± 2.8	19.8
4	65.2 ± 1.6	46.8 ± 2.5	18.4
5	64.6 ± 3.6	43.8 ± 4.3	20.8
6	62.4 ± 3.4	44.4 ± 5.1	18.0
alkylated glass (OTS)	111.2 ± 2.4	105.0 ± 1.4	6.2

[a] Polymerization conditions: initiator was AAPD; vesicle size was 600 nm; concentration was 1200 μM; fusion time was 24−48 h at 40 °C.

Table 2. Comparison of Free Radical Initiators (AIBN vs AAPD)

initiator	advancing contact angle (deg)	receding contact angle (deg)	hysteresis (deg)
AAPD	62.4 ± 3.4	44.4 ± 5.1	18.0
AIBN	76.2 ± 2.6	58.6 ± 5.3	17.6

Table 3. ESCA Results for Polymer

polymer	take-off angle 15°	take-off angle 45°	take-off angle 90°	approximate theoretical %
C	78.8 ± 0.7	69.2 ± 4.9	65.6 ± 6.3	79.3
P	0.7 ± 0.2	0.6 ± 0.3	0.8 ± 0.4	0.9
O	16.3 ± 0.8	22.2 ± 3.8	24.2 ± 4.3	16.5
N	0.4 ± 0.1	0.6 ± 0.1	0.6 ± 0.4	0.9
Si	3.8 ± 0.6	7.4 ± 1.1	8.8 ± 1.3	2.4

Table 4. ESCA Results for OTS Glass

OTS	take-off angle 15°	take-off angle 45°	take-off angle 90°	theoretical %
C	85.3 ± 1.7	62.6 ± 0.5	53.2 ± 0.4	81.8
Si	4.6 ± 0.8	12.5 ± 0.3	14.3 ± 1.0	4.6
O	10.0 ± 1.0	24.7 ± 0.4	32.5 ± 0.7	13.6

Table 5. Static Stability Contact Angles (deg) for the Optimal Polymer (Advancing/Receding)

polymer run	initial	1 day	2 days	1 week
1	65/44 (±4)	75/56 (±2)	77/55 (±5)	81/55 (±4)
2	65/45 (±3)	78/59 (±5)	76/56 (±4)	78/56 (±2)

Table 6. Shear Flow Contact Angle Results

polymer	advancing contact angle (deg)	receding contact angle (deg)	hysteresis (deg)
initial	71.7 ± 2.9	50.3 ± 7.7	21.4
10 min	76.8 ± 0.6	60.3 ± 0.2	16.5
60 min	84.5 ± 6.0	58.0 ± 5.0	33.5

characterization of PC-based surfaces and no angle-dependent data have been reported to date. However, our results are consistent with those of Hayward *et al.*[35] and Köhler *et al.*[36] of PC derivatized glass.

We have observed, as have others, that nonpolymerized substrate-supported phospholipid membranes are unstable on transfer from water to air, even for brief surface measurements.[37] Thus, following polymerization, serial contact angles were determined in order to characterize the stability profile of the lipid monolayer (Table 5). The largest increase in contact angle was noted within the first 24 h, likely due to the leaching of nonpolymerized lipid monomer and remained relatively unchanged for at least 2 weeks thereafter. Under operating conditions, blood-contacting surfaces are usually subjected to wall shear rates of 20 dyn/cm^2 or less. As a short term test, films were exposed to a shear force of 200 dyn/cm^2 at 37 °C for either 10 or 60 min (Table 6). Similarly, the largest change in the contact angle was noted early in the exposure period.

The limitations of short-term blood-contacting studies in predicting the risk of surface-induced clot formation are well-known. Nonetheless, they provide a convenient preliminary mechanism for screening the clinical performance of a biomaterial. In particular, the baboon is the animal of choice for blood compatibility testing since its blood-clotting system most closely resembles that of man.[38] Throughout a 2-h time period, minimal platelet deposition was observed on polymerized phospholipid surfaces, in contrast to the high level of reactivity exhibited by uncoated glass surfaces (Figure 2).

Most reports that have investigated the generation of phosphatidylcholine-derivatized surfaces have focused on the synthesis of a variety of copolymers containing the PC functional group in either side chains or, less frequently, the polymer backbone.[8−12] For example, in a series of studies from Nakabayashi's group, Kojima *et al.*[8] prepared a copolymer of MPC ((2-(methacryloyloxy)ethyl) phosphorylcholine) and styrene and Ueda *et al.*[9] prepared copolymers of MPC with various alkyl methacrylates (e.g., *n*-butyl methacrylate [BMA]). In the latter report, hysteresis values averaged 80°, which, presumably, was attributable to both surface roughness and the rearrangement of hydrophilic PC head groups on contact with water. Of interest, blood compatibility decreased as the length of the alkyl chain increased. In a more recent report from this same group, Ishihara *et al.*[10] synthesized a copolymer of MPC, BMA (*n*-butyl methacrylate), and a methacrylate with a urethane bond in the side chain, which were subsequently cast onto a segmented polyurethane. A similar strategy has been utilized by Campbell *et al.*[11] in which a copolymer of MPC and lauryl methacrylate has been synthesized and coated onto metal, glass, and polymer surfaces. In an interesting variation of these approaches, Nakaya has recently produced a polymer composed of PC groups in the main backbone chain.[12,13] The polymer self-organizes due to the presence of docosyl or stearyl side chains. In all cases, coating stability has been attributed to multipoint attachment of the alkyl chains to the underlying surface.

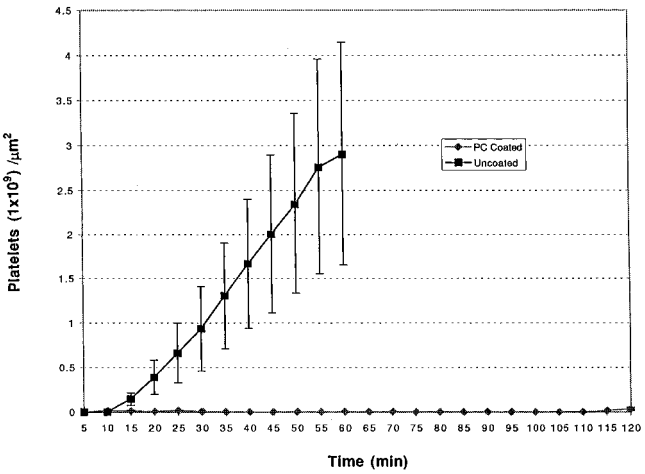


Figure 2. Platelet deposition on test surfaces in a baboon femoral arteriovenous shunt. Series 1 and 2 represent untreated glass and phospholipid-coated surfaces, respectively. Data are presented as mean ± standard error of three separate samples.

An extensive literature exists on the two-dimensional polymerization of lipids in the form of vesicles; however, there are notably fewer studies that have evaluated the feasability of *in-situ* polymerization of dialkyl amphiphiles at a solid−liquid interface. Regen *et al.* reported the polymerization of bis(methacrylate)- and bis(diacetylene)-containing phospholipids onto polyethylene.[24] However, they were unable to polymerize a mono(acrylate)-lipid monomer and concluded that the success of the bis polymer was due to the formation of a cross-linked network. Cross-linked lipid networks on a solid surface have also been produced by polymerization of bis(diacetylene)-containing phospholipids after Langmuir−Blodgett deposition.[23,25] Polymerization in this system proceeds most rapidly when the crystalline phase of the polymer most closely resembles that of the monomer. That is, preservation of the structural order displayed by the monomeric lattice is required. Consequently, a high degree of surface defects is common in cross-linked diacetylene monolayers.

Despite more than four decades of research, a clinically durable blood compatible surface remains an elusive goal. Nevertheless, scientists and engineers are recognizing with increasing frequency that paradigms

established in Nature offer new approaches to the design of biomaterials with selective and specific enhancement of structural and/or functional properties. In this regard, the biological membrane appears to be an ideal starting point for the generation of a synthetic blood compatible substrate. We report the use of *in-situ* polymerization of a mono(acrylate) phospholipid as a convenient means of stabilizing a monolayer at a solid−liquid interface with promising blood-contacting properties. Importantly, this approach offers a number of potential advantages over conventional surface modification strategies, including enhanced control over surface physiochemical properties and the capacity to incorporate diverse biomolecular functional groups into the membrane-mimetic surface. While the lipid chain may not be essential for biocompatibility, it is critical to the self-assembly process, which facilitates both two-dimensional polymerization and, potentially, the creation of heterogeneous biologically active surface structures that more closely mimic living cells. The generation of more complex cell mimicking or cytomimetic materials is currently underway, and the results of these studies will be reported in due course.

Summary

A stabilized, phosphatidylcholine-containing polymeric surface was produced by *in-situ* polymerization of 1-palmitoyl-2-[12-(acryloyloxy)dodecanoyl]-*sn*-glycero-3-phosphorylcholine at a solid−liquid interface. The phospholipid monomer was synthesized, prepared as unilamellar vesicles, and fused onto a close-packed monolayer of octadecyltrichlorosilane on glass. Free-radical polymerization was carried out in aqueous solution at 70 °C and was optimal using the water-soluble initiator 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPD). The supported monolayer displayed advancing and receding water contact angles of 64 and 44°, respectively, and ESCA data were consistent with theoretical predictions for the lipid membrane. In the absence of network formation, polymeric films demonstrated acceptable short-term stability and blood compatibility.

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