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Fabrication of a phospholipid membrane-mimetic film on the luminal surface of an ePTFE vascular graft

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Abstract

A stabilized, membrane-mimetic film was produced on the luminal surface of an ePTFE vascular graft by in situ photopolymerization of an acrylate functonalized phospholipid using a fiber optic diffusing probe. The phospholipid monomer was synthesized, prepared as unilamellar vesicles, and fused onto close-packed octadecyl chains that were components of an amphiphilic terpolymer anchored onto the polyelectrolyte multilayer (PEM) by electrostatic interactions. Scanning electron microscopy (SEM) confirmed that gelatin impregnation of the graft followed by the subsequent biomimetic film coating filled in the fibril and node structure of the luminal surface of the ePTFE graft and was smooth. The lipid film displayed an initial advancing contact angle of 44°, which increased to 55° after being subjected to a wall shear rate of 500 s^{-1} for 24 h at 37 °C in phosphate buffered saline (PBS). Fourier transform (FT-IR) spectroscopy was used to characterize the stages of biomimetic film assembly and confirmed the stability of the film under shear flow conditions. In vivo assessment using a baboon femoral arteriovenous shunt model demonstrated minimal platelet and fibrinogen deposition over a 1-h blood-contacting period. The results of this study confirm the versatility of a biomimetic film coating system by successfully transferring the methodology previously developed for planar substrates to the luminal surface of an ePTFE vascular graft.

Keywords: Vascular graft; Biomimetics; Membrane-mimetic; Blood-contacting materials; Thrombogenicity

1. Introduction

Supported lipid membranes can be produced by assembling a layer of closely packed hydrocarbon chains onto an underlying substrate followed either by controlled dipping through an organic amphiphilic monolayer at an air–water interface or by exposure to a dilute solution of emulsified lipids or unilamellar lipid vesicles [1]. In the process of membrane formation, the functional reconstitution of integral or membrane-anchored proteins can be readily achieved. As a consequence, supported lipid membranes have proven to be useful tools for characterizing both protein function and cell–cell interactions. Moreover, interest has been generated in the potential application of supported membranes as sensors or biofunctional coatings for artificial organs and other implanted medical devices [1,2].

A significant limitation in the widespread use of supported biomembranes, however, remains their limited stability for most applications outside of a laboratory environment. In order to generate more robust systems, strategies have been developed to tether membranes to an underlying substrate, such as gold or glass with or without an intervening flexible spacer or polymer cushion [3–8]. While membrane fluidity is critical for many of the functional responses of biological membranes, certain applications lend themselves to compromise in which a

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substantial increase in membrane stability may be achieved by in situ polymerization of the planar lipid assembly, admittedly at the expense of reducing lateral mobility. While not necessarily an ideal solution, the capacity to design surfaces with a high degree of molecular control over the *assembly* of diverse lipid and other membrane-associated constituents is retained. In this regard, we have reported the in situ polymerization of phospholipids on self-assembled monolayers of octadecyl mercaptan bound to gold [9], octadecyl trichlorosilane on glass [2,10], and on an amphiphilic polymer cushion [11,12].

We have previously described the formation of a stabilized, membrane-mimetic film on a polyelectrolyte multilayer (PEM) by in situ photopolymerization of an acrylate-functionalized phospholipid assembly at a solid–liquid interface [9,10,12,13]. Additionally, we have demonstrated the ability to functionalize this membrane-mimetic platform by creating glycocalyx-mimetic surfaces using streptavidin–biotin chemistry [14] and protein C activating surfaces by the functional reconstitution of thrombomodulin [15,16]. In this report, we further demonstrate the versatility of our membrane-mimetic film by applying it onto the luminal surface of a small diameter (4 mm i.d.) ePTFE vascular prosthesis. Briefly, the vascular graft surface was impregnated with gelatin and crosslinked using a dilute glutaraldehyde solution. An amphiphilic polymer with anionic anchoring groups was designed that provided a convenient means for surface alkylation with an intervening polymer cushion. Following the formation of a surface supported assembly of mono-acrylated lipids, photoinitiated polymerization was performed using a fiber optic diffusing probe coupled to an argon ion laser. The molecular properties of this system were characterized by contact angle goniometry, FT-IR spectroscopy, and SEM, and its inherent stability in a high shear rate environment confirmed. Excellent short-term blood contacting properties are demonstrated in a baboon ex vivo shunt model.

2. Materials and methods

2.1. Reagents

All starting materials and synthetic reagents were purchased from commercial suppliers unless otherwise noted. Poly L-lysine (PLL; >300 kD) and porcine type A gelatin was purchased from Sigma. Alginate (Pronova UP LVM) was obtained from NovaMatrix (Oslo, Norway) and used as received. *N*,*N*-dioctadecylcarbamoyl-propionic acid (DOD), poly(HEA-DOD)$_{6:3}$, and 1-palmitoyl-2-(12-(acryloyloxy)dodecanoyl)-*sn*-glycero-3-phosphorylcholine (mono-AcrylPC) were synthesized as previously described [2,11,12,17].

2.2. Instrumentation

Contact angles were obtained using a Rame-Hart goniometer, Model 100–00. Graft samples were cut into 5 × 5 mm sections and adhered onto glass slides using double-stick tape. Measurements are reported as the average value of advancing or receding contact angles of at least 12 data points (4 measurements each per three samples).

SEMs were obtained using an in-lens field emission SEM (ISI DS-130F Schottky Field Emission SEM) that was operated at 5 kV. Sample-containing ePTFE grafts were prepared using critical point drying and were subsequently mounted onto aluminum specimen stubs with double-stick tape, degassed for 30 min, and coated with a 1 nm chromium (Cr) film.

Infrared spectroscopy. Spectra were acquired using a BioRad FTS-4000 Fourier Transform Infrared spectrometer equipped with a wide band MCT detector, collected with 100 scans, and 2 cm^{-1} resolution. Attenuated total reflection (ATR) spectra were acquired using a Silvergate ATR anvil press accessory equipped with a germanium prism (Specac Inc., Woodstock, GA). The single beam spectrum of the ATR accessory was used as a background. Spectra manipulations performed on the data, such as baseline correction, CO_2 peak removal (from 2250 to 2405 cm^{-1}) and center-of-gravity frequency position determination of IR absorption bands were performed using the Grams/AI software package (Thermo Galactic Industries, Salem, NH). Infrared band assignments were obtained from reference values previously reported in the literature [18–21].

2.3. Statistical copolymerization of 2-hydroxyethyl acrylate (HEA) and styrene sulfonate (SS)

HEA (1.15 mL; 10 mmol) and SS (0.206 g; 1.0 mmol) were dissolved into 20 mL dimethyl sulfoxide. 2,2′-Azobisisobutyronitrile (AIBN; 18 mg; 0.11 mmol) was added, the solution purged with N_2 for 30 min, and the reaction mixture placed in a 70 °C oil bath for 20 h. The reaction mixture was slowly added to 400 mL of cold CH_2Cl_2 with vigorous stirring. A colorless glue-like precipitate formed and the solid rinsed twice with CH_2Cl_2 prior to overnight drying under vacuum. The final product was a white solid that was highly hygroscopic and was referred to as poly(HEA-SS)$_{10:1}$. Yield: (1.16 g, 85%). ^{1}H NMR (CD_3OD), δ: 3.99 (2 H, CH_2 of HEA), 7.50 and 7.04 (4 H, Ar-H of SS). ^{1}H NMR (DMSO-d6), δ: 7.50 (br, Ar-H); 7.04 (br, Ar-H); 3.99 (br, CH2–O–C=O); 3.54 (br, CH2–OH); 2.54 (br., CH); 2.25 (br, CH2). GPC (LLS): Mn = 25,300, PDI = 1.75.

2.4. Synthesis of an HEA-SS-DOD terpolymer

Poly(HEA-*co*-SS)$_{10:1}$ (0.410 g; 3.0 mmol of HEA mer-unit) was dissolved in 15 mL dimethylformamide (DMF) and later diluted with 10 mL of CH_2Cl_2. *N*,*N*-dioctadecylcarbamoyl propionic acid (DOD; 0.652 g; 1.05 mmol) in 10 mL of CH_2Cl_2 was slowly added to the above solution followed by the addition of *p*-dimethyl aminopyridine (12.8 mg; 0.11 mmol). A small volume of DMF (ca. 0.5 mL) was added to maintain clarity of the solution, the reaction mixture placed in an ice-water bath, and dicyclohexylcarbodiimide (0.284 g, 1.38 mmol) in 3 mL of CH_2Cl_2 was added dropwise. The reaction proceeded overnight under N_2. The reaction mixture was then evaporated to dryness and mixed with 20 mL of diethyl ether. The mixture was filtered, the filtrate evaporated to dryness, dissolved in 5 mL of CH_2Cl_2, and the polymer precipitated in 100 mL of acetonitrile. The product was a white powder and ^{1}H NMR and elemental analysis confirmed a monomer ratio in the terpolymer of HEA:DOD:SS of 6:3:1. This compound was referred to as poly(HEA_6:DOD_3:SS_1). Yield: 0.770 g, 73%. ^{1}H NMR ($CDCl_3$), δ:4.27 (br., CH_2–O–C=O); 3.76 (br. CH_2–OH); 3.22 (br. CH_2–N–C=O); 2.62 (br., CH_2–C=O, CH-Ar); 2.39 (br., CH_2, CH); 1.56 (br., CH_2); 1.47 (br., CH_2); 1.25 (br., CH_2); 0.87 (br., CH_3). IR (KBr pellet): 1732 cm^{-1} (O=C–O–), 1649 cm^{-1} (O=C–N). GPC (LLS): Mn = 49,800, PDI = 1.80. Elemental analysis: Calculated: 67.83% C, 10.20% H, 18.80% O, 1.37% N; 1.04% S; Observed: 67.54% C, 10.28% H, 18.77% O, 1.80% N, 0.96% S.

2.5. Gelatin impregnation and cross-linking on the luminal surface of ePTFE grafts

The ePTFE graft (4 mm i.d.) was initially impregnated with a gelatin base layer by clamping one end and infusing 5 mL of warm gelatin solution (7 w/v% in water, 37 °C) through the graft using a Luer-Lok syringe. Gelatin was extruded from the pores on the sides of the graft

during this process. The protocol was repeated from the other end of the graft, and the prosthesis was subsequently immersed in a gelatin bath at 43 °C for 2 h to insure uniform coating.

After the 2-h incubation, the graft was drained, 60 mL of air was pushed through the lumen to remove excess gelatin, and the graft was oriented vertically for 5 min to allow the gelatin to congeal. The graft was then immersed in a 1.5% v/v glutaraldehyde solution for a minimum of 24 h at room temperature to crosslink the gelatin for thermal and aqueous stability. After glutaraldehyde crosslinking, the graft was rinsed with 20 mL of DI water at a 2 mL/min flow rate using an Orion syringe pump.

2.6. Preparation of (PLL–alginate)$_5$-PLL-polymer films on glass

PLL and alginate were prepared at concentrations of 0.10 and 0.15 w/v% in PBS, respectively. In the generation of a polyelectrolyte multilayer on the luminal surface of the gelatin-impregnated ePTFE graft, polyelectrolyte solutions were perfused through the graft using a syringe pump at a flow rate of 1 mL/min for 2 min. This was followed by rinsing the graft with 10 mL of DI water at a flow rate of 2 mL/min between each coating solution. (PLL–alg)$_5$-PLL coated gelatin-impregnated vascular grafts were exposed to a 0.1 mM terpolymer solution (poly(HEA-DOD-SS)$_{6:3:1}$) in 1% DMSO/20 mM NaH_2PO_4, pH 7.4 that was passed through the graft at a flow rate of 0.1 mL/min for 15 min. The graft was subsequently rinsed with 10 mL of DI water at a flow rate of 2 mL/min.

2.7. Vesicle fusion

Large unilamellar vesicles (LUV) of 12 mM acrylate-PC in 20 mM sodium phosphate buffer (pH 7.4) were prepared by three successive freeze/thaw/vortex cycles using liquid N_2 and a 65 °C water bath. The LUVs were then extruded 21 times each through two back-to-back 2.0 μm and two back-to-back 600 nm polycarbonate filters using an Avanti Lipid Extruder (Alabaster, AL) and the solution diluted to 1.2 mM with 20 mM sodium phosphate buffer (pH 7.4) and 150 mM NaCl. The diluted lipid solution was purged with argon gas for 10 min. The alkylated gelatin-impregnated graft was flanked by stopcocks and placed into a custom-designed, form-fitting, graft holder (Fig. 1). A stock solution of co-initiators was prepared as 10 mM Eosin Y (EY), 225 mM triethanolamine (TEA), and 37 mM 1-vinyl-2-pyrrolidinone (VP) in water. A 10:1 (mol/mol) monomer/EY ratio was used for photo-polymerization. The photoinitiator (10 μL/mL of lipid; 2 mL of lipid total) was added to the diluted lipid solution and aspirated into a 3 mL Luer-Lok syringe. The graft was filled with the lipid solution and the stopcocks were closed off to the graft. The graft holder was then placed into a custom designed graft rotator housing (Fig. 1) and rotated at 250 rev/min. Vesicle fusion was allowed to proceed for 18–20 h at 43 °C in a dark incubator.

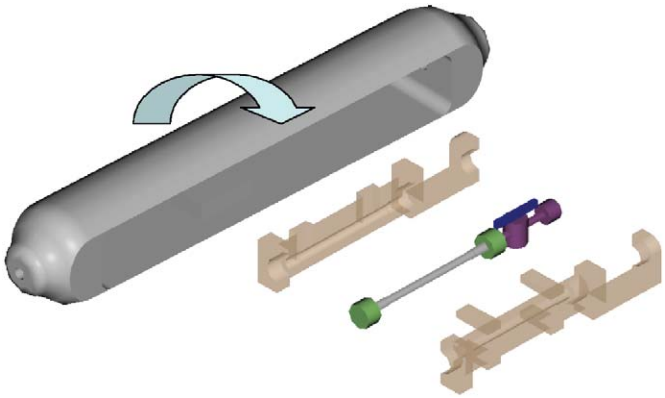

Fig. 1. A two-piece, form-fitting, graft holder supports the prosthesis and prevents moisture loss during lipid fusion. The holder snaps into the larger rotator housing, which is directly coupled to a miniature DC gearmotor.

2.8. In situ photopolymerization of a supported lipid film

Details of the photopolymerization of lipid films on alkylated glass have been reported elsewhere [10]. The photoinitiator was added to the lipid solution prior to lipid fusion in order to insure uniform mixing for photopolymerization. After the 18–20 h fusion period, the graft holder assembly was removed from the rotator housing and clamped vertically onto an optics table. A 360°, 1.6 cm long diffusing probe attached to a 600 μm dia. fiber optic with an SMA connector at one end (Romack Inc.) was attached to a 200 mW argon ion laser (Melles Griot). Approximately, 50–75 mW could be delivered to the surface of the graft with polymerization performed by advancing the laser at a rate of ~1.5 mm/min. After photopolymerization, the graft was washed with 20 mL of DI water at a flow rate of 2 mL/min. Surface properties were analyzed before and after the prosthesis was perfused for an additional 24 h at 180 mL/min ($\gamma_\omega = 500\,s^{-1}$) with 20 mM phosphate buffer saline at 37 °C.

2.9. Baboon model. Arteriovenous shunt

In vivo studies were performed as previously described [2,22]. Briefly, grafts were interposed into a permanent Silastic arteriovenous shunt that had been surgically implanted between the femoral artery and vein in male Baboons (Papio papio). Circulating platelet concentrations averaged 281,000 ± 19,000 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. Blood contacting studies were performed using three individual baboons.

2.10. 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 5 mL of 3.8% sodium citrate anticoagulant. The blood was centrifuged at 160*g* for 15 min and the platelet rich plasma (PRP) transferred to a 50 mL sterile tube to which was added PGE1 to a final concentration of 4 ng/mL PRP and centrifuged at 1500*g* for 25 min. The platelet pellet was resuspended in plasma to a platelet concentration of 1×10^{10} and 1000 μCi of indium-111 tropolone (^{111}InCl3, 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. A small aliquot was removed to determine labeling efficiency and the PRP was centrifuged at 1500*g* for 10 min to remove excess ^{111}In. The platelets were resuspended in 5 mL of reserved plasma and 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 labeling efficiency ranged between 80% and 90%.

2.11. Platelet deposition measurement

Platelet uptake on test surfaces was monitored over a 60 min period using scintillation camera imaging of the 172 keV ^{111}In g photon peak. A high-sensitivity ^{99}Tc collimator was utilized, and images were acquired with a GE 400 T scintillation camera (General Electric, Milwaukee, WI) interfaced with Nuquest Imaging Software. Immediately before imaging, a 5 min image was acquired of a 3 mL blood sample (blood standard). Images were obtained continuously with data storage at 5 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:

$$\text{Platelets/unit surface area} = \frac{[\text{test surface area (cpm)} - \text{background activity (cpm)}] \times \text{platelet/mL}}{\text{blood specific activity (cpm/mL)}},$$

where

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

2.12. Total fibrin accumulation

Homologous baboon fibrinogen was purified and labeled with ^{125}I as described [23]. The labeled fibrinogen preparation was >90% clottable. In total, 5 μCi of ^{125}I-fibrinogen was injected intravenously 10 min prior to shunt studies. After blood exposure for 1 h, the prosthesis was thoroughly rinsed with isotonic saline. After allowing at least 30 d for the ^{111}In to decay ($t_{1/2} = 2.8$ d) ^{125}I-activity was measured using a gamma counter. Total fibrin accumulation was calculated by dividing the deposited ^{125}I-radioactivity (cpm) by the clottable fibrinogen radioactivity (cpm/mL) and multiplying by the circulating fibrinogen concentration (mg/mL) as measured in each experiment [23,24].

3. Results and discussion

3.1. Layer-by-layer assembly of a membrane-mimetic film on a gelatin-impregnated vascular graft

We have demonstrated in prior reports that membrane-mimetic films, which are stable in air and under static and dynamic flow conditions in an aqueous environment can be produced by in situ polymerization of a planar lipid assembly on a variety of alkylated supports [2,10,12]. Significantly, an alkylated terpolymer, electrostatically coupled to a polyelectrolyte multilayer, allows film fabrication on a hydrophilic cushion that facilitates the incorporation of transmembrane proteins [15]. Additionally, we have shown that mixed vesicles, comprised of polymerizable lipids with either biotin or phosphatidylcholine head groups, could be successfully fused onto an alkylated terpolymer bound to a poly L-lysine/alginate multilayer, and by using streptavidin–biotin chemistry, a glycocalyx-mimetic surface could subsequently be produced [14]. In this investigation, we report the successful application of our membrane-mimetic film coating strategy to a gelatin-impregnated ePTFE vascular graft.

Contact angles were measured at various stages of film coating (Fig. 2A). As expected, advancing contact angles for the bare ePTFE graft were extremely high (140°) and decreased after gelatin impregnation and cross-linking (50°). In agreement with prior studies, advancing contact angles for the ALG/PLL multilayer were low (35°) and increased after the addition of the terpolymer (110–115°) [12]. After in situ photopolymerization of the deposited phospholipid layer an advancing contact angle of 44±1° was observed, in good agreement with previous studies [10,12]. These values are also consistent with prior reports of Hayward et al. [25] and Köhler et al. [26] of phosphorylcholine derivatized glass, as well as with our own data in other polymerized lipid systems. After exposing the film to phosphate buffered saline for 24 h at a 37 °C and at a shear rate of 500 s^{-1} the mean advancing contact angle increased to 55±2°. Contact angles were consistent when examined on samples obtained at 5 equidistant positions along the axial length of the prosthesis (Fig. 2B).

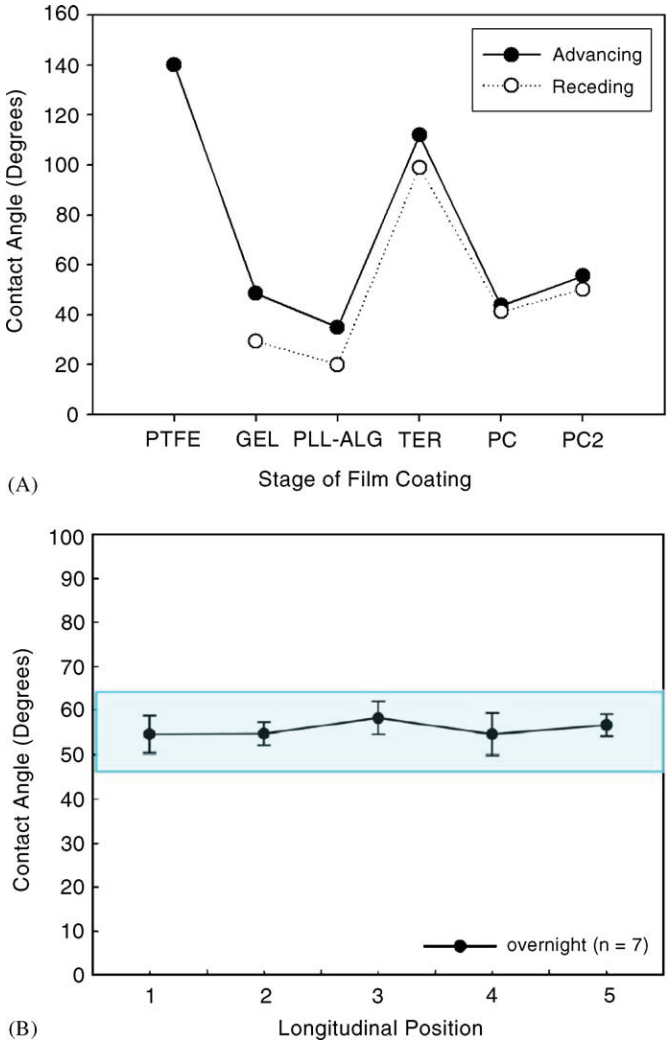

Fig. 2. (A) Advancing and receding contact angle measurements of various stages of film coating onto ePTFE vascular grafts. Plain ePTFE graft (PTFE), after gelatin impregnation (GEL), after poly L-lysine and alginate coating (PLL–ALG), after terpolymer self-assembly (TER), after acrylate-PC fusion and photopolymerization *before* flow conditioning (PC), after acrylate-PC fusion and photopolymerization *after* flow conditioning (PC2). Flow conditioning experiments were performed at a shear rate of 500 s^{-1} at 37 °C for 24 h in PBS. (B) Advancing contact angle measurements (mean ±SE) at various longitudinal positions on ePTFE vascular grafts after membrane-mimetic film coating and flow conditioning.

3.2. Surface imaging characterization of a supported lipid assembly on gelatin-impregnated vascular grafts using scanning electron microscopy

SEM images of the various stages of biomimetic film assembly on the luminal surface of an ePTFE graft are presented in Figs. 3–5. Prior to gelatin impregnation, the classic fibril and node structure of ePTFE was observed (Fig. 3). After gelatin impregnation and crosslinking, the

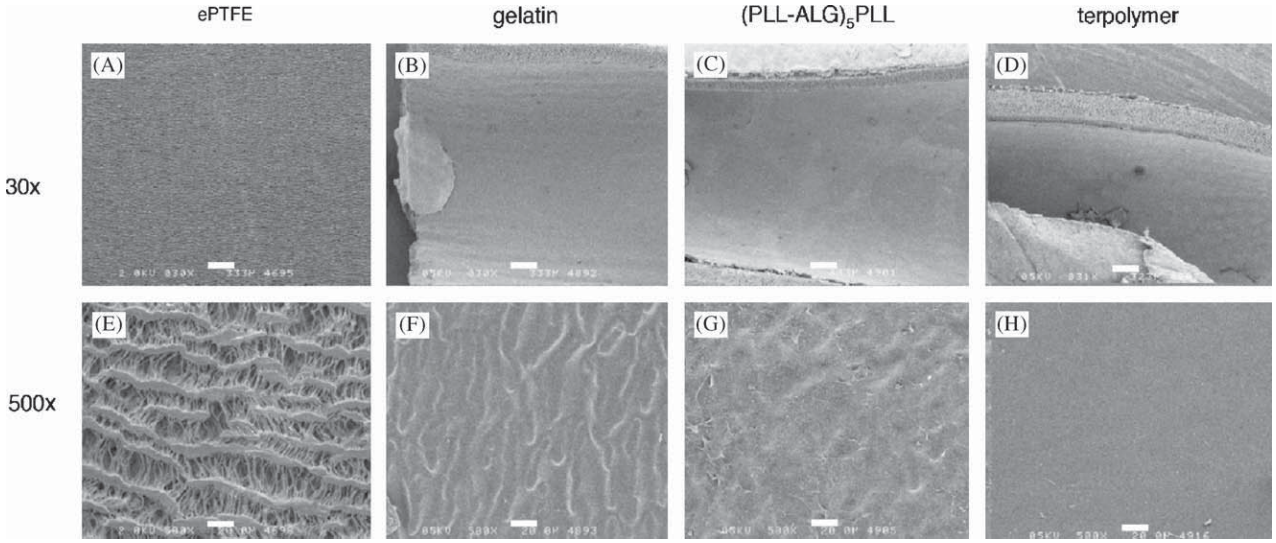

Fig. 3. Scanning electron microscopy of various stages of film coating onto ePTFE vascular grafts. Scale bar in A–D is equal to 333 μm and in E–H is equal to 20 μm.

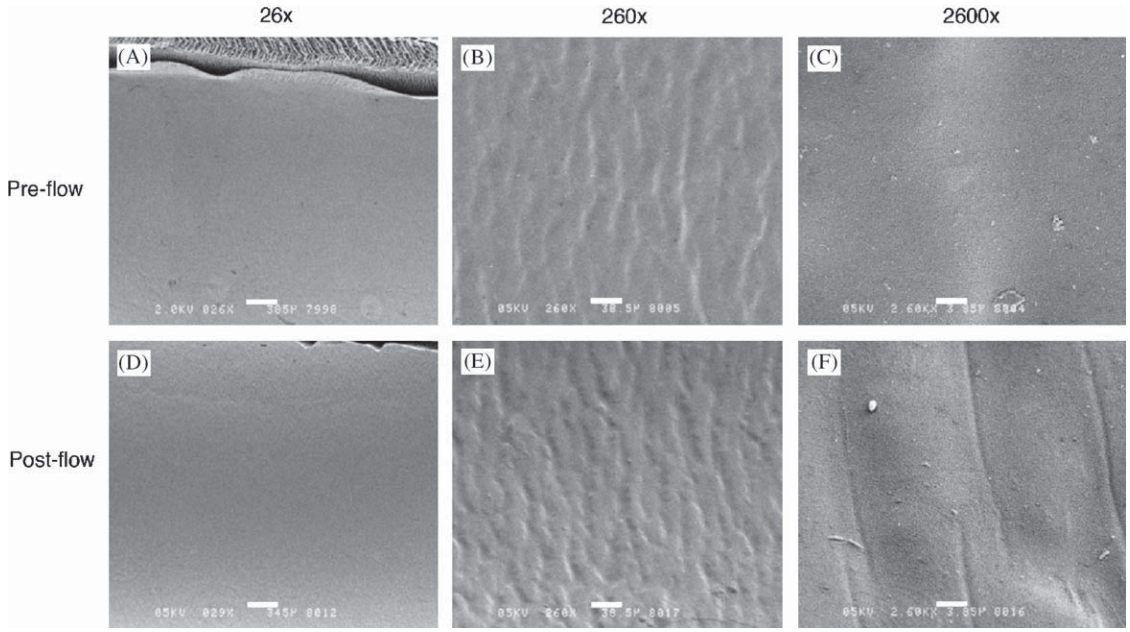

Fig. 4. Scanning electron microscopy of ePTFE vascular grafts after acrylate-PC coating. Scale bar in A is 385 μm, B, 38.5 μm, C, 3.85 μm, D, 345 μm, E, 38.5 μm and F, 3.85 μm.

luminal surface of the graft was filled in, with only small ripples in the surface apparent due to underlying fibrils of the graft (Fig. 3F,G). Continued smoothing of the surface continues after PLL–ALG multilayer terpolymer coating. After lipid fusion and photopolymerization, the surface appears to be smooth both prior to and after flow (Fig. 4). High resolution SEM at 63 kx magnification reveals a cobblestone-like surface, which may be due to segregation of the hydrophilic HEA cushion from the hydrophobic alkyl chains of the terpolymer film induced by critical point drying (Fig. 5).

3.3. Structural characterization of the supported lipid assembly on gelatin-impregnated vascular grafts by infrared spectroscopy

Infrared spectra were acquired during each stage of film construction in order to identify functional group characteristics and induced structural changes that were unique to each film component. This analysis also provides a framework for characterizing film stability under varying environmental conditions. Specifically, infrared spectroscopy provides detailed information regarding

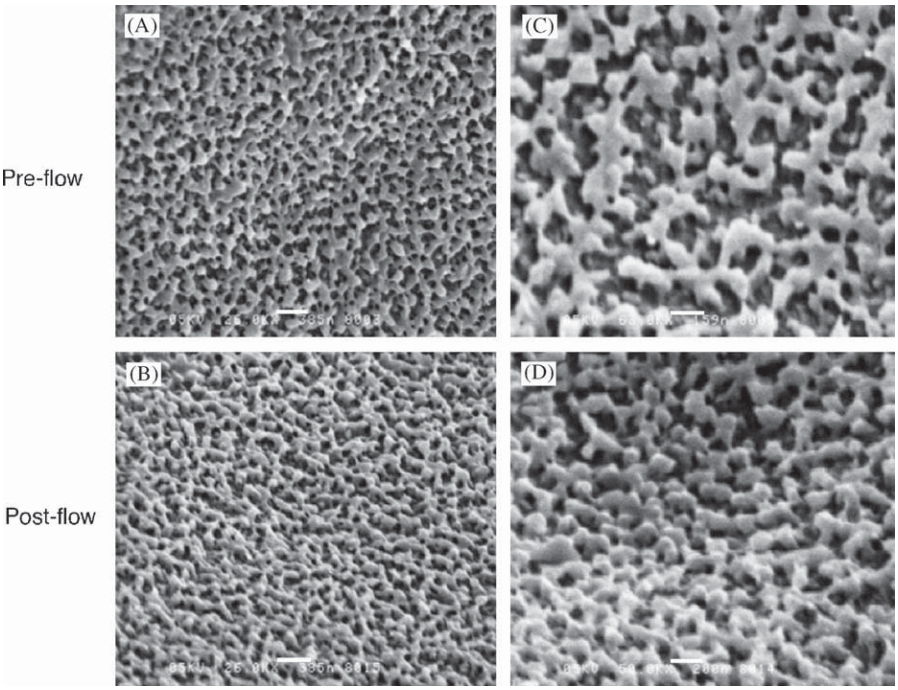

Fig. 5. High resolution scanning electron microscopy of membrane-mimetic coating on ePTFE vascular grafts after critical point drying: (A) 26 kx, bar 385 nm, (B) 26 kx, bar 385 nm, (C) 63 kx, bar 159 nm, (D) 50 kx, 200 nm.

hydrocarbon chain conformation and order, and by using polarized spectra, molecular orientation. The level of disorder may influence film properties, such as the orientation and assembly of membrane-associated proteins. Characteristic IR absorption bands of the fabricated membrane-mimetic film applied to the surface of a gelatin-impregnated graft are summarized in Table 1.

Infrared spectra of the various stages of film coating are presented in Fig. 6I, A–E. Before the graft is coated, characteristic CF_2 antisymmetric and symmetric stretching modes at 1208 and 1147 cm^{-1}, respectively, are observed from the bare ePTFE graft (Fig. 6A). After gelatin impregnation and crosslinking, amide I and amide II stretching modes at 1646 and 1542 cm^{-1} appear (Fig. 6I, A vs. B). The addition of the PLL–ALG polyelectrolyte multilayer film revealed subtle changes in the infrared spectrum (Fig. 6I and 6II, C). Notably, there is PLL-dependent sharpening of the amide I and amide II stretching vibrations when compared to the spectrum of the gelatin impregnated base layer (Fig. 6II, B vs. C). The symmetric (1402 cm^{-1}) and antisymmetric (1602 cm^{-1}, shoulder) stretching vibrations of the carboxylate ion (COO^-) are unique to the alginate component of the film and were resolved in the infrared spectrum (Fig. 6II, C).

Addition of the terpolymer (Fig. 6I, D) was associated with the appearance of intense symmetric and antisymmetric methylene stretches, located from 3000 to 2800 cm^{-1}, as well as an ester carbonyl band centered at 1735 cm^{-1}. These vibrations can be attributed to DOD and HEA groups, respectively. The spectral intensity of unique PLL (amide I stretch) and alginate (antisymmetric and symmetric COO^- stretches) vibrations were reduced, but remain identifiable. Vibrational bands assigned to the C–O–C antisymmetric (~1225 cm^{-1}), C=O–O–C symmetric (1100 cm^{-1}), and C–O–C symmetric (1040 cm^{-1}) stretching modes were also observed after terpolymer self-assembly.

Dramatic changes in the IR spectra were seen after fusion and lipid photopolymerization using a fiber optic

Table 1
Infrared band assignments for components present in a biomimetic film coated onto an ePTFE vascular graft

Absorption mode	Frequency (cm^{-1})	Component of film
CH_2 stretch (antisymm)	2926–2918	TER, PC
CH_2 stretch (symm)	2853–2850	TER, PC
C=O (ester)	1735	TER, PC
Amide I	1646	GEL, PLL
COO^- stretch (antisymm) (SH)	1602	ALG
Amide II	1542	GEL, PLL
CH_2 bend (scissoring)	1456	TER, PC
COO^- stretch (symm)	1402	ALG
C–O–C stretch (antisymm)	~1225–1217	TER, PC
CF_2 stretch (antisymm)	1208	ePTFE
C=O–O–C stretch (antisymm)	~1171	TER, PC
CF_2 stretch (symm)	1147	ePTFE
C = O–O–C stretch (symm)	~1100	TER, PC
C–O–C stretch (symm)	~1040–1030	TER, PC
$(CH_3)_3N^+$ bend (antisymm)	975	PC

Abbreviations: antisymm—antisymmetric, symm—symmetric, SH—shoulder, PLL—poly L-lysine, ALG—alginate, TER—poly(HEA_6–AOD_3–SSS_1) terpolymer, GEL—cross-linked gelatin, ePTFE—ePTFE vascular graft, and PC—mono-acrylPC.

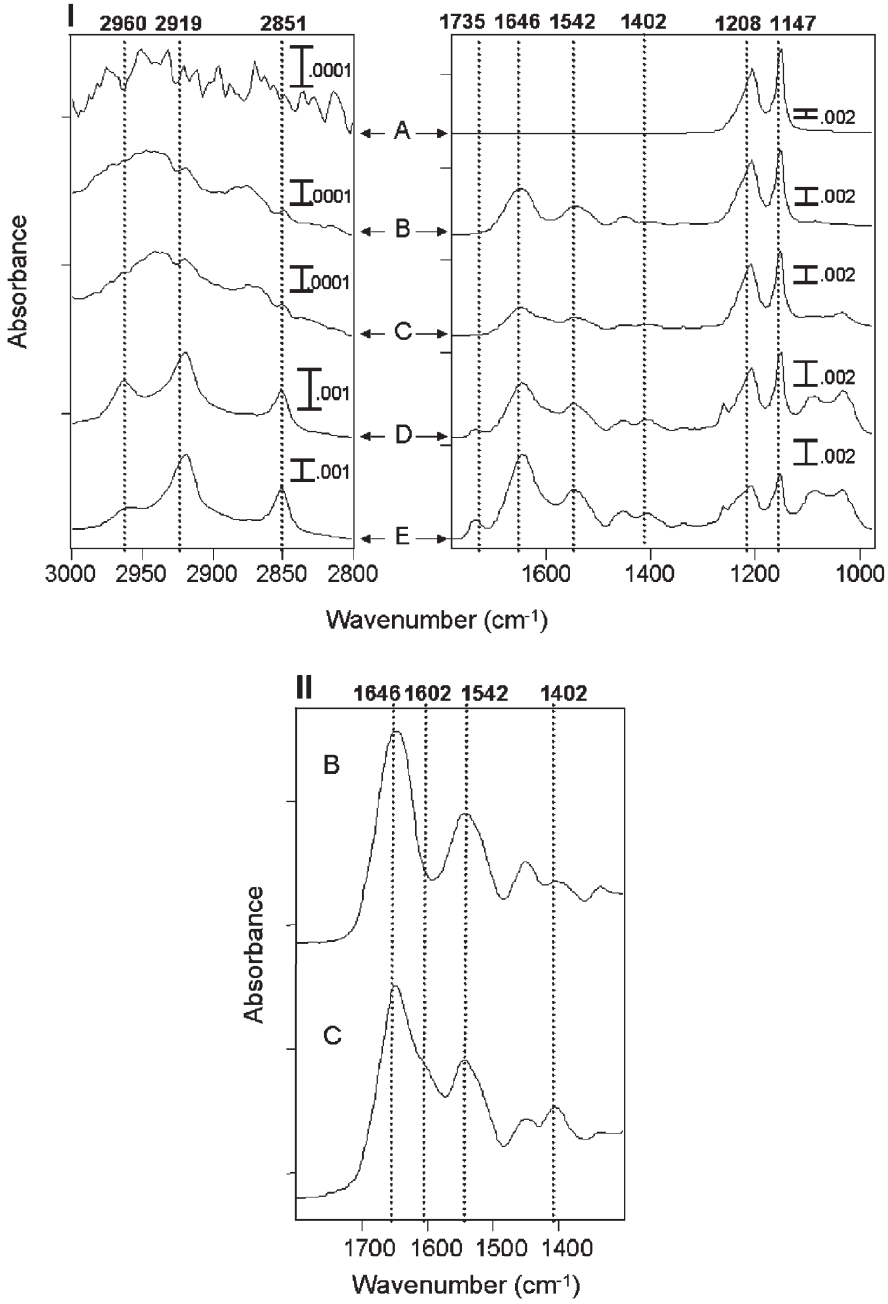

Fig. 6. (I) Infrared ATR spectra of various stages of film coating onto ePTFE vascular grafts: (A) Plain ePTFE graft, (B) after gelatin impregnation, (C) after poly L-lysine and alginate coating, (D) after terpolymer self-assembly, and (E) after acrylate-PC fusion and photopolymerization. (II) Expanded view of infrared ATR spectra from 1750 to 1350 cm^{-1} acquired after gelatin impregnation (B) and after poly L-lysine and alginate coating (C).

diffusing probe coupled to an argon ion laser (Fig. 6I, E). The most noticeable differences included marked increases in the methylene antisymmetric and symmetric stretching modes from 3000 to 2800 cm^{-1} and the ester carbonyl stretching mode at 1735 cm^{-1}. A small increase in the methylene scissoring mode at 1460 cm^{-1} was also detected. Of note, we have recently reported in a concurrent investigation that near-infrared Raman microscopy provides a useful tool for the in situ analysis of the extent of photopolymerization of an acrylate lipid membrane on a PEM film coating of an ePTFE vascular graft. This method was based on the ratio of the integrated areas of the C=C and C–N vibrations and confirmed that a polymerization efficiency of >90% is achieved [27].

3.4. A polymeric lipid film, assembled on a polyelectrolyte multilayer-gelatin impregnated graft, is stable in a high shear flow environment

Films after lipid fusion and photopolymerization were exposed to phosphate buffer at a shear rate of 500 s^{-1} for a 24-h contact period at 37 °C. Except for slight variation in

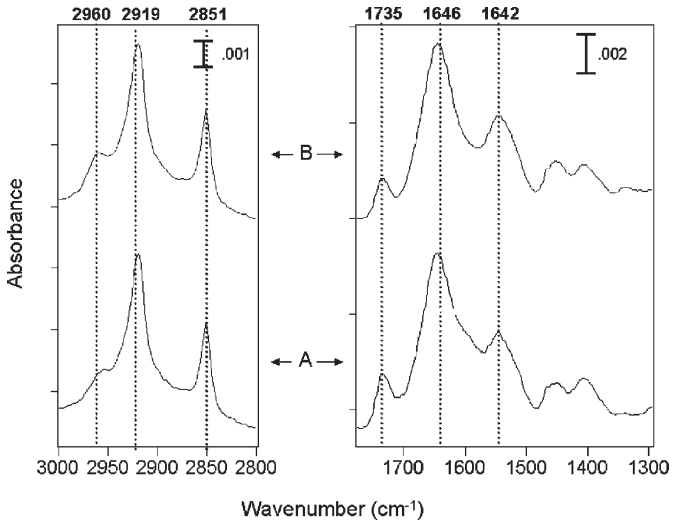

Fig. 7. Infrared ATR spectra acquired of biomimetic film construct coated onto ePTFE vascular grafts after (A) and before (B) flow conditions at a shear rate of 500 s^{-1} at 37 °C for 24 h.

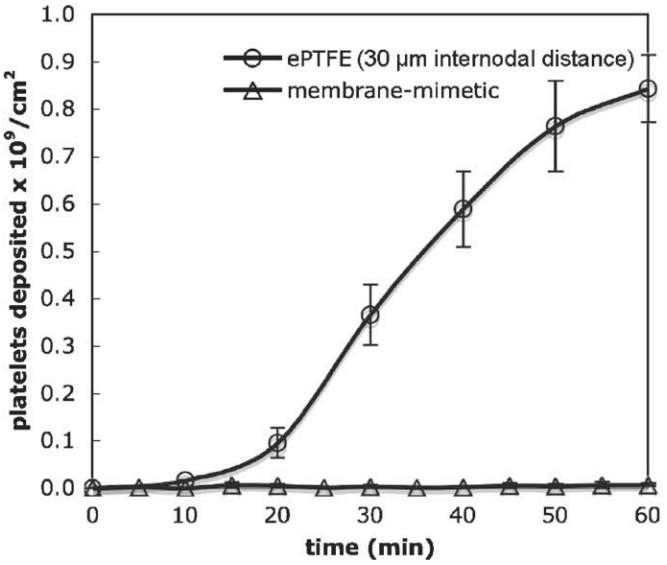

Fig. 8. Platelet deposition on 4 mm id vascular prostheses over a 1 h period of blood contact at a flow rate of 100 mL/min ($n = 3$).

lipid band spectral intensity, the IR spectra were unchanged, as shown in Fig. 7. This observation was consistent with contact angle measurements (Fig. 2), which showed a small increase in the advancing contact angle from 44° to 55° after flow tests. This may represent the removal of intact non-fused vesicles from the graft surface. These results are in agreement with previous studies of biomimetic film stability on planar substrates subjected to high shear conditions [12].

3.5. Blood-contacting properties of a small diameter ePTFE graft coated with a membrane-mimetic film in a baboon ex vivo shunt model

^{111}In-labeled platelet deposition was monitored in real time over a 1-h period at a blood flow rate of 100 mL/min and compared to responses observed to a reference set of uncoated ePTFE grafts. Both sets of vascular prostheses had an internal diameter of 4 mm. Significant platelet deposition was observed on the uncoated ePTFE graft, but not on the prosthesis coated with a membrane-mimetic film (Fig. 8). Likewise, total adsorption of ^{125}I-labeled fibrinogen onto membrane-mimetic coated grafts during the test period was $0.76 \pm 1.3\,\mu g/cm^2$ consistent with negligible thrombus formation. This compares with fibrinogen adsorption of $1.44 \pm 0.75\,mg/cm^2$ on uncoated ePTFE grafts.

It is recognized that the adverse events leading to the failure of small diameter vascular prostheses are related to maladaptive biological reactions that occur at both blood– and tissue–material interfaces. In response to these problems, vascular prostheses have been coated with matrix proteins or integrin-selective peptide sequences as a mechanism to promote the growth of endothelial cells onto the luminal graft surface, either from perigraft tissue or from the intima of the adjacent host vessel. Unfortunately, a substantial risk for thrombus formation exists in the period prior to complete endothelial regeneration. We believe that membrane-mimetic thin films represent an optimal thromboresistant platform for presentation of ligands that drive endothelial cell growth in vivo in order to create a durable blood contacting surface.

4. Conclusions

A stabilized, polymeric membrane-mimetic film was produced on an alkylated polyelectrolyte multilayer by in situ photopolymerization of a lipid assembly on the luminal surface of a gelatin-impregnated ePTFE graft using a fiber optic diffusing probe. The stages of film coating were visualized using high resolution SEM and confirmed that gelatin impregnation of the graft followed by subsequent biomimetic film coating filled in the fibril and node structure of the ePTFE graft and created a smooth surface. Contact angle and infrared spectroscopic measurements confirmed the presence of each component during film assembly and showed that the film was stable when subjected to a wall shear rate of 500 s^{-1}. The results of this study confirm the versatility of our biomimetic film coating system by successfully transferring the methodology previously developed for planar substrates to the luminal surface of an ePTFE vascular graft. Promising short-term blood contacting properties were noted.

Acknowledgments

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