Fabrication and Characterization of Glycocalyx-Mimetic Surfaces†

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A glycocalyx-mimetic film was created by the assembly of biotin chain-terminated glycopolymers onto a polymeric lipid membrane electrostatically coupled to a polyelectrolyte multilayer. Varying molar compositions (10, 25, and 50 mol %) of acrylate-derivatized biotin−phosphoethanolamine/phosphorylcholine lipid mixtures were prepared as unilamellar vesicles, fused onto the alkyl chains of an amphiphilic terpolymer, and photopolymerized in situ as a planar assembly. Polarized external reflection infrared spectroscopy confirmed the presence of streptavidin. Notably, IR spectroscopy revealed an increase in the conformational and orientational disorder of the lipid hydrocarbon chains with increasing mole fraction of biotinylated lipid. Correlative images obtained by confocal fluorescence microscopy demonstrated that biotinylated lipids cluster at high surface density. Despite the presence of biotin microdomains, the size and hydrophilic characteristics of coupled glycopolymer chains produced a uniform carbohydrate surface coating.

Introduction

Cell surface proteoglycans and glycolipids collectively form a membrane-bound carbohydrate coating, often referred to as a *glycocalyx*. Membrane-associated polysaccharides are critical mediators of molecular recognition events via the interactions of unique oligosaccharide sequences with specific protein epitopes that may be found on bacteria, viruses, and other cells, as well as on a variety of soluble and matrix-bound factors. Significantly, membrane-bound carbohydrates also exert a steric repulsive effect that may improve the specificity of these interactions, by limiting nonspecific cell−cell and protein−cell binding. We believe that in fabricating stable, substrate-supported model membranes, which emulate the structural features of the glycocalyx, new surface modification strategies will evolve to enhance the functionality of biosensors, biochips, and microfluidic devices. In addition, this design strategy offers the potential to improve the clinical performance characteristics of blood-contacting artificial organs and other implantable medical devices by modulating maladaptive processes at the blood−material and tissue−material interfaces.[1,2] Finally, glycocalyx-mimetic films may provide additional tools that complement existing approaches directed at the study of carbohydrate-dependent interactions under static or flow conditions.[3−7]

We have recently 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.[8−10] In this report, biotin chain-terminated polymers were synthesized bearing pendant lactose units. These glycopolymers were used to fabricate a glycocalyx-like structure on a membrane-mimetic film composed of mixed polymerizable lipids containing phosphatidylcholine or biotin headgroups (Figure 1). Polarized external reflection infrared spectroscopy in combination with confocal fluorescence microscopy was utilized to characterize the detailed molecular structure of this system and the uniformity of the thin film coating after streptavidin and glycopolymer binding. Biotinylated lipids appear to cluster in microdomains within the membrane-mimetic film, particularly at high surface density. Nonetheless, a uniform carbohydrate coating was achieved after glycopolymer binding to the membrane-mimetic film.

Materials and Methods

Reagents. All starting materials and synthetic reagents were purchased from commercial suppliers unless otherwise noted. Poly-L-lysine (PLL, ∼400 kDa) was purchased from Sigma. Alginate (ALG; low viscosity, ca. 60% mannuronic acid) was obtained from Pronova Biomedical (Norway) and used as received. Streptavidin and fluorescein isothiocyanate (FITC) labeled streptavidin were purchased from Calbiochem (San Diego, CA). FITC-labeled lectin (from *Psophocarpus tetragonolobus*) with binding specific to the galactose residues of the glycopolymer was purchased from Sigma. The synthesis and characterization of a terpolymer (TER) composed of 2-hydroxyethyl acrylate (HEA), sodium styrene sulfonate (SSS), and *N*,*N*-dioctadecylcarbamoylpropionic acid (DOD), poly(HEA−SSS−DOD)$_{6:3:1}$, 1-palmitoyl-2-(12-(acryloyloxy)dodecanoyl)-*sn*-glycero-3-phosphorylcholine (mono-acrylPC), and 1-palmitoyl-2-(12-(acryloyloxy)dodecanoyl)-*sn*-glycero-3-phosphoethanolamine (mono-acrylPE) and its biotin

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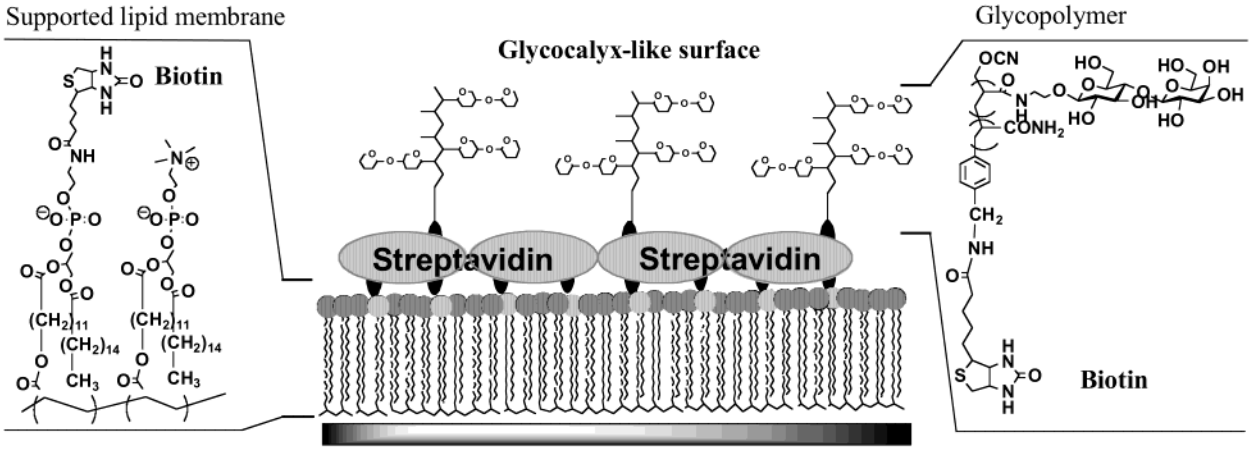

Figure 1. Schematic representation of a glycocalyx-mimetic thin film produced by coupling biotin-terminated glycopolymers to a supported lipid membrane.

derivative (mono-acrylPE−biotin) have been described in detail elsewhere.[10−14]

The biotin chain-terminated glycopolymer was generated by cyanoxyl-mediated free radical polymerization, as previously reported.[6] Briefly, 4-aminobenzyl-biotinamide was used as the initiator for the polymerization of 2-acrylaminoethyl lactoside with acrylamide. Treatment of the initiator with HBF_4 and $NaNO_2$ in deoxygened H_2O−THF (1:1) generated an arenediazonium cation, which upon reaction with NaOCN at 50 °C afforded a biotinyl aryl free radical and a cyanoxyl free radical (•OC≡N) as the initiating system. A biotin chain-terminated glycopolymer was generated in 75% conversion as a white spongy powder. The molar mass (M_n) of the polymer was 12 kDa with a polydispersity index (M_w/M_n) of 1.3 determined from size-exclusion chromatography coupled with both refractive index and laser-light scattering detectors. The mole fraction of the lactose-bearing repeat units in the biotinylated glycopolymer is 1:7 lactose/acrylamide with a total of 10 repeat units.

Instrumentation. *Contact angles* were obtained using a Rame-Hart goniometer, model 100-00. Measurements are reported as the average value plus or minus standard deviation of advancing or receding contact angles of at least 15 data points (5 measurements each per 3 samples).

Infrared Spectroscopy. Spectra were acquired using a Digilab/BioRad FTS-4000 Fourier transform infrared (FT-IR) spectrometer (Randolf, MA) equipped with a wide-band MCT detector, collected with 512 background scans, triangular apodization, and 4 cm^{-1} resolution. Polarized infrared external reflection spectra were acquired using a Thermo Spectra-Tech 510 external reflection accessory (60° angle of incidence; Shelton, CT) with infrared (IR) radiation polarized perpendicular or parallel to the surface normal using a Thermo Spectra-Tech ZnSe wire grid polarizer. Perpendicular (R_s) and parallel (R_p) polarized external reflection spectra were acquired using the spectrum of a clean silicon wafer as a background. Samples were acquired with 300−450 sample scans depending on the amount of water vapor present in the sample spectrum. Reference spectra of biotin−acrylate-PE and acrylate-PC bulk powders were obtained with a Specac Silvergate (Smyrna, GA) single bounce attenuated total reflection anvil press accessory. Conditions were similar to those described above, with the exception that the spectra were obtained in an unpolarized format and with 100 background and sample scans. Spectral 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/32 software package (Thermo Galactic Industries, Salem, NH). Infrared band assignments were obtained from reference values previously reported in the literature.[15−18]

Confocal Fluorescence Microscopy. Fluorescence images were acquired using a Zeiss LSM 510 epifluorescence microscope under 63× (oil) magnification and illuminated using an argon ion laser at 485 nm. Lower magnification images acquired in air look similar, albeit with less detail compared to those obtained using oil immersion. The oil was added to the film surface immediately prior to the initiation of imaging. Images were acquired using eight coadded scans in line mode for films coated onto silicon substrates. No post-image processing was performed.

Preparation of Silicon Substrates. Prime grade silicon wafers were purchased from Si-Tech Inc. (Topsfield, MA), were 4 in. in diameter, type P/b ⟨100⟩, with a 4−8 Ω cm resistivity and 500−550 μm thickness, and were polished on one side. Slides were cut using a diamond-tipped glass cutter in dimensions of approximately 1.0 in. × 1.5 in. for infrared external reflection spectroscopy and 0.5 in. × 0.5 in. for confocal fluorescence microscopy. After cutting, the slides were cleaned by sonication in a 1:8 mixture of Multi-Terge detergent: DI water for 15 min followed by rinsing the samples 10 times in deionized water. The sonication and rinsing procedure was then repeated twice using deionized water alone.

Fabrication of Biotin-Functionalized Supported Lipid Membranes. *Preparation of a (PLL−ALG)$_5$−PLL Terpolymer Film on Silicon*. PLL and ALG were prepared at concentrations of 0.10 and 0.15 w/v % in phosphate-buffered saline (PBS; 20 mM NaH_2PO_4, 0.9 w/v % NaCl, pH 7.4), respectively. PLL and ALG alternating monolayers were deposited on silicon using 60 s contact times for each solution, followed by three rinses with deionized water (∼10 s/rinse) between each coating solution. The (PLL−ALG)$_5$−PLL-coated silicon substrates were then exposed to a 0.1 mM solution of poly(HEA−DOD−SSS)$_{6:3:1}$ dissolved in a mixture of 20 mM NaH_2PO_4/DMSO (99:1 v/v), pH = 7.41, for 90 s. The (PLL−ALG)$_5$−PLL terpolymer coated samples were then rinsed 7−10 times with deionized water.

Vesicle Fusion. Large unilamellar vesicles (LUVs) totaling 12 mM lipid (in either 0, 10, 25, or 50 mol % biotin−mono-acrylPE/mono-acrylPC mixtures) 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 2.0 μm and 600 nm polycarbonate filters (Millipore), and the solution was diluted to 1.2 mM with

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20 mM sodium phosphate buffer (pH 7.4) and 750 mM NaCl. The $(PLL-ALG)_5-PLL$ terpolymer coated substrates were then incubated with the vesicle solution at 43 °C overnight for 14−16 h.

In Situ Photopolymerization of a Supported Lipid Film. Details of the photopolymerization of lipid films on alkylated glass and silicon have been reported elsewhere.[9,10] Briefly, a stock solution of coinitiators was prepared as 10 mM Eosin Y (EY), 225 mM triethanolamine (TEA), and 37 mM VP in water. A 10:1 (mol/mol) monomer/EY ratio was used for photopolymerization. After lipid fusion, the samples were placed into a N_2-purged atmosphere at 30% relative humidity and 10 μL of initiator was added per 1 mL of sample solution. The initiator was gently mixed by slowly rotating the vial in a horizontal circular motion without lifting it from the bench surface. The sample was then irradiated with a Dynalume visible light lamp at an intensity of 50 mW/cm^2. Following photopolymerization, the samples were washed with deionized water 6−8 times and stored for analysis.

Generation of a Glycocalyx-Mimetic Surface on a Supported Lipid Membrane. *Streptavidin Binding onto a Biotin-Functionalized Lipid Membrane.* Streptavidin and FITC-labeled streptavidin, for confocal fluorescence microscopy studies, were prepared in PBST (150 mM NaCl, 50 mM NaH_2PO_4, pH 7.34) at a concentration of 5 μg/mL. Streptavidin-containing solutions were incubated with biotin-functionalized substrates for 15 min with horizontal shaking, followed by rinsing 10 times with deionized water.

Glycopolymer Binding to a Streptavidin-Functionalized Surface. Streptavidin-coated lipid membranes were incubated with biotin-terminated glycopolymer (25 μg/mL) in PBST for 1 h at room temperature with horizontal shaking. The membrane was then washed 10 times with deionized water. For confocal fluorescence microscopy studies, the film was incubated with FITC-labeled *P. tetragonolobus* lectin (100 μg/mL) in PBST for 1 h at room temperature with horizontal shaking and then washed 10 times with deionized water.

Results and Discussion

Synthesis of a Biotin Chain-Terminated Glycopolymer. Glycopolymers with surface anchoring groups located along the polymer backbone have been synthesized to generate carbohydrate-functionalized surfaces with potential utility in the design of novel immunochemical assays,[19] in biocapture analysis,[20] and for coatings of implanted biomedical devices.[1,2] Nonetheless, glycopolymers bearing a series of pendant anchoring groups characteristically demonstrate reduced bioactivity due to steric hindrance, which is only partially offset through the introduction of a spacer arm between the anchor and the polymer backbone. Avidin (streptavidin)/biotin-based surface engineering has the advantage of being rapidly completed in a mild aqueous environment, with simple washing and purification steps. Thus, potential damage to candidate surface ligand groups due to the conjugation process is limited and any moiety that can be biotinylated can be immobilized onto an avidin/streptavidin surface. As such, polymers with biotinylated endgroups have recently been used to generate self-organizing protein−polymer hybrid amphiphiles[21] and molecularly engineered surfaces.[6]

In this report, a chain-end-functionalized glycopolymer was synthesized in which multivalent lactose units serve as ligands for lectins and/or antibodies,[22] and a single biotin group on the initiating species provides a convenient anchor to avidin or streptavidin.[23] Modulating lactose density and polymer solubility was achieved by using acrylamide as a comonomer. Binding specificity to streptavidin has been previously verified using a SDS-PAGE gel shift assay and confocal fluorescence imaging of the glycopolymer adsorbed onto a streptavidin-patterned surface.[6]

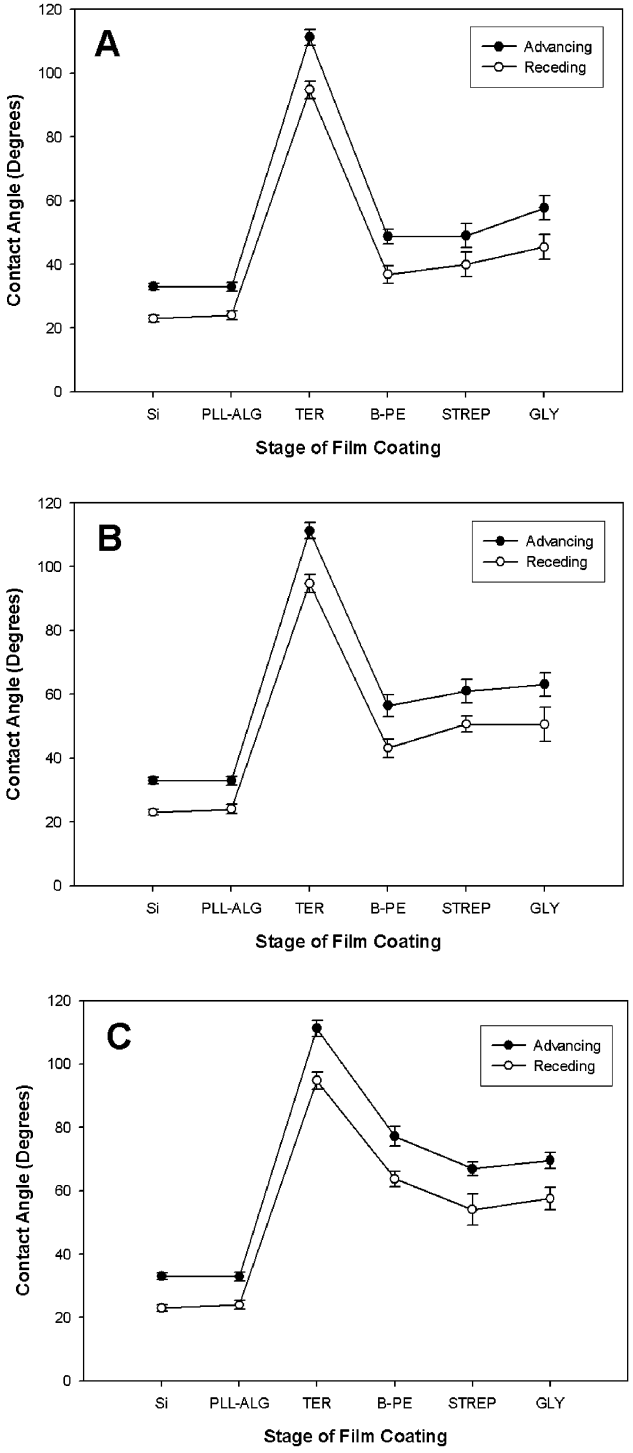

Figure 2. Advancing and receding contact angles of successive assemblies of an alginate/poly-L-lysine (ALG/PLL) multilayer, terpolymer (TER), biotin−PE/PC lipid vesicles (B−PE/PC), streptavidin (STREP), and glycopolymer (GLY): (A) 10 mol % biotin−PE, (B) 25 mol % biotin−PE, and (C) 50 mol % biotin−PE.

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Table 1. Infrared Band Assignments for Components Present in a Glycocalyx-Mimetic Thin Film[a]

absorption mode	frequency (cm^{-1})	component of film
OH stretch	3600−3000	ALG, GLY
amide A (mostly N−H)	∼3300	PLL, STREP
amide B	∼3100	STREP
CH_2 stretch (antisymm)	2926−2918	TER, B−PE, PC
CH_2 stretch (symm)	2853−2850	TER, B−PE, PC
C═O (ester)	1735	TER, B−PE, PC
amide I	1650	PLL, STREP
COO^- stretch (antisymm)	1602	ALG
amide II	1550	PLL, STREP
CH_2 bend (scissoring)	1456	TER, B−PE, PC
COO^- stretch (symm)	1402	ALG
PO_2^- stretch (antisymm)	∼1263−1245	B−PE, PC (stronger)
C−O−C stretch (antisymm)	∼1225−1217	TER, B−PE, PC, GLY
C═O−O−C stretch (antisymm)	∼1171	TER, B−PE, PC
C═O−O−C stretch (symm)	∼1100	TER, B−PE, PC
PO_2^- stretch (symm)	∼1090	B−PE, PC (stronger)
C−O−C stretch (symm)	∼1040−1030	TER, B−PE, PC, GLY
$(CH_3)_3N^+$ bend (antisymm)	975	PC
$(CH_3)_3N^+$ bend (symm)	925	PC
P−O stretch (symm)	816	B−PE, PC

[a] Abbreviations: antisymm, antisymmetric; symm, symmetric; PLL, poly-L-lysine; ALG, alginate; TER, poly(HEA_6−AOD_3−SSS_1) terpolymer; B−PE, biotin−mono-acrylPE; PC, mono-acrylPC; STREP, streptavidin; GLY, glycopolymer.

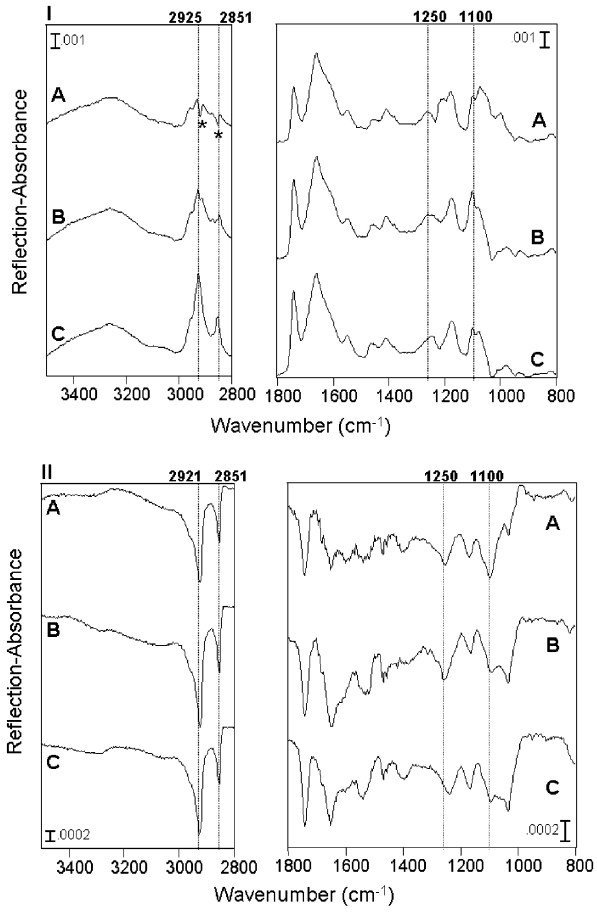

Figure 3. Parallel (R_p) (I) and perpendicular (R_s) (II) polarized external reflection infrared spectra of polymerized 10 mol % biotin−PE/PC (A), 25 mol % biotin−PE/PC (B), and 50 mol % biotin−PE/PC (C) films. IR bands labeled with an asterisk in R_p polarized spectra indicate CH_2 absorption modes that are changing in intensity with increasing biotin−PE content as shown in part I, spectra A−C.

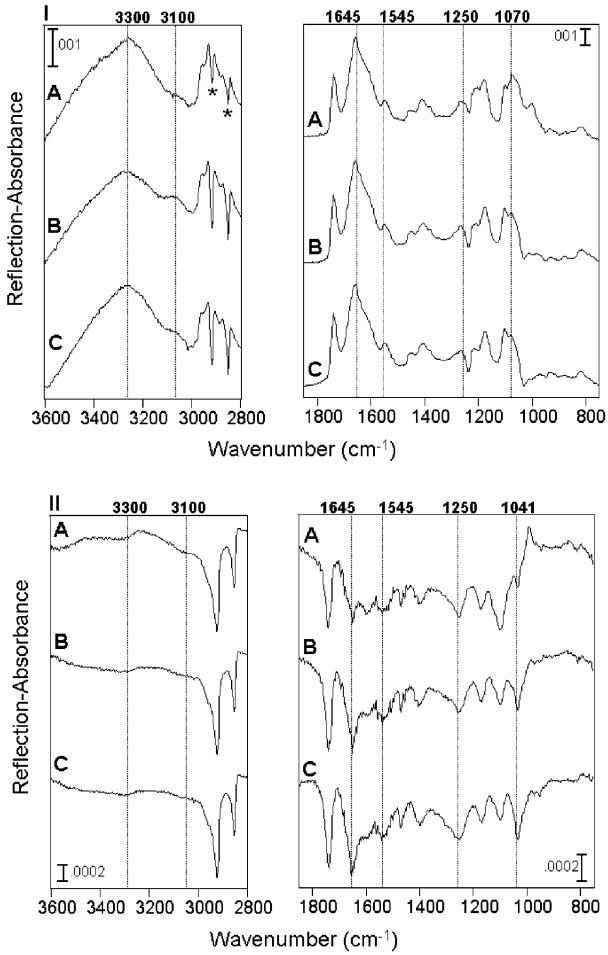

Figure 4. Parallel (R_p) (I) and perpendicular (R_s) (II) polarized external reflection infrared spectra of a polymerized 10 mol % biotin−PE/PC film (A) and spectra following successive absorption of streptavidin (B) and a glycopolymer (C). IR bands labeled with an asterisk in R_p polarized spectra indicate CH_2 absorption modes that are changing in intensity after streptavidin binding to the biotin−PE-functionalized surface as shown in part I, spectra A and B.

Fabrication of a Glycocalyx-Mimetic Surface using Biotin−Streptavidin Coupling to a Supported Lipid Film. We have demonstrated in prior reports that membrane-mimetic films that 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.[10] Significantly, an alkylated terpolymer, electrostatically coupled to a polyelectrolyte multilayer, allows film fabrication on a hydrophilic cushion that facilitates the incorporation of transmembrane proteins. In this investigation, mixed vesicles, comprised of polymerizable lipids with either biotin or phosphatidylcholine headgroups, were fused onto an alkylated terpolymer bound to a poly-L-lysine/alginate multilayer. Streptavidin and subsequent glycopolymer coating of the polymerized lipid film was then performed to produce a glycocalyx mimic.

Contact angles were measured at various stages for films containing 10, 25, or 50 mol % biotin−PE/PC lipids (B−PE/PC) (Figure 2). In agreement with prior studies, advancing contact angles for the ALG/PLL multilayer were low (35°) and increased after the addition of the terpolymer (105−109°).[10] Contact angles for films composed solely of lipids containing a phosphatidylcholine headgroup char-

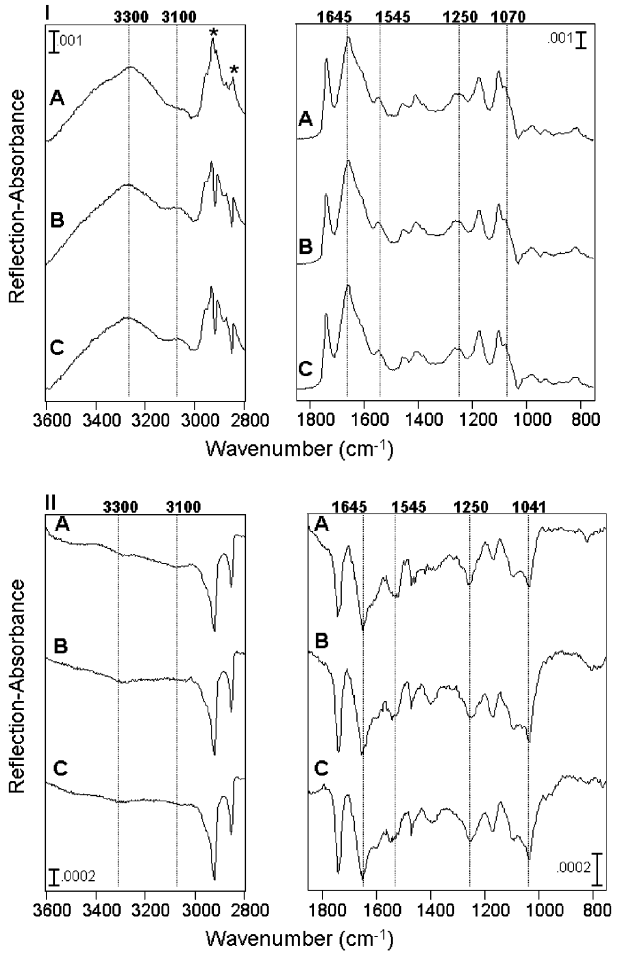

Figure 5. Parallel (R_p) (I) and perpendicular (R_s) (II) polarized external reflection infrared spectra of a polymerized 25 mol % biotin−PE/PC film (A) and spectra following successive absorption of streptavidin (B) and a glycopolymer (C). IR bands labeled with an asterisk in R_p polarized spectra indicate CH_2 absorption modes that are changing in intensity after streptavidin binding to the biotin−PE-functionalized surface as shown in part I, spectra A and B.

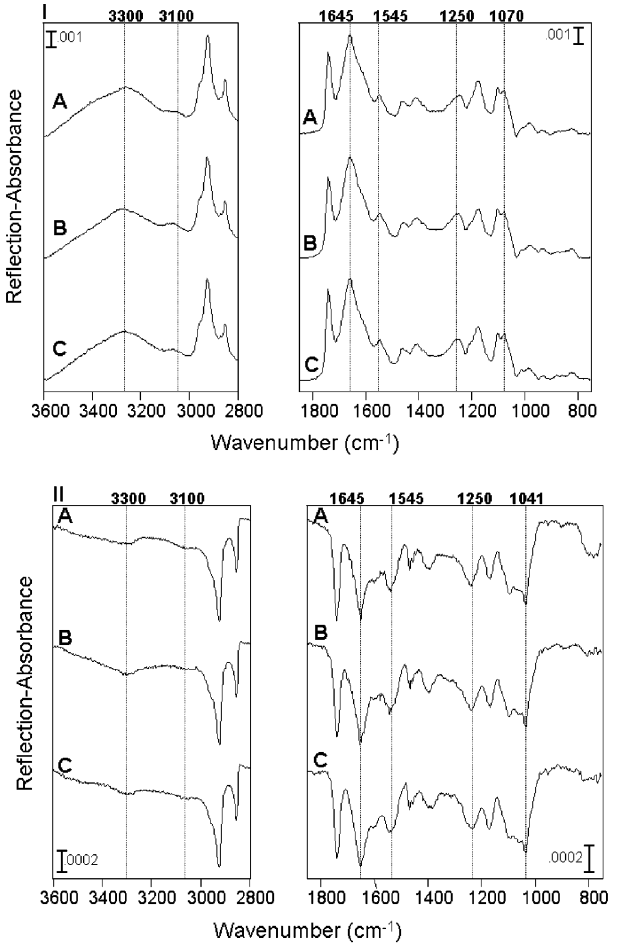

Figure 6. Parallel (R_p) (I) and perpendicular (R_s) (II) polarized external reflection infrared spectra of a polymerized 50 mol % biotin−PE/PC film (A) and spectra following successive absorption of streptavidin (B) and a glycopolymer (C).

acteristically range between 50 and 60°.[9,10,24,25] The addition of biotinylated lipids, however, produced a small but noticeable increase in film hydrophobicity, with contact angles of 51, 59, and 77° for films containing 10, 25, and 50 mol % of biotin−PE, respectively.

Advancing contact angles were unchanged upon addition of streptavidin or glycopolymer to 10 or 25 mol % biotin−PE/PC films. A reduction in film hydrophobicity from 77° to 65° was observed after incubation of the 50 mol % biotin−PE/PC sample with streptavidin. Glycopolymer coating, even at high surface binding density, was not associated with further reduction in contact angle.

Structural Characterization of a Glycocalyx-Membrane-Mimetic Film Using Polarized Infrared External Reflection Spectroscopy. *The Effect of Increasing Biotin−PE Film Concentration.* Polarized external reflection infrared spectra were acquired during each stage of film construction in order to identify functional group characteristics and induced structural changes unique to each film component. Specifically, infrared spectroscopy provides detailed information regarding hydrocarbon chain conformation and order and, by using polarized spectra, molecular orientation. The level of disorder may influence film properties, such as diffusion across a lipid membrane, as well as the orientation and assembly of membrane-associated proteins. Characteristic IR absorption bands of the component structures of the fabricated glycocalyx-mimetic film are summarized in Table 1.

Varying the surface concentration of biotin−PE yields several changes in R_s and R_p spectra (Figure 3). On increasing biotin−PE content from 10 to 25 mol %, a decrease is observed in the combination of symmetric C═O−O−C and PO_2^- stretches at ∼1100 cm^{-1} in the R_s polarized spectrum (Figure 3II, A vs B). In addition, increasing biotin−PE composition is associated with a broadening of the antisymmetric PO_2^- headgroup band at 1250 cm^{-1} that appears to shift to a lower wavenumber in both R_p and R_s polarized spectra (Figure 3I,II, A−C). Reference ATR-IR spectra of acrylate-PC and biotin−acrylate-PE in powder form demonstrate that both compounds possess PO_2^- antisymmetric (∼1245 cm^{-1}) and symmetric (1090 cm^{-1}) stretches, although the intensities of these bands are significantly reduced in biotin−PE. Thus, it is likely that the observed reduction in intensity at 1100 cm^{-1} in the R_s polarized spectra results from a direct change in film composition (i.e., a reduction in acrylate-PC). Likewise, the shift and broadening of the band at ∼1250 cm^{-1} to a lower wavenumber is due to both a reduction of the PO_2^- antisymmetric stretch and a simultaneous increase of the C−O−C stretch at 1217 cm^{-1}.

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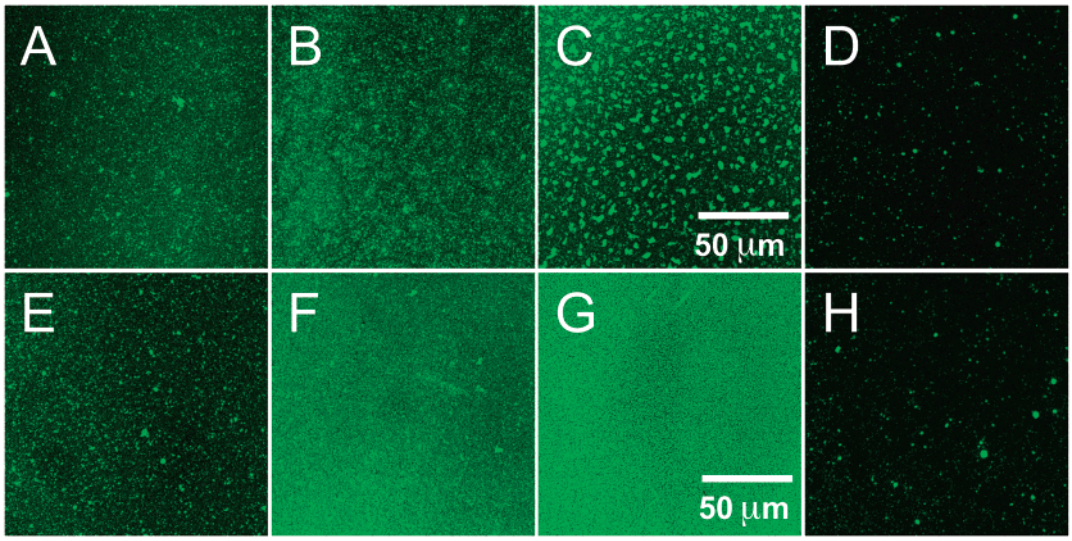

Figure 7. Confocal fluorescence microscopic images of FITC−streptavidin bound to films comprised of 10 mol % B−PE/PC (A), 25 mol % B−PE/PC (B), 50 mol % B−PE/PC (C), and PC only (D). Confocal fluorescence microscopic images of FITC-labeled lectin bound to glycopolymer-coated surfaces produced on films of 10 mol % B−PE/PC (E), 25 mol % B−PE/PC (F), 50 mol % B−PE/PC (G), and PC only (H) films. All images were acquired using a 63× oil immersion lens.

The most notable difference in the polarized IR spectra on varying biotin concentration occurs in the ν_a and ν_s CH_2 stretching modes from 3000 to 2800 cm^{-1} of the R_p polarized spectra, which shift from negative to positive with increasing biotin−PE concentration (Figure 3I, A−C). The observation of positive and negative absorption bands in polarized spectra has been previously reported in theoretical and experimental studies of monomolecular thin films coated onto silicon substrates.[26−29] We have also observed this behavior in polymeric lipid films deposited onto OTS/Si[9] and onto $(PLL/ALG)_5$−PLL−poly-$(HEA_6−DOD_3−SSS_1)$ terpolymer/Si[10] coated substrates. Using the Fresnel reflection equations for a three-phase system,[26,28,30,31] we correlated, qualitatively and quantitatively, the positive and negative methylene absorption modes with the molecular orientation of the alkyl chains in the OTS, terpolymer, and supported lipid monolayers.[9,10] From these investigations, we correlated the positive intensity of the R_p polarized ν_a and ν_s CH_2 stretching modes with a random alkyl chain orientation that is tilted away from the surface normal and negative CH_2 stretching modes with more ordered alkyl chain packing. In this regard, the negative CH_2 absorption bands observed in the 10 mol % B−PE/PC film (Figure 3I, A) are indicative of ordered alkyl chains. An ordered alkyl chain orientation is also supported by the frequency positions of the methylene stretching modes, which can be utilized to assess hydrocarbon chain order.[32−34] The ν_a and ν_s CH_2 positions of 2919 and 2851 cm^{-1}, respectively, indicate that the alkyl chains are only slightly disordered and possess a predominance of trans conformers.

Increasing the concentration of biotin−PE induces alkyl chain disorder. Specifically, in films containing 25 mol % of biotin−PE, the intensity of methylene absorption modes shifts from negative to positive, indicating an increase in random chain orientation (Figure 3I, B). In support of this notion, the ν_a CH_2 band also increases from 2919 to 2920 cm^{-1}. Further, in films containing 50 mol % of biotin−PE (Figure 3I, C), methylene bands are positive and the ν_a CH_2 band shifts to 2925 cm^{-1}, consistent with disordered hydrocarbon chains containing large numbers of gauche conformers.

Binding of Streptavidin and Glycopolymer to Biotin-Functionalized Surfaces. Polarized IR spectra obtained after streptavidin binding are presented in Figures 4−6, spectra B. The presence of streptavidin is represented by the appearance of amide A (∼3300 cm^{-1}) and amide B (∼3100 cm^{-1}) bands. An additional observation is the increase in the negative intensity of the R_p polarized methylene absorption bands for both the 10 and 25 mol % B−PE/PC films that may represent induced alkyl chain orientation. Further investigations using deuterated components are ongoing in order to fully define these events. No appreciable changes were noted in IR spectra after glycopolymer binding (Figures 4−6, spectra C).

Fluorescence Imaging of Streptavidin and Glycopolymer Bound onto Membrane-Mimetic Films. Confocal fluorescence images obtained after incubation of biotin-derivatized films with FITC-labeled streptavidin are presented in Figure 7A−C. As expected, surface fluorescence increases with increasing concentrations of biotin−PE. Of interest, microdomains of FITC-labeled streptavidin appear on films containing 50 mol % biotin−PE and may indicate a clustering of biotin−PE. It is possible that the inhomogeneity of the observed fluorescence is related to microphase separation of the PC and biotinylated lipids. Moreover, it is also plausible that increasing alkyl chain disorder along with a small but noticeable increase in film hydrophobicity may be indicative of incomplete vesicle fusion at high biotin−PE surface concentrations. Additional investigations are ongoing to further address this possibility.

Fluorescence images after incubation with FITC-labeled lectin are presented in Figure 7E−G. Notably, glycopolymer-modified surfaces demonstrate a much greater degree of homogeneous fluorescence at 25 and 50 mol % biotin−PE labeling when compared to the corresponding strepta-

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vidin-decorated surfaces. Undoubtedly, this can be attributed to the large amplification of lectin binding sites over the film surface due to the presence of several biotin binding sites on each streptavidin molecule, as well as the availability of approximately 10 pendant lactose units per glycopolymer chain. Relatively little nonspecific lectin or streptavidin binding was noted on membranes composed of phosphatidylcholine lipids alone (Figure 7D,H).

Conclusions

The fabrication of glycocalyx-mimetic surfaces was achieved by designing a biotin chain-terminated glycopolymer, which was incorporated onto streptavidin-functionalized polymeric lipid films. By variation of carbohydrate type and density, the production of structurally heterogeneous surfaces is facilitated. Significantly, increasing the surface concentration of biotinylated lipids was associated with some increase in hydrocarbon chain disorder and the formation of biotin microdomains. Nonetheless, the size and hydrophilicity of the bound glycopolymer chains led to the generation of a uniform carbohydrate surface coating on a membrane-mimetic thin film.

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