pH-dependent lipid vesicle interactions with plasma polymerized thin films

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Model lipid vesicle and supported lipid bilayer (SLB) systems are used in a variety of applications including biosensing, cell membrane mimics, and drug delivery. Exposure of a surface to a vesicle solution provides a straightforward method for creating such systems via vesicle adsorption and collapse. However, this process is complex and the relationship between the surface physicochemical properties and vesicle collapse is poorly understood. Plasma polymers are thin conformal films that can be applied to a variety of materials to modify surface properties. This paper uses quartz crystal microbalance with dissipation and fluorescence recovery after photobleaching (FRAP) to explore lipid vesicle interactions with plasma polymerized acrylic acid (ppAAc), allylamine (ppAAm), and ppAAc/ppAAm micropatterns. Vesicle interactions were dependent on plasma polymer chemistry and pH of the buffer solution. Vesicles readily and stably adsorbed to ppAAm over a wide pH range. ppAAc demonstrated limited interactions at pH 7 and vesicle adsorption at pH 4. Vesicle collapse and SLB formation could be induced using a pH change. FRAP was used to explore the fluidity of the lipid structures on both the patterned and unpatterned plasma polymer films. On ppAAm/ppAAc micropatterns, pH transitions combined with the presence of chemically distinct regions on the same substrate enabled immobile lipid islands on ppAAc to be surrounded by fluid lipid regions on ppAAm. This work demonstrates that plasma polymer films could enable spatially controlled vesicle adsorption and SLB formation on a wide variety of different substrates. © 2017 American Vacuum Society. [<http://dx.doi.org/10.1116/1.4984261>]

I. INTRODUCTION

The cell membrane encases and protects cellular components and therefore plays an important role in transport, signaling, and disease. The complexity of these processes has driven the demand for model systems capable of mimicking cell membrane environments. Two such systems are supported lipid bilayers (SLBs) and adsorbed vesicle layers. These models are ideal candidates for the development of high throughput sensing technologies, which are required in the fields of biotechnology and medicine. Furthermore, the three-dimensional structure of vesicles makes them useful for applications such as encapsulation and drug release¹ and the development of artificial tissues.²

A simple method for generating vesicle layers and SLBs is via the exposure of a substrate to a solution containing lipid vesicles. Under specific conditions, the vesicles will

adsorb to the surface and in some cases will collapse spontaneously forming a bilayer. Traditionally, a limited number of surfaces have reliably promoted the formation of fluid lipid bilayers including SiO₂, glass, and mica.^{3–5} The interactions governing this process are complex and in some cases poorly understood.³ However, the need for increasingly complex cell membrane models for a wide range of applications including biosensing⁶ has driven demand for surfaces that will support both vesicle attachment and induce collapse to form SLBs. A variety of experimental conditions affect the adsorption and collapse processes of lipid vesicles on surfaces.^{3,7} Electrostatic interactions play an important role, and therefore, pH and ionic strength have emerged as useful tools to regulate lipid vesicle interactions with surfaces. On TiO₂, pH has been used to promote either vesicle adsorption at intermediate pH or bilayer formation at low pH.⁸ Vesicle collapse has also been promoted at low pH on polymer cushions.⁹

Plasma polymers are thin films (<100 nm) deposited at low pressure onto almost any solid substrate in a one-step, dry process. A variety of monomers and deposition parameters can be used to control surface functionality. One of the

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major advantages of plasma polymers is that they can be used to modify the surface properties of a wide variety of substrates from gold and silica through to polymers.¹⁰ It is therefore surprising that there is little literature exploring the interactions of lipid vesicles with plasma polymerized surfaces. Plasma polymerized maleic anhydride has been used previously to immobilize vesicles in the development of smart wound dressings for pathogenic bacteria detection¹¹ and as a surface modification for both supported lipid bilayers¹² and adsorbed vesicle layers.¹³ One of the challenges with maleic anhydride is the limited shelf stability due to the reactivity of the chemistry. There are a wide range of other plasma polymer chemistries currently used in biomaterials and biomedical applications that could be used to provide specific chemical reactivity and flexibility for lipid immobilization.

The ability to spatially control the presentation of lipids on surfaces is also of significant interest in the research and development community. Typical patterning methods include chromium¹⁴ and protein¹⁵ barriers to control lipid diffusion, photochemical patterning of silanes,¹⁶ dip-pen lithography placement of lipid vesicles,¹⁷ and a range of grafted polymer pattern approaches.¹⁸ It is also possible to spatially control plasma polymers using a range of techniques including physical and photolithographic processes.^{19,20} This not only enables more than just physical barriers to be placed within the SLB but also opens up the opportunity for placing the same patterned surface chemistry onto virtually any substrate. This independence of the substrate makes plasma polymers particularly interesting platforms for exploring vesicle adsorption and collapse as well as the spatial control of membrane-mimetic architectures for a wide variety of technology and analysis platforms.

In this work, we explore the use of pH to control lipid vesicle interactions with commonly used plasma polymers to produce novel surfaces capable of controlling lipid vesicle adsorption and SLB formation. Plasma polymerized acrylic acid (ppAAc) and allylamine (ppAAM) films were selected for this study as they are well established and well characterized systems capable of creating acid^{21,22} and amine-rich^{23–25} surface chemistries. Quartz crystal microbalance with dissipation (QCM-D) was used to observe the lipid vesicle interactions with the plasma polymers, while x-ray photoelectron spectroscopy (XPS) and contact angle were used to verify the surface chemistry and hydrophilicity, respectively. Photolithography lift-off techniques were then used to pattern the two plasma polymers, and the chemistry was confirmed using time-of-flight secondary ion mass spectrometry (ToF-SIMS) imaging. The spatial control of both vesicle adsorption and lipid structure on ppAAc/ppAAM patterned surfaces was studied using fluorescence recovery after photobleaching (FRAP). The results show that by varying the pH, it was possible to control lipid vesicle interactions on ppAAc and ppAAM surfaces and induce the formation of SLBs under selected conditions. These outcomes demonstrate the versatility of plasma polymers as coatings for the

wide range of substrates used in lipid arrays and other biotechnology applications.

II. MATERIALS AND METHODS

A. Materials

Acrylic acid (99% purity) was purchased from Sigma-Aldrich, USA. Allylamine (98+%, extra pure) was purchased from Acros Organics, USA. 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (14:0 NBD PE), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-N-(lissamine rhodamine B sulfonyl) (ammonium salt) (14:0 Liss Rhod PE) were supplied in powder form by Avanti Polar Lipids Inc., USA. QCM-D crystals (QSX301 gold coated quartz, 100 nm Au) were purchased from Q-Sense, Sweden. Silicon wafers (orientation (1-0-0), thickness 500–550 μm , single side polished, resistivity 1–10 Ωcm) were purchased from Micro Materials and Research Consumables Pty Ltd, Australia. Plain circular No. 5 19 mm diameter glass coverslips were purchased from ProSciTech Pty Ltd, Australia. Isopropyl alcohol (IPA, 99.5% purity) and acetone (99.8% purity) were purchased from Chem-Supply Pty Ltd, Australia. Decon 90 (Decon Laboratories Limited, UK) and Milli-Q grade water (18.2 $\text{M}\Omega\text{-cm}$, Millipore, Australia) were used for the purpose of cleaning. Tris(hydroxymethyl)aminomethane (trometamol) was purchased from Merck, USA. Sodium chloride was purchased from Riedel-de Haën, Germany. All buffers were prepared using Milli-Q grade water (18.2 $\text{M}\Omega\text{cm}$, Millipore, Australia) and pH-adjusted using 1 M HCl and NaOH as required. Both photoresist AZ1505 and developer AZ726 were purchased from Micro Materials and Research Consumables Pty Ltd, Australia.

B. Surface preparation

Silicon wafers and glass coverslip substrates were cleaned using the following sonication steps: 2% Decon 90 (30 min), Milli Q water (10 min), and IPA (10 min). Substrates were then rinsed with fresh IPA and dried under a stream of nitrogen before being treated in a UV Ozone Cleaner (Bioforce Nanosciences, Inc., USA) for 10 min. Gold QCM-D crystal sensors were wet cleaned in a heated solution of 30% ammonia, 30% hydrogen peroxide, and Milli-Q water (18.2 $\text{M}\Omega\text{-cm}$) at a volume ratio of 1:1:5, respectively, for approximately 10 min or until vigorous bubbling could be seen on the surface of the crystals. Following submersion in Milli-Q water, the crystals were rinsed with IPA before being dried using a flow of nitrogen. All crystals were subjected to a 10 min ozone treatment before use.

C. Plasma polymerization

Plasma polymerization was performed in a stainless steel T-piece reactor with an internal aluminum disk electrode (17 cm in diameter). The plasma was ignited using a 13.56 MHz radio frequency power source generator (Coaxial Power Ltd, UK) coupled to the internal disc electrode by

means of an impedance matching network. Prior to coating, monomers were degassed using a minimum of three freeze-thaw cycles. The monomer flow rate was controlled using a fine (CMV-VFM-2-P-KK) and medium (CMV-VFM-3-P-KK) flow needle valve (Chell Instruments Ltd, UK) for the allylamine and acrylic acid monomers, respectively. The internal reactor pressure was measured using a Pirani Gauge (Edwards, UK). An aluminum sample stage was used to elevate samples within the reactor chamber to a height of 4.35 cm. The substrates were positioned 3 cm away from the electrode, which was perpendicular to the horizontal stage. Prior to coating, the chamber was pumped down to a base pressure of 1×10^{-3} mbar and the plasma was ignited at a pressure of around 1.2×10^{-2} mbar. All coatings were deposited for 20 min at a deposition power of 20 W and a flow rate of 1.5 standard cubic centimeters per minute (sccm).

D. Contact angle

The sessile drop method was used to measure the static contact angles of Milli-Q water on plasma polymerized films deposited onto silicon wafers. Drops were deposited onto each sample using an FTA1000 instrument (First Ten Ångströms Inc.). Images were captured using a Pelco Model PCHM 575-4 camera and analyzed using a spherical fit in the FTA Windows Mode 4 software.

E. Ellipsometry

Film thicknesses were determined using an M-2000XI[®] Spectroscopic Ellipsometry system (J.A. Woollam Co., Inc.) at ambient temperature. Measurements were taken at 60°, 65°, 70°, and 75° over wavelengths of 210–1650 nm. The data were fitted with a B-Spline model on top of a silicon substrate with a native oxide layer using COMPLETE[®] software version 4.92 (J.A. Woollam Co., Inc.). The mean squared error was typically less than 10.

F. XPS

XPS spectra were collected using a Kratos Axis Nova instrument (Kratos, Manchester, UK) with a monochromated Al K_α source (Source energy 1486.69 eV) at a power of 150 W. Identification of elements was carried out using survey spectra collected at a pass energy of 160 eV and a 1 eV step size. Coating chemistries were investigated using high resolution spectra collected at a pass energy of 20 eV and a step size of 0.1 eV. Data were converted into VAMAS format and analyzed using CASAXPS software, version 2.3.15 (CASA Software Ltd., UK). Charge correction was applied by placing the hydrocarbon peak at a binding energy of 285 eV. The chemical shifts related to specific functional groups were taken from Beamson and Briggs Database of Polymer XPS spectra²⁶ unless otherwise stated. Instrument specific sensitivity factors were used for quantification.

G. Vesicle preparation

DOPC powder was dissolved by the addition of chloroform in a glass vial to a concentration of 25 mg/ml. For the FRAP studies, DOPC lipids were doped with 1 wt. % 14:0 NBD PE (green) or 1 wt. % 14:0 Liss Rhod PE (red). DOPC/chloroform solution of 200 μ l was left to evaporate completely in the fume hood to form a lipid cake on the base of a glass vial. Residual chloroform was removed by drying in a vacuum desiccator for a minimum of 2 h. For consistency, the stock vesicle solutions were made by hydrating the lipid cake using 10 mM Tris solution (pH 6.8–7.3) to a concentration of 5 mg/ml. The mixture was vortexed until the cake was fully hydrated. Extrusion was conducted mechanically by passing the lipid mixture through 100 nm polycarbonate filters (Whatman, UK) using either an Avanti Mini-Extruder (Avanti Polar Lipids Inc.) or a Liposofast-Basic (Avestin Inc., Canada) with 21–31 passes. Stock vesicle solutions were stored at 4 °C for no longer than one week. Immediately before use, the stock vesicles were diluted to a final concentration of 0.5 mg/ml in 10 mM Tris + 150 mM NaCl buffer at the required pH. This strategy was used to form a difference in salt concentration across the wall of the vesicle, inducing an osmotic stress and thus lowering the barrier for vesicle rupture. It has been observed that the presence of an increased salt concentration on the outside of the vesicle (as opposed to inside the vesicle) has the greatest effect on lowering the critical coverage required for vesicle collapse on SiO₂.³

H. QCM-D

QCM-D measurements were conducted using a Q-Sense E4 instrument (Q-Sense, Sweden). Freshly cleaned or plasma coated sensor crystals were placed in the measurement chambers, and prior to the measurement period, resonant frequencies (overtones 3, 5, and 7) for all crystals were measured in air and then in liquid. Data from the fifth overtone were used for comparison. The temperature of the QCM-D chamber was set to 37 °C with a constant flow rate of 50 μ l/min. Once the baseline for both frequency and dissipation had stabilized in Milli-Q water, the buffer and vesicle solutions were introduced into the measurement chambers.

I. Photolithography

Dual plasma polymer patterns were prepared using photolithography, as previously described.^{19,20} Positive photoresist AZ1505 was spin coated onto ppAAm plasma coated (20 W, 1.5 sccm, 20 min) round glass coverslips at 8000 rpm. Coverslips were heated to 200 °C for 3 min to harden the resist and remove any residual solvent. Substrates were then brought into contact with a chromium photomask (Bandwidth Foundry International, ANFF, Sydney Australia) and exposed to ultraviolet radiation using a Series 200 mask aligner (OAI) for 2–3 s depending on the measured light intensity. Exposed regions were removed via immersion in AZ726 developer solution for 6 s. Samples were rinsed with Milli-Q water

before drying in a stream of N₂ gas. Following ppAAc deposition (20 W, 1.5 sccm, 20 min), the underlying photoresist was removed using sonication in acetone for 3 min.

J. Optical profilometry

Samples were first coated with 10 nm of Au using an AXXIS instrument (Kurt J. Lesker). Micropatterned topographies were characterized using a Contour GT-K1 3D Optical Profiler (Bruker, USA). Imaging was performed using phase shifting interferometry (PSI) with a 50× objective. Images were flattened by applying a Terms mask using the VISION software (v.4.20, Bruker, USA). Further image preparation and processing was conducted using PHOTOSHOP CS6 (Adobe) and IMAGE J (v.1.45s, National Institutes of Health).

K. Atomic force microscopy

Topography images were obtained using a FastScan atomic force microscope (AFM) (Bruker, USA) under ambient conditions in air. The quantitative nanomechanical mapping (PeakForce QNM) mode was used together with ScanAsyst-Air cantilevers (nominal spring constant, 0.4 N m⁻¹). Images were flattened before analysis using the NANOSCOPE ANALYSIS software (v.1.40, Bruker, USA). The height measurements of the individual ppAAc patterned features were measured using the depth analysis tool in the NANOSCOPE ANALYSIS software, and the average was calculated from all the features in the image.

L. ToF-SIMS imaging

ToF-SIMS images of the plasma polymer micropatterns on glass were collected using an ION-TOF ToF-SIMS IV instrument (ION-TOF GmbH, Germany). The analysis chamber was typically held at $\sim 1.9 \times 10^{-10}$ mbar. Positive ion images were acquired for all the samples. Images were collected in the spectroscopy mode using a Bi₃⁺⁺ ion source at a kinetic energy of 50 keV over a 100 × 100 μm scan area (256 × 256 pixel). Before analysis, the positive ion spectra were calibrated using the CH₃⁺ (m/z 15.0229), C₂H₃⁺ (m/z 27.0229), C₃H₅⁺ (m/z 41.0385), and C₇H₇⁺ (m/z 91.0542) peaks.

Multivariate analysis of ToF-SIMS image data was performed using MATLAB R2012b (MathWorks, Inc.), NBToolbox, and ImageGUI (NESAC/BIO, The University of Washington). NBToolbox can be accessed from the following link: <http://www.nb.uw.edu/mvsa/multivariate-surface-analysis-homepage>.

Raw measurement (.itm) files were opened in Surface Lab 6.4 (ION-TOF GmbH, Germany). Peaks with an intensity greater than 100 counts, over the mass range typically between m/z 0 and 100, were selected to create a peak list. The integration limits of each peak were checked to ensure that suitable areas were assigned to each peak. The peak list was then used to reconstruct ToF-SIMS image data which were exported as a .bif6 file. The .bif6 file was exported into the ImageGUI of the NBToolbox operating within MATLAB. Before performing multivariate analysis, data were normalized to total ion counts for each spectrum/image

and mean centered using the ImageGUI as described previously.²⁰

M. Confocal laser scanning microscopy and fluorescence recovery after photobleaching

Dual 100 μl chamber Ibidi Culture Inserts (ibidi GmbH, Germany) were mounted on the top of plain or patterned plasma polymer coated glass No. 1 coverslips. For plain plasma polymer films, 10 mM Tris + 150 mM NaCl buffer (pH dependent on experiment) was first incubated on the surface for 10 min before applying the vesicle solution (diluted to 0.5 mg/ml immediately before use with 10 mM Tris + 150 mM NaCl buffer of appropriate pH for the experiment) for 10 min. The individual 100 μl chambers were then rinsed by removing 50 μl of the solution and replacing it with 50 μl of fresh buffer solution of appropriate pH ten times before imaging. For the ppAAc/ppAAm micropatterned surfaces, the following steps were followed as illustrated in Fig. 1: 10 mM Tris + 150 mM NaCl buffer, pH 7 (incubate 10 min) → 14:0 NBD PE doped vesicle solution, pH 7 (incubate 10 min) → 10 mM Tris + 150 mM NaCl buffer, pH 7 (rinse then image) → 10 mM Tris + 150 mM NaCl buffer, pH 4 (rinse) → 14:0 Liss Rhod PE doped vesicle solution, pH 4 (incubate 10 min) → 10 mM Tris + 150 mM NaCl buffer, pH 4 (rinse then image) → 10 mM Tris + 150 mM NaCl buffer, pH 7 (rinse then image). Confocal microscopy imaging with FRAP was conducted using an FV1000 IX81 confocal microscope (Olympus, Japan) using a 40× oil immersion lens (UPLAPO, NA 1.00, Olympus, Japan) and a 300 μm pinhole. Images were collected using FLUOVIEW software (v. 1.7c, Olympus, Japan) and analyzed using FLUOVIEW viewer (v. 4.0.a, Olympus, Japan). Areas were bleached using the Tornado scan setting at 100 μs/pixel, and images were typically collected using a fast scan speed (2.0 μs/pixel) to minimize bleaching of the imaging area over time.

Unpatterned plasma polymer substrates were incubated with DOPC vesicles doped with 1 wt. % 14:0 NBD PE, and excitation was conducted using a 488 nm laser which was used for both bleaching (100% laser intensity, 40 s) and imaging (2% laser intensity). Substrates incubated with DOPC vesicles doped with 1 wt. % 14:0 Liss Rhod PE were excited using a 543 nm laser which was used for both bleaching (100% laser intensity, for 60 s) and imaging (4% laser intensity). For unpatterned plasma polymer surfaces, the bleach spot was kept constant throughout (diameter, ~ 50 μm). Line profiles were generated from exported greyscale images using IMAGE J software (v.1.45 s, National Institutes of Health, USA).

For patterned substrates, areas were bleached using both the 488 and 543 nm laser simultaneously (100% intensity, 60 s). Images were then collected sequentially by first exciting using the 488 nm laser (2% intensity) and then using the 543 nm laser (2% intensity). Images were typically collected every minute and up to 10 min after the initial bleach. Recovery kinetics of the lipids was analyzed using the simFRAP plugin of IMAGEJ.²⁷ The analysis algorithm

FIG. 1. (a) Illustration showing the structure of the ppAAc/ppAAm patterned surfaces. (b) Illustration of the steps of the lipid patterning protocol used to deposit 1 wt. % 14:0 NBD PE (green) and 1 wt. % 14:0 Liss Rhod PE (red) doped DOPC vesicles onto the ppAAc/ppAAm patterned surfaces and the collection points for the fluorescence imaging and FRAP experiments.

simulates the 2D random diffusion of fluorescent probes and compares this with the experimental FRAP data.

III. RESULTS AND DISCUSSION

A. Characterization of plasma polymer thin films and patterns

Ellipsometry, XPS, and contact angle analysis were used to characterize the plasma polymer film properties prior to exposure to the lipid solutions. As shown in Table I, variations in the film thicknesses demonstrated that the deposition rate of ppAAc was almost twice that of ppAAm. The ppAAc film was slightly more hydrophilic than the ppAAm, attributed to the higher oxygen content originating from the acrylic acid monomer. Although the allylamine monomer does not contain any oxygen, the ppAAm films showed low-level oxygen incorporation due to the rapid and well established oxidation from postdeposition atmospheric exposure.^{24,28} The surface chemistries determined by XPS indicated that the ppAAm and ppAAc film compositions were in line with the literature, with significant oxygen present in the ppAAc film and nitrogen incorporation in the ppAAm film.

The high resolution C1s spectra of ppAAm and ppAAc are shown in Fig. 2. High resolution C 1s spectra of the ppAAm films were fitted with four component peaks, accounting for the variety of chemical species known to be produced in plasma polymerization of ppAAm.²⁵ The ppAAc spectra were fitted with five component peaks associated with various carbon-oxygen species known to occur in these films including the indicative carboxylic acids and esters (COOH/R) at ~ 289.2 eV and β -shift associated with these species (C-COOH/R) at ~ 285.7 eV.

A 3D profilometry image of ppAAc micropatterned on ppAAm, fabricated using a photolithography mask consisting of $10\ \mu\text{m}$ circles separated by $10\ \mu\text{m}$ horizontal and vertical spacings, is shown in Fig. 3. The deposition of circular features approximately $10\ \mu\text{m}$ in diameter is clearly visible. Using the depth tool, heights of the individual features on the image were analyzed. An average feature height of 47 ± 1 nm for ppAAc patterned on ppAAm was observed. Both the optical profilometry and AFM analysis (Fig. S1)³⁷ demonstrated that the patterns of ppAAc on ppAAm were sometimes irregular and not fully circular in shape.

ToF-SIMS imaging of the patterned surfaces followed by principal component analysis (PCA) clearly showed the

TABLE I. Film thickness (as measured by ellipsometry), XPS atomic concentrations, XPS atomic ratios, and water contact angles for ppAAm and ppAAc films. The mean values ($\pm$ standard deviation) are calculated from three analyses per sample for XPS and ellipsometry and three individual samples from the same run (one analysis spot per sample) for the contact angle.

Surface	Film Thickness (nm)	Contact angle (deg)	XPS atomic concentration (%)			Atomic ratio	
			C 1s	O 1s	N 1s	O/C	N/C
ppAAm	30 ± 1	61 ± 1	84.6 ± 0.2	3.2 ± 0.2	12.0 ± 0.1	0.04	0.14
ppAAc	58 ± 1	50 ± 1	78.4 ± 0.1	21.2 ± 0.1	—	0.27	—

differentiation of the surface chemistry on the patterned surface as evident in the score image [Fig. 4(a)]. The loading plots from PC1 shown in Fig. 4(b) identify the positive loading peaks as nitrogen containing secondary ion fragments associated with the ppAAm base polymer layer. The negative loading plot contains various hydrocarbon and oxygen containing secondary ion fragments associated with the patterned ppAAc regions.²⁰ These results confirmed

that the lithographic processes were successful in producing defined plasma polymer patterns with distinct chemical properties.

B. Lipid interactions with plasma polymers at pH 7

To investigate DOPC lipid vesicle interactions with ppAAm and ppAAc plasma polymer films, QCM-D

FIG. 2. High resolution XPS C 1s spectra of (a) ppAAm and (b) ppAAc deposited 3 cm away from the electrode at 20 W at 1.5 sccm for 20 min.

FIG. 3. 3D optical profilometer image of 10 μm diameter ppAAc circles patterned on ppAAm.

experiments were initially conducted at pH 7. The plasma polymer coated QCM crystals were first hydrated in Milli-Q water to establish a stable baseline for both frequency and dissipation. The crystals were then exposed to 10 mM Tris solution (step 1) followed by 10 mM Tris with 150 mM NaCl solution (step 2). These two buffer concentrations were introduced in a stepwise manner to investigate the changes associated with the plasma polymer film properties at different ionic strengths. The vesicle solution was then introduced

(step 3) followed by a final buffer rinse (step 4). Time markers show the point at which the pump was started with each new solution. The delay in response is due to the solution traveling through tubing prior to entering the chamber. As illustrated in Fig. 5(a), at pH 7.0, the frequency changes observed when transitioning from Milli-Q water to 10 mM Tris and subsequently 10 mM Tris with 150 mM NaCl were less than -1 and -3 Hz, respectively, for the gold control surface. Both ppAAc [Fig. 5(b)] and ppAAm [Fig. 5(c)] demonstrated similar minor frequency changes.

For the gold sensor, the addition of the DOPC vesicle solution [Fig. 5(a), step 3] resulted in large changes in both frequency and dissipation with Δf and ΔD stabilising at -250 Hz and 48×10^{-6} , respectively. As vesicles adsorbed to the crystal, the mass of the lipid, buffer trapped inside the vesicles and any water associated with the vesicular layer, increased at the surface causing a decrease in resonant frequency. The transition from the highly rigid environment of the gold crystal to the highly viscous vesicular layer caused an increase in dissipation.²⁹ No changes were observed when rinsing with buffer solution, suggesting the presence of a stable vesicular layer at the gold surface, which is consistent with previous literature.^{3,29,30} For the ppAAc surface, the introduction of DOPC vesicles in step 3 [Fig. 5(b)] resulted in a small frequency change of ~ -6 Hz and dissipation change of $\sim 2 \times 10^{-6}$, indicative of sparsely adsorbed vesicles. Upon rinsing, the Δf and ΔD values began returning to prevesicle levels, indicating the gradual removal of lipid materials from the ppAAc surface and only weak

FIG. 4. PCA of ToF-SIMS images collected from ppAAc/ppAAm micropatterns deposited onto silicon wafer substrates. (a) Reconstructed PC 1 score image and (b) positive and (c) negative loadings plots for PC1. Images were $125 \times 125 \mu\text{m}$. The score color bar corresponds to secondary ion fragments with positive loading (bright) and negative loading (dark).

interactions between the lipid vesicles and ppAAc. In contrast, when the DOPC vesicles were introduced to the ppAAm surface, there were significant and rapid changes in both frequency ($\Delta f = -270$ Hz) and dissipation ($\Delta D = 44 \times 10^{-6}$). These values are indicative of a tightly packed layer of adsorbed vesicles at the surface.³ The vesicular layer was stable upon rinsing, suggesting a strong attraction between the lipid and nitrogen-rich ppAAm surfaces. At pH 7 each of the components in the system are expected to display different surface charge characteristics. ppAAc would be

negatively charged due to the dissociation of carboxylic acid groups in the film,¹⁰ while ppAAm would be positively charged due to protonation of the amine species in the film.³¹ The lipid DOPC head, despite being zwitterionic, exhibits an overall net negative charge.³² This suggests that the behavior observed was driven by electrostatic interactions, with vesicles being repelled by ppAAc and attracted to the ppAAm surfaces.

C. Lipid interactions with ppAAc and ppAAm at varying pH values

Previous studies on vesicle collapse on TiO₂ have shown that varying solution pH can be used to control vesicle/surface interactions and in some cases drive vesicle collapse.^{8,32} At pH 7, very weak interactions between DOPC vesicles and the ppAAc surface were observed; however, as ppAAc surfaces have a surface isoelectric point at pH 3–4.4,¹⁰ vesicle interactions at pH 4 were investigated. As shown in Fig. 6, a large mass increase was observed upon addition of the vesicle solution (step 3) with a frequency shift close to -290 Hz. A simultaneous increase in dissipation rising to over 50×10^{-6} was also observed. These changes can be attributed to the adsorption of a tightly packed and highly viscous vesicular layer on the surface, as previously observed with ppAAm at pH 7. Upon rinsing at pH 4, it was observed that the vesicular layer remained stable on the surface (step 4). The substrate was then rinsed a second time at pH 7 (step 5). This triggered a distinct spike in dissipation followed by a rapid decrease in ΔD which stabilized at $\sim 7 \times 10^{-6}$. The Δf values simultaneously increased stabilizing at $\Delta f = -30$ Hz, indicating substantial mass loss from the surface of the crystal.

The spike in dissipation following the pH 7 rinse solution entering the chamber (Fig. 6, step 5) suggested that there was a conformational change in the vesicle layer induced by the change in pH as this feature was not present on the control ppAAc surface without vesicles [Fig. S2(a)]. However, the resting dissipation of 7×10^{-6} was much greater than

FIG. 5. QCM-D frequency and dissipation plots at pH 7 for (a) untreated, (b) ppAAc coated, and (c) ppAAm coated gold sensors. Liquid additions: (1) 10 mM Tris, (2) 10 mM Tris + 150 mM NaCl, (3) vesicles in 10 mM Tris + 150 mM NaCl, and (4) 10 mM Tris + 150 mM NaCl. Tris of 10 mM (pH 6.8–7.3) and 10 mM Tris + 150 mM NaCl (pH 7.0–7.3).

FIG. 6. QCM-D frequency and dissipation plot for ppAAc coating when using a two-step pH technique. Liquid additions: (1) 10 mM Tris pH 4.0, (2) 10 mM Tris + 150 mM NaCl pH 3.9, (3) DOPC vesicles, (4) 10 mM Tris + 150 mM NaCl pH 3.9, and (5) 10 mM Tris + 150 mM NaCl pH 7.0.

the expected value of 0.2×10^{-6} for a planar bilayer.⁸ In order to confirm if this value was due to the presence of film swelling or the presence of some intact vesicles at the surface, a ppAAc control surface was exposed to identical solutions without vesicles in an adjacent chamber. There was little evidence of film swelling at pH 4 [Fig. S2(a), step 2], and upon addition of the 10 mM Tris 150 mM NaCl solution (without vesicles) at pH 7, the ppAAc coating swelled with $\Delta f = -15$ Hz, but there was little change in the dissipation [Fig. S2(a), step 3]. This suggested that the larger than expected dissipation value was associated with the presence of some intact vesicles at the surface of the ppAAc and not film swelling.³

For SiO₂ surfaces, lowering the pH has been observed to promote a one-step vesicle collapse process,⁸ but this was not observed at the ppAAc surface. This suggests that there needs to be a specific interaction available to induce collapse of the vesicle layer on the plasma polymers. At pH 4, both the surface and the lipid head group will be close to or neutral in charge.^{8,10} These conditions appear to have enabled vesicles to adsorb readily and pack at the ppAAc surface (Fig. 6, step 3), where it can be assumed that shorter range attractive forces such as van der Waals would dominate the interactions. Introduction of the pH 7 rinse caused significant changes to occur at the ppAAc surface. The exact mechanism is unclear; however, it was likely caused by increased electrostatic repulsion between the negatively charged ppAAc and lipid headgroups at pH 7.

FRAP experiments were conducted on the ppAAc surfaces where QCM-D data indicated the presence of vesicle collapse with some intact vesicles following a pH 4 to 7 transition. During a 10 min recovery period, the results indicated that the adsorbed lipid layer was immobile [Fig. S2(b)].

Having established that pH could be used to control lipid vesicle interactions on the ppAAc surface, it was then investigated if the same process could be used to induce rupture on the nitrogen-rich ppAAm surface. ppAAm exhibited a

large Δf indicative of film swelling upon introduction of 10 mM Tris + 150 mM NaCl at pH 3.7 (Fig. 7 step 2). The swelling occurred in two stages, with a slower initial stage lasting for around 5 min before a more rapid uptake of mass into the film with Δf reaching more than -200 Hz. The swelling behavior did not appear to inhibit vesicle adsorption onto the ppAAm surface, as shown by a large increase in dissipation and simultaneous reduction in Δf in step 3. The vesicles adsorbed strongly and packed tightly at the surface with Δf values increasing over -500 Hz and ΔD reaching 55 Hz before a rinse buffer at pH 7.3 entered the chamber (Fig. 7, stage 4). The rinse stage caused a slight frequency increase related to mass loss, with no associated change in dissipation, indicating that the vesicle layer remained intact (Fig. 7, step 4).

Given that the isoelectric point of ppAAm had previously been measured in the range of 8.3–9.2,¹⁰ a final QCM-D study was conducted at pH 10 to investigate vesicle interactions in a neutral charge regime. Increasing the pH to 10 did not cause any swelling of the plasma polymer film (Fig. 8 step 2) or affect the rapid adsorption of the vesicles onto the ppAAm film (step 3). Following addition of the vesicle solution, both the frequency and dissipation stabilized at -280 Hz and 50×10^{-6} , respectively. Rinsing with a pH 10 buffer (step 4) followed by solutions at pH 7.3 (step 5) and 3.7 (step 6) had little effect on the QCM-D responses with the vesicles remaining adsorbed to the surface, demonstrating stability on ppAAm over a large pH range. The drop in frequency upon rinsing at pH 3.7, by approximately -40 Hz, was attributed to swelling of the underlying plasma polymer layer as observed previously at low pH (Fig. 7 step 2).

The ready adsorption of vesicles on the ppAAm surface over this wide range of pH may be explained by the surface charge behavior of ppAAm. Unlike ppAAc, ppAAm remains positively charged until a pH in the range of 8.3–9.2 where it

Fig. 7. QCM-D frequency and dissipation plot for the ppAAm film with a two-step pH transition. Liquid additions: (1) 10 mM Tris pH 3.7, (2) 10 mM Tris + 150 mM NaCl pH 3.7, (3) DOPC vesicles, and (4) 10 mM Tris + 150 mM NaCl pH 7.3.

Fig. 8. QCM-D frequency and dissipation plot for the ppAAm film with a three-step pH transition. Liquid additions: (1) 10 mM Tris pH 10.1, (2) 10 mM Tris + 150 mM NaCl pH 10.0, (3) DOPC vesicles, (4) 10 mM Tris + 150 mM NaCl pH 10.0, (5) 10 mM Tris + 150 mM NaCl pH 7.3, and (6) 10 mM Tris + 150 mM NaCl pH 3.7.

FIG. 9. Fluorescence imaging and FRAP experiment conducted on the patterned surface of ppAAc circles on ppAAm after incubation with 1 wt. % 14:0 NBD PE and 99 wt. % DOPC (green) vesicles at pH 7. Confocal microscopy image of the surface immediately (a) before and (b) after bleaching (0 min) and (c) 10 min later. The scale bars are 20 μm in (a) and 10 μm in (b) and (c).

transitions into a neutral regime.¹⁰ The vesicle head group is expected to have a slightly negative surface potential at pH 4 becoming more negative as the pH increases to 10.³² Across the pH range investigated, favorable electrostatic interactions between the lipid and the surface would be expected. Using a pH change from 10 to 7 or 4 did not give rise to the same drastic changes in frequency and dissipation observed on ppAAc. Repulsion between the vesicles due to changes in surface potential was not sufficient to initiate collapse on ppAAm despite a high vesicle density at the surface. This suggested that the changes in f and D observed on ppAAc were initiated by interactions between the surface and the vesicles and not vesicle–vesicle interaction alone.

D. Vesicle interactions with ppAAc/ppAAm micropatterns

Having shown that different lipid vesicle interactions could be induced by different plasma polymer chemistries and buffer pH, the next step was to investigate if spatially controlling plasma polymer chemistry using patterning could produce different lipid structures on a single substrate.

As described in Sec. III C, stable vesicle layers could be formed on ppAAm under a variety of pH conditions. In contrast, ppAAc showed limited vesicle interactions at pH 7 and vesicle adsorption at pH 4. An experimental protocol was therefore devised to exploit this pH-dependent behavior to

FIG. 10. FRAP experiment conducted on the patterned surface of ppAAc circles on ppAAm after incubation with 1 wt. % 14:0 NBD PE and 99 wt. % DOPC (green) DOPC vesicles for 10 min at pH 7 followed by incubation with 1 wt. % 14:0 Liss Rhod PE, 99% DOPC (red) vesicles for 10 min. at pH 4. Confocal microscopy image of the surface immediately (a) before and (b) after bleaching (0 min) and (c) 10 min later. The scale bars are 20 μm in (a) and 10 μm in (b) and (c).

investigate spatially controlled lipid vesicle deposition on patterned surfaces of 10 μm diameter ppAAc circles on ppAAm (see Fig. 1 in the methods).

The ppAAc/ppAAm patterned surface was initially exposed to 1 wt. % 14:0 NBD PE, 99 wt% DOPC (green) vesicles at pH 7. As expected, the fluorescent lipid material could be imaged on the ppAAm patterned area but not on the ppAAc circles. A FRAP experiment was conducted on the surface to explore the fluidity of the adsorbed vesicles on the ppAAm surface at pH 7. As shown in Fig. 9(b), a bleach spot is clearly visible immediately after exposure (0 min). Lipid mobility was then monitored by imaging the area every minute throughout a 10 min recovery period. Unexpectedly, fluorescence recovery in the bleach region was observed, with a diffusion coefficient of $6.98 \times 10^{-10} \text{ cm}^2/\text{s}$, indicating that a mobile supported lipid bilayer had formed at the ppAAm surface. This result was somewhat surprising given the presence of a stable vesicular layer on the unpatterned ppAAm surface at pH 7 as indicated by QCM-D [Fig. 5(c)] and FRAP (see Fig. S3). This suggested

that either the physical or chemical presence of the pattern was able to induce vesicle collapse. The surface was then exposed to 1 wt. % 14:0 Liss Rhod PE and 99% DOPC (red) vesicles for 10 min at pH 4. As expected, lowering the pH to 4 enabled vesicle adsorption on the ppAAc regions (Fig. 10). The vesicles doped with the red fluorescent lipids were highly localized to the ppAAc areas with little evidence of adsorption to the pre-existing ppAAm/lipid regions. For FRAP, the exposure area was placed over two of the ppAAc coated features and part of the ppAAm surface. As before, mobility was observed on the ppAAm surface (green channel; diffusion coefficient $8.58 \times 10^{-10} \text{ cm}^2/\text{s}$) after 10 min. On the ppAAc features, limited mobility was observed at the edges of the bleached areas.

Finally, the surface was rinsed at pH 7 (Fig. 11), with the intent of inducing rupture of any vesicles on the ppAAc regions of the substrate. This caused a reduction in the signal-to-noise ratio in the red channel, suggesting the loss of the lipid material from the ppAAc domains. This observation supported the QCM-D data (Fig. 6) during a pH 4 to 7

FIG. 11. FRAP experiments conducted on a patterned surface of ppAAc circles on ppAAm after incubation with 1 wt. % 14:0 NBD PE, 99 wt. % DOPC (green) DOPC vesicles for 10 min and rinsing at pH 7, followed by incubation with 1 wt. % 14:0 Liss Rhod PE and 99% DOPC (red) vesicles for 10 min and rinsing at pH 4 and finally rinsing at pH 7. Confocal microscopy images of the surface were collected after bleaching (0 min) and 10 min later. Scale bar: 10 μm .

TABLE II. Summary of the vesicle adsorption and collapse observed on homogenous and patterned plasma polymerized surfaces exposed to different pH regimes.

Coating type	Surface	pH	Lipid vesicle interaction	Mobility
Homogenous	ppAAm	7	Adsorbed vesicles	Immobile
		4	Adsorbed vesicles	Immobile
		4 → 7	Adsorbed vesicles	Immobile
	ppAAc	7	Limited if any adsorption	N/A
		4	Adsorbed vesicles	Immobile
		4 → 7	SLB	Immobile
Patterned	ppAAc on ppAAm	7	ppAAc: No lipid interaction	N/A
			ppAAm: SLB	Mobile
	ppAAc on ppAAm	7 → 4	ppAAc: Adsorbed vesicles	Immobile
			ppAAm: SLB	Mobile
	ppAAc on ppAAm	7 → 4 → 7	ppAAc: Adsorbed vesicles/SLB	Immobile
			ppAAm: SLB	Mobile

transition, which showed significant mass and dissipation losses from the surface of ppAAc with some lipid material remaining at the surface. Lipid mobility was retained on the ppAAm surface with a diffusion coefficient of $1.07 \times 10^{-9} \text{ cm}^2/\text{s}$; however, the lipid material on ppAAc remained immobile during the 10 min observation period as shown in Fig. 11. On ppAAm, a dark “shadow” could still be observed in the green channel after the 10 min recovery period (at the location of the initial bleach area), which was associated with partial recovery at this time point.

To investigate bleaching of the plasma polymer films, as plasma polymers show auto-fluorescence associated with the presence of unsaturated carbon bonds within their structure,^{20,33} FRAP on the unpatterned control surfaces was undertaken. The experiments showed that the FRAP conditions used in this study could partially bleach the plasma polymer (Fig. S4), leading to low signal to noise ratios. In contrast, on the surfaces where the labeled lipids were present, the bleach areas were clearly defined with little residual fluorescence signal.

The lipid vesicle interactions with the plasma polymerized surfaces in different pH regimes are summarized in Table II. As only vesicle adsorption, not vesicle collapse, was observed on unpatterned ppAAm surfaces, these results suggested that it is the presence of the surface pattern that induced fluid bilayer formation. Two features of the patterned surface may be inducing vesicle rupture: (1) the difference in chemistry between the patterned areas³⁴ and (2) the presence of nanoscale topographical features due to the pattern edges^{35,36} or a combination of both the effects.

IV. CONCLUSIONS

Lipid vesicle interactions with ppAAc and ppAAm films have been investigated to explore the possible use of plasma polymers as interfaces for lipid presenting biosensors and other technologies. The interaction of DOPC lipid vesicles with plasma polymers was shown to be highly dependent on surface chemistry and pH. At pH 7, vesicles readily adsorbed

to ppAAm which was attributed to electrostatic attraction between the positively charged surface and the negatively charged lipid. In contrast, minimal vesicle adsorption occurred on the ppAAc surface, likely a result of the repulsion between the negatively charged ppAAc surface and the negatively charged lipid. Stable and immobile vesicular layers could be formed on ppAAm at pH 4, 7, and 10, demonstrating potential for use in applications such as cell membrane models and encapsulation/delivery platforms.

Vesicle interactions with ppAAc surfaces proved to be far more complex, providing a more versatile coating. At pH 7, the surface provided a largely lipid-resistant surface; however, reducing the pH to 4 enabled vesicle adsorption. This is postulated to be due to the reduced electrostatic repulsion between the neutral ppAAc surface (at pH 4) and the vesicles. QCM-D indicated that a pH 4 to 7 transition could initiate vesicle rupture, which demonstrates its potential as a pH-controlled release system. Although ppAAm films demonstrated immobile vesicle adsorption across a wide range of pH, the patterning of ppAAm with ppAAc regions appeared to initiate rupture of lipid vesicles adsorbed to the ppAAm regions at pH 7. This was significant as it enabled the deposition of immobile lipid islands on ppAAc, surrounded by fluid lipid regions on ppAAm, formed on a single substrate. This study demonstrates the versatility of plasma polymers as coatings that can be used to pattern chemically distinct regions on a wide variety of substrates to enable the spatial control of both the lipid location and the mobility, which is required in a wide range of research and industrial applications.

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