Poly(dimethylsiloxane)-Coated Sensor Devices for the Formation of Supported Lipid Bilayers and the Subsequent Study of Membrane Interactions

Tamar Shahal,[†] Kathryn A. Melzak,[‡] Christopher R. Lowe,[†] and Electra Gizeli*[,‡]

Institute of Biotechnology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QT, U.K., and Institute of Molecular Biology and Biotechnology, FO.R.T.H., and Department of Biology, University of Crete, Vassilika Vouton, 71110 Heraklion, Greece

Received January 23, 2008. Revised Manuscript Received June 5, 2008

The development of smooth hydrophilic surfaces that act as substrates for supported lipid bilayers (SLBs) is important for membrane studies in biology and biotechnology. In this article, it is shown that thin films of poly(dimethylsiloxane) (PDMS) formed on a sensor surface can be used as a substrate for the deposition of reproducible and homogeneous zwitterionic SLBs by the direct fusion of vesicles. Poly(dimethylsiloxane) solution (1% w/v) was spin coated on Love acoustic wave and surface plasmon resonance devices to form a thin PDMS layer. Acoustic, fluorescence, and contact angle measurements were used for the optimization of the PDMS film properties as a function of plasma etching time; parameters of interest involve the thickness and hydrophilicity of the film and the ability to induce the formation of homogeneous SLBs without adsorbed vesicles. The application of PDMS-coated sensor devices to the study membrane of interactions was demonstrated during the acoustic and fluorescence detection of the binding of melittin and defensin Crp4 peptides to model supported lipid bilayers.

Introduction

Supported lipid bilayers (SLBs) serve as models for the lipid bilayer component of cellular membranes and provide a controlled environment for characterizing membrane interactions. Membrane-related processes that have been studied using SLBs include signal transduction events,[1] cell−cell interactions,[2] and membrane disruption by membrane-lytic peptides.[3] Additional areas of application for SLBs include their use as a means to modify surfaces with a variety of groups that are readily available as modifications of lipid headgroups. The advantages of using SLBs to modify surfaces include resistance to protein adsorption conferred by the SLB and the potential to regenerate the original surface by a simple detergent rinse. Surfaces modified with SLBs have been used as supports for immunoassays,[4] chromatography,[5] and nucleic acid hybridization studies.[6]

One of the advantages of SLBs is that they lie flat on a surface, which makes them compatible with surface-sensitive or surface-based methods of analysis. Analytical techniques that have been used to characterize SLBs include both atomic force[7,8] and fluorescence[2,5,9] microscopy as well as procedures based on acoustic,[4,10–12] optical,[13,14] and electrochemical[15] transducers. The analytical technique to be employed in the characterization will determine the exposed surface that is available to support the lipid bilayer. Substrates that have been used for SLBs include glass (in combination with fluorescence microscopy[1,2]), mica (in combination with atomic force microscopy[8,16]), thermally evaporated silica films (in combination with acoustic techniques[5,12]), thiol-modified gold (in combination with surface plasmon resonance and acoustic sensors[3,4,17]), xerogel (in combination with an acoustic sensor[18]), and polymers (in combination with acoustic sensors,[11,19] fluorescence microscopy,[20,21] and electrochemical measurements[15]). The analysis of SLBs by several procedures that provide complementary information can therefore involve the deposition of the SLB on different surfaces. This has the potential to affect the SLB organization and thus the results obtained; it would therefore be advantageous to have a method for producing uniform surface chemistry on different initial substrates, which is one of the goals of the work described in this article.

The simplest and most convenient method for the preparation of SLBs is by the fusion of lipid vesicles from suspension, which will happen spontaneously on surfaces that are smooth, hydrophilic, homogeneous, and of suitable charge. This will result in the formation of fluid SLBs with a 1−3-nm-thick reservoir between the substrate and the bilayer, allowing the accommodation of small extramembrane domains. The major disadvantage of SLB formation by vesicle fusion is that the process can result in incomplete fusion that leaves intact lipid

* Corresponding author E-mail: gizeli@imbb.forth.gr.
[†] University of Cambridge.
[‡] University of Crete.

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10.1021/la800211v CCC: $40.75 © XXXX American Chemical Society
Published on Web 08/26/2008

vesicles present on the surface.[2,9,18,22,23] Surfaces that are rough[11] or insufficiently hydrophilic[17] will induce the adsorption of intact vesicles. Analytes can interact differently with SLBs, vesicles, and exposed substrates; supported lipid layers that contain some unfused vesicles or that are incomplete, so that the underlying substrate is exposed, are therefore undesirable because of the variety of potential interactions that can take place. It is therefore desirable to have a surface-modification procedure that induces the reliable formation of homogeneous SLBs.

The goal of the work described here was to develop a surface-modification procedure that induces the reliable formation of homogeneous SLBs by vesicle fusion. As mentioned above, one additional consideration was that the modification should be compatible with different analytical techniques. The procedure that was developed is based on the formation of a thin layer of poly(dimethylsiloxane) (PDMS) on top of the surface of interest. The PDMS is oxidized to give the hydrophilicity[24,25] required for SLB formation; this occurs through the conversion of surface methyl to hydroxyl groups. Bulk PDMS has been shown previously to be a good substrate for the formation of stable, homogeneous SLBs.[21] The PDMS surfaces have also been shown to be versatile; they have been used to stamp SLBs on a second hydrophilic surface,[26] and they have been oxidized selectively to produce hydrophilic surfaces in localized areas, which results in a patterned formation of SLBs.[20]

The challenge associated with the use of PDMS for modifying surfaces was to produce a PDMS layer that was sufficiently thin to be compatible with optical and acoustic analytical techniques while retaining the properties and stability of the bulk polymer. Thin PDMS layers were successfully formed on the polymer waveguide coating of an acoustic sensor and on the thiol-modified surface of a gold layer used for SPR detection. Subsequent acoustic experiments supported by fluorescence microscopy demonstrated that PDMS films are suitable for inducing the formation of homogeneous egg-phosphatidyl choline SLBs. The same techniques were used to detect the selective interaction of membrane-lytic peptides, melittin and Crp4, with the egg-phosphatidyl choline SLBs supported on oxidized PDMS films.

Experimental Section

Materials. Phosphate-buffered saline (PBS) containing tablets (pH 7.4, 0.01 M phosphate, 2.7 mM potassium chloride, and 137 mM sodium chloride), 1,2-diacyl-*sn*-glycero-3-phosphocholine from egg yolk (egg-PC), synthetic melittin, medium-molecular-weight poly(methylmethacrylate) (PMMA), 1-hexadecanethiol, 2-ethoxyethyl acetate, Triton X-100, glycerol, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. (2-(12-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (NBD C_{12}-HPC) was from Molecular Probes, and *n*-octane was from Fisher Scientific. Gold powder, Premion-20 mesh, was obtained from Alfa Aesar, Johnson Matthey, and Hellmanex II from Hellma, Mullheim Germany. The polydimethylsiloxane (PDMS)-Sylgard 182 silicone elastomer was purchased from Dow Corning.

Surface Acoustic Wave (SAW) Device. The 155 MHz device was fabricated on a 0.5-mm-thick piezoelectric Y-cut (42.5°) quartz substrate. The surface of the quartz substrate was patterned with input and output interdigitated transducers (IDTs) produced by the photolithographic technique. The IDTs consisted of 92 pairs of split fingers with a periodicity of 32 μm. The separation distance between the IDTs was 9.4 mm (center to center), and the finger length was 1.6 mm. An initial 20 nm chromium flash was deposited, followed by a 100 nm gold overlayer.

Love Wave Device. Love waves were produced on the SAW device described above by the addition of a PMMA waveguide. An approximately 1-μm-thick PMMA waveguide layer was deposited on the surface of the acoustic device by spin coating (Specialty Coating Systems P6700) a 17% (w/w) PMMA solution in 2-ethoxyethyl acetate (Sigma-Aldrich) at 4000 rpm for 40 s, followed by heating the polymer-coated device at 190 °C for 2 h. Prior to spin coating, the PMMA solution was filtered through a 0.45 μm PTFE filter to eliminate particles; filtering the polymer solution was found to be important for producing smooth PMMA layers.

Instrumentation and Experimental Setup for Acoustic Measurements. A Hewlett-Packard 8753ES network analyzer was used to measure the amplitude (energy dissipation) and phase (velocity) of the wave at room temperature. The Fourier transform/time gating function of the device was used for biosensing measurements to minimize unwanted interference with electromagnetic feed-through and triple transit signals. The acoustic device and flow-through system were mounted in a special custom-made holder. Liquid samples were pumped by a peristaltic pump through poly(vinyl chloride) (PVC) tubing and a Perspex flow cell at a flow rate of 17 μL/min. A silicone rubber gasket was used to seal the flow cell to the device surface, exposing an area of 0.06 cm^2.

Instrumentation and Experimental Setup for SPR Measurements. SPR experiments employed the Kretschmann configuration using a hemicylindrical lens as described in detail elsewhere.[27] For the SPR device, an approximately 0.5 nm chromium layer (Megatech, Royston, U.K.) followed by a 32 nm gold (Alfa, Johnson Matthey, Royston, U.K.) film was evaporated on a microscope slide using an Edwards Auto306 evaporator. Glass slides were coupled to the hemicylindrical lens (BK7 glass; $n = 1.51509$ at 632.8 nm) using dimethylphthalate (Aldrich) as an index-matching oil.

PDMS Deposition and Activation. For acoustic measurements, the PMMA surface of the acoustic waveguide device was coated with a thin PDMS overlayer by spin coating from a 1% (v/v) PDMS solution in *n*-octane at 4000 rpm for 40 s. Viscous Sylgard 182 was transferred to a separate 10 mL Pyrex container with a plastic syringe, and the curing agent was added in a ratio of 10:1 (v/v) (Sylgard 182 curing agent). A diluted 1% (v/v) PDMS solution was obtained by adding *n*-octane solvent to the container. The reagents were mixed with the solvent by a magnetic stirrer until a uniform solution was obtained (after approximately 2 min), and the solution was filtered through a 0.45 μm PTFE filter using a Pyrex syringe. Following the PDMS coating, the device was transferred into the oven, and the PDMS coating was cured at 150 °C for 1 h. The presence of the PDMS film did not have any effect on the Love wave transfer function. A schematic representation of the Love wave device coated with a thin PDMS layer is shown in Figure 1A.

For SPR measurements, gold-coated glass slides were immersed in 1 mM 1-hexadecanthiol (HDT) solution in ethanol for 2 days to be rendered hydrophobic. Then the slides were washed with ethanol and dried under N_2. A solution of 0.5% (v/v) PDMS was spin coated on the modified gold slides and cured as described above. SPR measurements revealed that the thickness of a PDMS film, spin coated from 1% (v/v) PDMS solutions, on 1-hexadecanthiol-activated gold-coated glass slides was (18 ± 2) nm.

Pure (100%) PDMS was formed by slowly mixing Sylgard 182 with a curing agent in a ratio of 10:1 for at least 45 min in a Denley Spira MIX5 rotary mixer. When the mixture was uniform with only one liquid phase visible, it was centrifuged for 15 min

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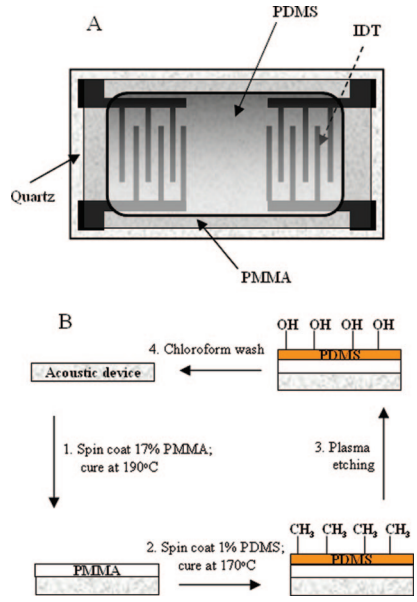

Figure 1. Schematic presentation of (A) the PDMS-coated Love wave device and (B) the procedure used for PDMS coating, activation, and surface regeneration.

at 10 000 rpm in an accuspin microcentrifuge (Fisher Scientific) to eliminate air bubbles. Following that, the PDMS mixture was spin coated on the surface of the acoustic device and cured in an oven at 70 °C for 80 min. PDMS films (both 1 (v/v) and 100%) were rendered hydrophilic by air plasma treatment, carried out in an Edwards Auto306 evaporator. The pressure inside the evaporator chamber was kept constant at 1.5×10^{-1} mbar by letting a small amount of air leak into the chamber during the etching process.

Contact Angle Measurements. The hydrophilicity of the plasma-treated PDMS surfaces was determined using an OCA contact angle system from Dataphysics. Drops of 3 μL of ultrahigh purity (UHP) water were applied to the surface at a flow rate of 0.1 μL/s through a thin metal tube. Photographs of the water drops were taken by a video camera immediately after the drops were applied. The advancing contact angle was measured from the image of the drop by SCA 20 software for OCA.

Preparation of Lipid Vesicles. Small unilamellar vesicles (SUV) were prepared by extrusion with an Avestin Liposofast Basic extrusion apparatus. Chloroform solutions of egg-phosphatidyl choline (egg-PC) and fluorescently labeled lipid NBD-C12-HPC were mixed to a final concentration of 1% (w/w) NBD-C12-HPC total lipids. The chloroform was evaporated under a stream of nitrogen for 1 h; the dried lipid films were resuspended by 30 s of vortex mixing in 1 mL of UHP water to a total lipid concentration of 2 mg/mL. The suspensions were extruded 21 times through a 50-nm-pore membrane (Glen Creston). Light-scattering measurements revealed that the average diameter of the extruded vesicles was 71 ± 19 nm.

Vesicle Fusion and Peptide-Binding Assay. A 5 mM Tris buffer, pH 8, with 50 mM NaCl was pumped over the acoustic device surface until a steady signal was obtained. An egg-PC vesicle suspension of 0.2 mg/mL diluted in the buffer solution was applied to the device surface until the signal reached a steady state, followed by buffer rinse. For peptide binding, PBS buffer, pH 6.8, was first applied to the SLB-coated surface for 10 min, followed by the sequential addition of peptide aliquots. After each peptide addition, the device was washed with PBS buffer.

Fluorescence Microscopy. PDMS-coated surfaces (prepared by spin coating a 1% v/v solution and pure PDMS) were plasma etched for various times and then fixed at the bottom of a static or flow-through cell. Generally, 70 μL suspensions of 2.5 mg/mL egg-PC lipid vesicles in UHP water, fluorescently labeled with 1 (w/w) % NBD-C_{12}-HPC (of total lipids) were applied. After 2 min of incubation with the vesicle suspension, the PDMS surfaces were washed extensively with 5 mM Tris buffer, pH 8, 50 mM NaCl and transferred onto a microscope glass slide to be examined under the microscope. Care was taken to keep the surfaces in contact with buffer; at this stage, it is essential that the PDMS surfaces be kept covered with liquid to avoid drying and disrupting the lipid bilayer. The fluorescence measurements were carried out using a Nikon microscope with a B-2A filter block (λ_{ex} 450−490 nm and λ_{em} >505 nm). A sample area of approximately 1×10^4 μm^2, defined by a partially closed diaphragm, was exposed to UV and left to bleach for approximately 20 s until the entire lipid layer turned dark. After bleaching, the samples were left to recover in the dark for 10 min. The shutter was then opened, and the extent of recovery was observed. Photographs were taken within 3 s of exposing the samples to UV for observation. The percentage of fluorescence recovery was calculated from the brightness of the recovered photograph relative to the brightness of the photograph before photobleaching.

The number of bright spots observed on images describing nonhomogenous SLBs was determined using the ImageJ program from the National Institute of Mental Health, Bethesda MD (http://rsbweb.nih.gov/ij/index.html). The spots were selected according to their brightness in comparison to the background layer. Three different areas on each lipid layer were defined by the partially closed diaphragm. On each diaphragm marked area, three selected areas of 441 μm^2 were chosen, and the number of bright spots was calculated as the average number of spots from the three areas. The above procedure was always repeated on two devices per experiment.

Results and Discussion

Effect of PDMS Thickness on Love Wave Device Operation. PDMS films deposited on the Love wave device should be compatible with sensor's operation. The Love wave device incorporates a polymer (PMMA) guiding layer (~1 μm thick) so that the acoustic wave is confined to the device surface rather than being dispersed in the bulk of the crystal.[28,29]

The confinement of the wave close to the sensing surface is detected as an increase in amplitude by ~9 dB compared with that of the uncoated device. Using PDMS films as a guiding layer resulted in high amplitude loss. For this reason, PDMS films of various thicknesses were deposited on top of PMMA; full regeneration of the quartz device was achieved through rinsing with an organic solvent. A schematic representation of the complete procedure used for PDMS coating and activation as well as surface regeneration is shown in Figure 1B. The effect of PDMS thickness on energy dissipation was evaluated through monitoring the amplitude of the wave. Figure 2 shows that only very thin films derived with 1% (v/v) PDMS gave a measureable acoustic signal allowing the successful application of the Love wave device for further studies.

Hydrophilicity of Plasma-Etched PDMS. For a surface to induce the fusion of vesicles, it must be hydrophilic and smooth.[10,17] In this study, the hydrophilicity of PDMS surfaces after exposure to air plasma etching was investigated for PDMS films prepared from a 1% (v/v) solution and pure PDMS. Films formed from undiluted PDMS were used as a positive control because such surfaces have been shown to support uniform lipid bilayers.[21] Figure 3 shows that the longer the exposure time, the lower the advancing contact angle on both surfaces. Interestingly, for each given plasma etching exposure, the contact angles measured for PDMS layers made from 1% (v/v) PDMS solutions were lower than those obtained from pure PDMS films.

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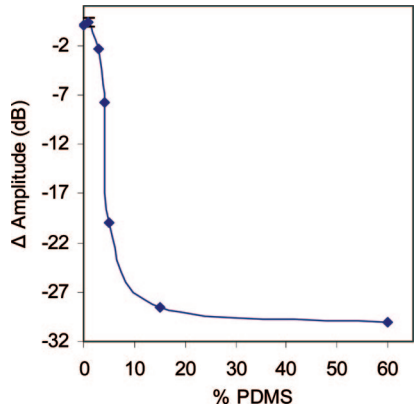

Figure 2. Amplitude change of the PMMA-coated Love wave device as a function of the PDMS solution used for spin coating a PDMS layer. The blue diamonds represent experimental points; the line was drawn for clarity.

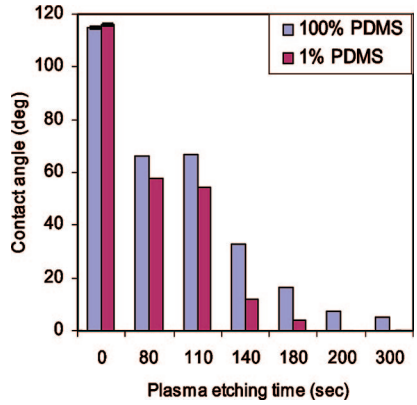

Figure 3. Contact angle as a function of plasma etching time on PDMS surfaces prepared from a 1% (v/v) solution and pure PDMS solutions.

The morphology of the PDMS layer may account for this difference; films prepared from diluted PDMS solutions may be less densely packed and ordered than PDMS films prepared from pure PDMS. As a result, fewer methyl silane groups are exposed on the 1% (v/v) PDMS surface, which would be rendered hydrophilic faster than surfaces prepared from pure PDMS films. This assumption is in agreement with Hillborg et al., who showed that PDMS films with lower cross-linking densities have lower advancing contact angles than films with high cross-linking densities after plasma etching both surfaces in air for 180 s.[30]

Fluorescence Detection of Supported Lipid Layers Formed on PDMS Surfaces. PDMS films prepared from a 1% (v/v) solution and from pure PDMS and plasma etched for 0 to 300 s were exposed to fluorescently labeled vesicle suspensions and were subsequently tested for their ability to induce the formation of SLBs that had fluorescence recovery after photobleaching (FRAP). Images displaying a uniform green color were attributed to the formation of homogeneous SLBs. Images consisting of a green background with bright dots were attributed to nonhomogenous SLBs; bright dots were attributed to clusters of nonfused vesicles because the spots did not recover after photobleaching.

As shown in Table 1, homogeneous SLBs with a negligible number of dots and high fluorescence recovery (~90%) were formed on hydrophilic films prepared from pure PDMS, which was plasma etched for 200 and 300 s (with corresponding contact angles of 7 and 5°, see Figure 3). Similarly, PDMS films prepared from 1% solutions and plasma etched for 180 and 200 s (with corresponding contact angles of 4 and 0°) induced the formation of homogeneous SLBs with a negligible number of dots and a high FRAP percentage (~92%) (Figure 4A and Table 1). The high degree of FRAP is attributed to the formation of complete, defect-free SLBs under these plasma-etching conditions.

Of interest is the fact although that a high degree of PDMS hydrophilicity is necessary it is not sufficient as a requirement for vesicle fusion. Table 1 shows that excess (300 s) plasma etching of the 1% (v/v) PDMS surface results in the formation of an SLB with many spots that gave only 66 ± 1% FRAP (Figure 4B). The PDMS surface roughness, which increases with plasma etching, could account for this behavior because vesicles can be trapped within the cavities of rough surfaces or within cracks formed on extensively plasma-etched surfaces.[24,31] In contrast, on films prepared from pure PDMS that was plasma treated for 300 s, a homogeneous SLB (82% FRAP) was formed. It is reasonable to assume that the roughness of films prepared from 1% (v/v) solutions that were plasma etched for 300 s may increase faster than that of films prepared from pure PDMS because of their poorer packing. Incomplete FRAP and a slower diffusion coefficient of lipids have been associated with the coexistence of unfused vesicles and SLBs in previous studies.[2,9] Fluorescence images of SLBs containing unfused vesicle aggregates have also been observed on xerogel thin films and are related to the relatively high roughness of the xerogel surfaces.[18]

Shorter etching times led to the formation of supported vesicle layers (SVLs) on both thick (from pure PDMS) and thin (1% (v/v)) PDMS surfaces, plasma etched for 110 s, with corresponding contact angles of 67 and 54°. Homogenous SVL formation was confirmed by detecting no FRAP. The formation of SVLs on surfaces with contact angles of around 60° has also been observed by other investigators.[20,32]

Acoustic Detection of SLB Formation on PDMS. Acoustic measurements involve monitoring phase and amplitude changes, which, in turn, are related to mass and viscosity perturbations occurring near the surface/liquid interface.[29] Figure 5 shows the acoustic response obtained following the application of egg-PC vesicles onto a freshly 180 s plasma-etched PDMS layer made from 1% (v/v) PDMS (blue diamonds). Lipid layers formed on freshly plasma-etched surfaces gave an acoustic response similar to that obtained for SLBs formed on hydrophilic silica surfaces;[11,12,33] this result is consistent with the fact that the plasma treatment of PDMS surfaces produces silanol chemistry like that found on hydrophilic silica. As shown in Figure 5, the amplitude and phase of the acoustic signal obtained on freshly plasma-treated PDMS film decrease upon addition of the vesicle suspension until a minimum value is observed. Briefly, this part of the kinetics has been associated with the adsorption of intact vesicles until a critical surface coverage is reached.[11,12] The recovery of both signals toward the original baseline has been attributed to vesicle fusion induced by lateral and surface interactions and the transition from a soft vesicle layer to an elastic lipid bilayer.[11,12] As expected, the amplitude signal recovers almost completely to the initial baseline, indicating that SLBs behave as an elastic layer, whereas the phase recovers only

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Table 1. Fluorescence Recovery and Homogeneity of Lipid Layers Formed on 1% (v/v) and Pure PDMS (100%), Plasma Etched for Different Durations (s)

plasma etching time (s)		no. of dots per 441 μm^2		% of FRAP recovery		type of bilayer	
1%	100%	1%	100%	1%	100%	1%	100%
110		SVL	SVL	0	0	SVL	SVL
140		5 ± 8	SVL	65 ± 8	0	SLB	SVL
180		2 ± 2	1 ± 1	95 ± 7	69	SLB	SLB
200		2 ± 2	2 ± 2	89 ± 13	91	SLB	SLB
300		99 ± 50	0 ± 0	66 ± 1	82	SVL	SLB

slightly, indicating the addition of mass to the surface of the acoustic device. Very good reproducibility was observed for the acoustic detection of SLB formation with a standard deviation for both signals of less than 10% from 20 depositions.

The existence of a uniform SLB on a plasma-etched PDMS surface was confirmed by observing good recovery during FRAP experiments. In addition, BSA, which was shown to adsorb onto both bare and plasma-etched PDMS surfaces, did not give an acoustic signal change when applied to an SLB-covered PDMS surface, confirming the formation of defect-free lipid bilayers (data not shown). Previous experiments have shown that resistance to BSA adsorption can be used to distinguish SLBs from SVLs.[3,17]

Figure 5 also shows the acoustic response obtained following the addition of an egg-PC vesicle suspension onto the same PDMS layer that was used for SLB formation; however, in this case, the PDMS surface was exposed to successive depositions of melittin aliquots (up to 50 μg/mL) and was rinsed with 2% Hellmanex prior to the egg-PC addition (pink squares). In this case, both acoustic signals decreased until a steady state was reached. This response was attributed to the adsorption of vesicles as confirmed by observing no FRAP. The formation of an SVL on this surface may be attributed to surface contamination by melittin and to the Hellmanex rinse; adsorbed melittin (which is positively charged) would affect the surface charge and the wetting properties, and the Hellmanex rinse could affect the surface roughness because of the alkalinity of the detergent. These results show that surfaces that have been exposed to melittin are not suitable for subsequent deposition of SLBs. (Attempts to regenerate surfaces that had been exposed to melittin using milder detergents were also unsuccessful; data not shown).

Regeneration of the PDMS Surface. Figure 6 shows the phase response following three cycles of egg-PC vesicle deposition on oxidized PDMS and surface regeneration by a Triton X-100 wash. The addition of Triton results in the solubilization and removal of the bilayer as indicated by changse in both acoustic signals toward the baseline obtained prior to SLB deposition. Upon the first Triton X-100 rinse, the phase signal does not recover fully up to the original baseline, probably as a result of lipid and/or detergent molecules irreversibly adsorbed to the surface. This effect is less pronounced during the second deposition and disappears in the third; interestingly, the amplitude signal is less sensitive in monitoring such changes. Lipid molecules remaining attached to the surface after SLB washing with a detergent solution were observed by Fisher et al., who

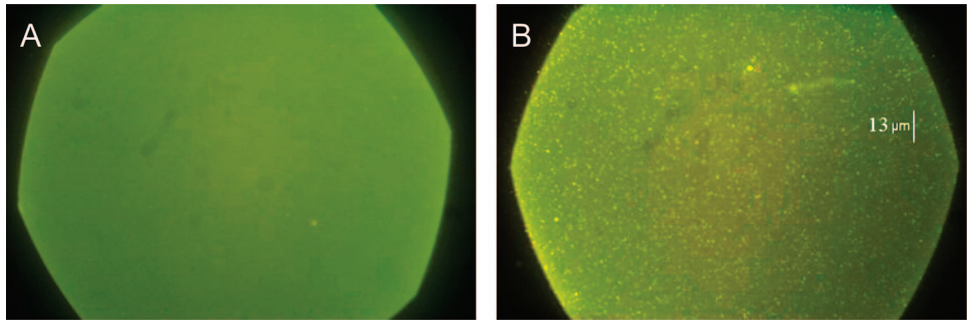

Figure 4. Photographs of fluorescently labeled lipid layers deposited on 1% (v/v) PDMS surfaces that were plasma etched for (A) 180 s, resulting in a homogeneous SLB, and (B) 300 s, resulting in a heavily dotted SLB. (The contrast in B was enhanced for clarity).

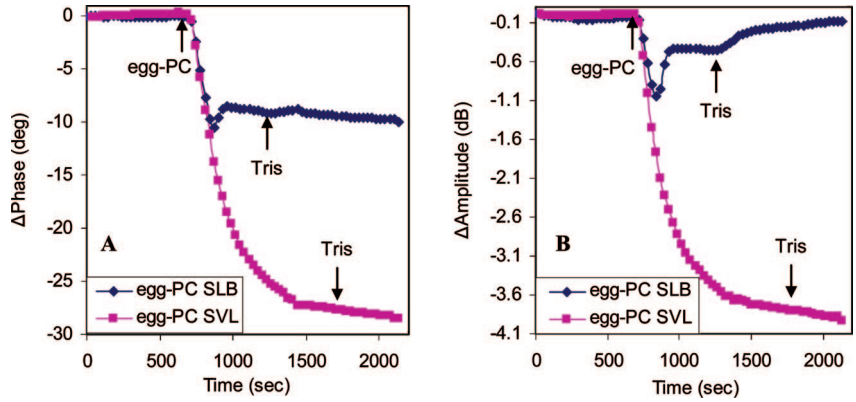

Figure 5. Phase (A) and amplitude (B) changes of the acoustic signal obtained during the formation of an SLB and an SVL on a 1% (v/v) etched PDMS-coated device. The SLB was formed on a freshly etched PDMS surface (180 s etching time); the SVL was formed on the same PDMS surface after it was exposed to successive melittin depositions (up to 50 μg/mL) and a 2% Hellmanex rinse.

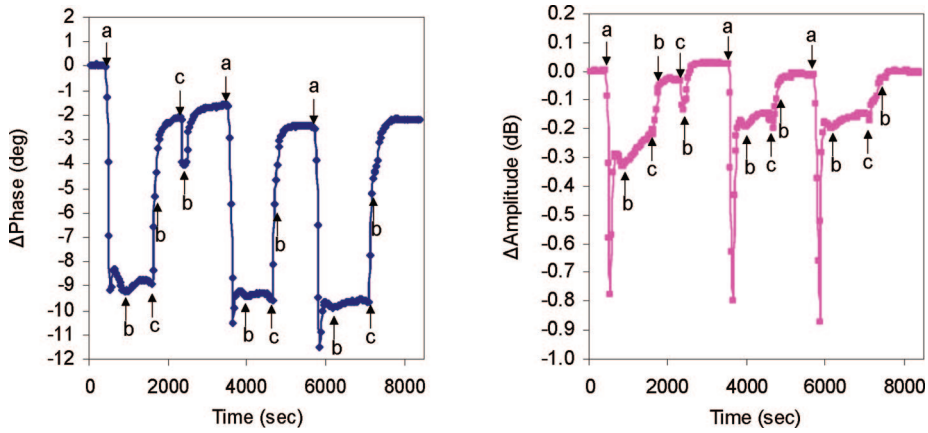

Figure 6. Change in phase and amplitude of the acoustic signal following three cycles of SLB formation with surface regeneration by Triton X-100 on a plasma-etched PDMS surface. The arrows indicate the times at which egg-PC vesicles (a), PBS buffer (b), and detergent (c) were added. After each vesicle and detergent addition, the surface was washed with PBS buffer.

also argued that these molecules promote the fusion of vesicles in the following deposition.[34] Washing the SLB surface with more alkaline solutions such as Hellmanex resulted in a more complete removal of the lipid bilayer. However, in most cases SLBs could not be formed after the Hellmanex rinse, implying that Hellmanex changed the characteristics of the etched PDMS surface (data not shown).

Previous studies have shown that the hydrophobicity of plasma-treated PDMS recovers when the surface is aged in gas, probably because of the diffusion of polar groups from the surface into the bulk PDMS, the migration of nonoxidized low-molar-mass species to the surface, and the condensation and consequent cross linking of surface silanol.[24,25] Conflicting studies regarding plasma-treated PDMS aged in water show that PDMS can maintain its wettability[25] or recover its hydrophobic nature.[35] The latter would introduce a threat to the integrity of SLBs on hydrophilic PDMS films when used for long experiments. Our experiments showed that the SLB remains intact and homogeneous for at least 7 h after its first deposition (which is the longest time tested), indicating that the PDMS surface maintains its hydrophilicity when covered with a lipid membrane.

PDMS-Coated SPR Device. Figure 7 shows the SPR reflectivity curves of a gold surface with a self-assembled layer of hexadecanethiol (HDT), the same surface with a PDMS thin film obtained after spin coating a 0.5% (v/v) PDMS solution, and the above surface after rendering it hydrophilic and exposing it to egg-PC vesicles. Fluorescence images of labeled lipid layers formed by vesicle fusion on gold slides modified with HDT and spin-coated with 0.5% (v/v) PDMS were homogeneous and showed good FRAP (data not shown). No decrease in reflectivity was observed for s-polarized light, indicating that the dip in the reflectivity of the p-polarized light is due to a pure SPR effect. These results show that the PDMS coating is thin enough to be compatible with SPR measurements and that it does not interfere with the detection of bilayer formation. Furthermore, the PDMS-coated SPR device could be readily used for fluorescence measurements because PDMS-supported lipid bilayers are placed a certain distance away from the gold surface to quench fluorescence.

Acoustic Study of SLB/Lytic Peptide Interactions. The goal of this work is to produce a surface modification that will induce the formation of SLBs and be compatible with diverse sensors. The first part of this work can be accomplished by an analysis

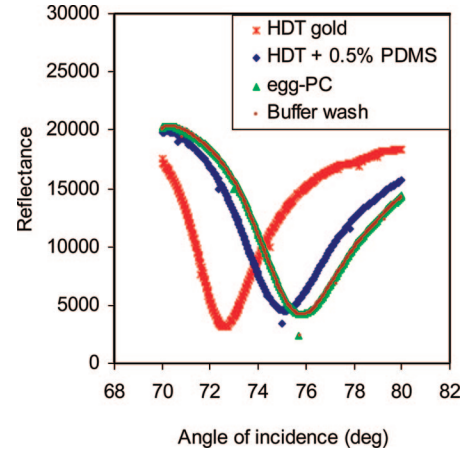

Figure 7. SPR reflectance curves of p-polarized light applied to a gold-coated slide activated with an HDT (red), spin coated with a 0.5% (v/v) PDMS solution (blue), exposed to egg-PC vesicles (green), and washed with buffer (brown).

of the SLBs by FRAP; the demonstration of the second requirement requires that the SLBs be used for an application. Accordingly, sample peptides having different membrane-lytic activities were selected and applied to SLBs to confirm the potential applications of the PDMS modification described here. The SLBs formed on the oxidized (180 s plasma-etched) PDMS surface were exposed to the membrane-lytic peptides melittin, secreted from bee venom, and cryptdin 4 (Crp4), a mouse paneth cell α-defensin. The interaction of the peptides with the egg-PC SLB was recorded with an acoustic sensor by following the phase and amplitude of the wave in real time (Figure 8). Melittin, a positively charged peptide with a net charge of +6, is a nonselective membrane-lytic peptide (i.e., it interacts with both mammalian and bacterial cells[36]), and Crp4, with a positive charge of +9, exerts its bactericidal effect through its affinity for electronegative phospholipids and acts against different pathogenic bacteria by the disruption of their membranes.[37]

Figure 8 clearly shows that there is a distinct difference in the acoustic response obtained upon multiple additions of the two peptides to egg-PC SLBs formed on PDMS. In the case of melittin, both acoustic signals change following peptide addition in a concentration-dependent manner up to 40 μg/mL (14 μM).

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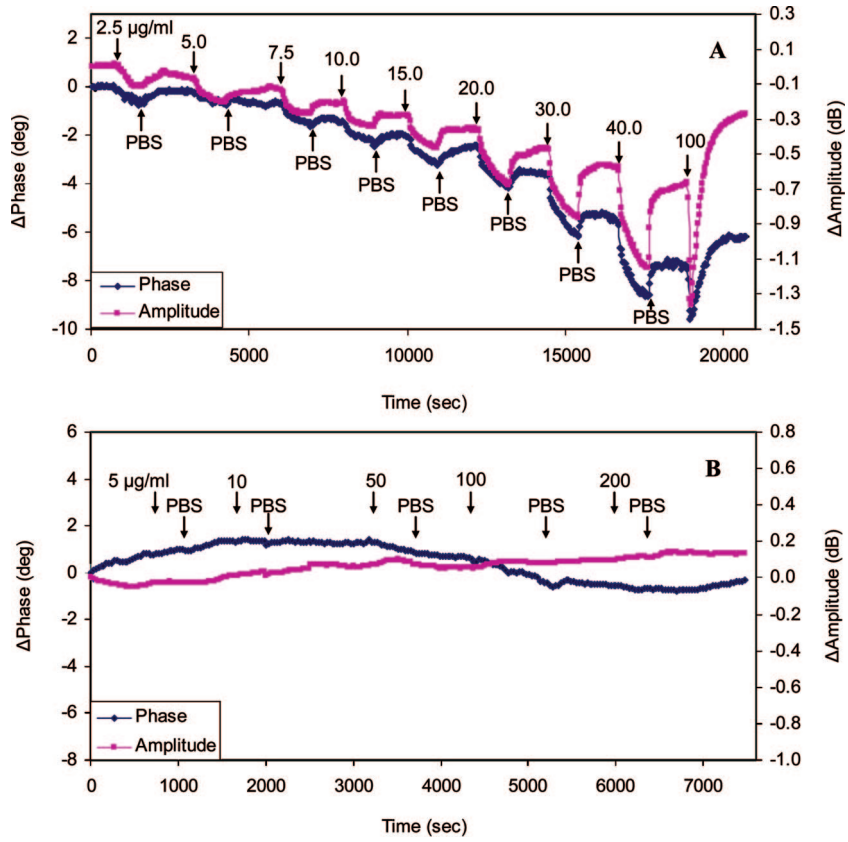

Figure 8. Phase and amplitude changes obtained following successive additions of melittin (A) and Crp4 (B) solutions at different concentrations to a PDMS-supported egg-PC lipid bilayer. The arrows indicate the time at which the peptide was added; numbers beside the arrows indicate the peptide concentration. In the case of melittin application (A), each peptide solution was applied for 15 min following a 30 min buffer wash until a stable signal was obtained.

Furthermore, the phase and amplitude responses clearly indicate a two-state interaction mechanism of the peptide with the lipid bilayer, one reversible and one irreversible. The former probably arises from weak electrostatic interactions of melittin with the top surface of the lipid bilayer, and the latter may be due to a stronger association of the peptide with the bilayer or the PDMS surface. It has been shown that melittin adsorbed on the outer surface of the membrane can translocate across the bilayer through transient pores via a mechanism known as torroidal pore formation.[38] It is reasonable to assume that following translocation in the inner membrane leaflet the positively charged peptide binds to the negatively charged PDMS surface; this is supported by the 1.2 ± 0.1 nm [39] cross section of the melittin molecule, which would fit in the 1−3 nm reservoir of water[40,41] known to exist between the SLB and the hydrophilic PDMS support. The adsorption of melittin to bare oxidized PDMS was indeed observed, utilizing the acoustic device (data not shown). The application of a sample of 100 μg/mL (35 μM) melittin shows that both signals first decrease and then increase toward the initial baseline obtained prior to SLB deposition; this is in agreement with lipid removal upon membrane solubilization as demonstrated by the peptide's known ability to lyse the plasma membrane of erythrocytes.[42] The removal of the lipid bilayer after the addition of a 100 μg/mL melittin solution was confirmed by fluorescence images of the layer indicating that most of the fluorescently labeled lipid molecules were washed off of the surface. Similar solubilization behavior of melittin has been reported using a QCM device and impedance spectroscopy.[43]

As expected, Crp4 did not appear to interact with the zwitterionic egg-PC SLBs. Successive additions of increasing concentrations of Crp4 from 5 to 200 μg/mL (1.75 to 70 μM) resulted in no change in the two signals. Additionally, fluorescently labeled SLB exposed to aliquots of Crp4 from 2.5 to 200 μg/mL appeared to be intact and uniform under the fluorescence microscope and showed good fluorescence recovery (data not shown).

A change in the acoustic signal may also be related to acoustoelectric interactions.[29] The fact that no significant acoustic response was recorded when Crp4, with a net charge of +9, was applied to the egg-PC SLB indicates that the acoustic response observed for melittin (net charge of +6) is due to the adsorption and interaction of melittin with the lipid bilayers and not to changes in the bulk of the solution.

Conclusions

We have demonstrated that films of PDMS can be used for the development of biosensor devices. Thin PDMS films, spin coated from diluted solutions, were found to be compatible with both acoustic and optical sensors. The advantage of surface modification with PDMS is that the PDMS layer can be easily formed without going through laborious procedures. This method

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is suitable for modifying any smooth surface on which the octane solution of PDMS will spread evenly (i.e., on any hydrophobic surface).

The high quality of the PDMS-supported egg-PC lipid bilayers in terms of their homogeneity and sensors signal reproducibility indicated that PDMS is an ideal substrate for developing model membranes. Furthermore, the potential of PDMS-coated SLBs for studying membrane events was shown during the discrimination between the binding pattern of two different peptides—hemolytic, pore-forming melittin and antibacterial defensin Crp4. This work indicates that PDMS-coated devices could find broad applications as biosensors for membrane studies.

Acknowledgment. We thank Professor A Ouellette, UCI, for donating the Crp4 peptide. The financial support of the HFSP and the Greek Ministry of Education (Pythagoras II) is acknowledged. T.S. also acknowledges St John's College, Bna'i Brith, The University of Cambridge, and the Anglo Israeli Association for financial help.

LA800211V