

A Silicate Gel for Promoting Deposition of Lipid Bilayers

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A simple method for modifying a polymer surface to induce lipid bilayer formation by vesicle fusion is described. A silicate gel was prepared by condensation of tetraethyl orthosilicate (TEOS) in the presence of acid. When applied to a poly(methylmethacrylate) substrate, either a rough or a smooth layer could be produced, depending on the method used for the application. The smooth surface induced formation of a supported lipid bilayer by fusion of lipid vesicles; the rough silicate surface induced adsorption of a vesicle layer. A high-frequency acoustic waveguide device was used to follow the initial adsorption of vesicles, the transition from a vesicle layer to a bilayer, and the formation of a complete bilayer; the time required to form a bilayer was determined as a function of lipid concentration in suspension. The presence of a bilayer on the smooth silicate surface was confirmed by fluorescence recovery after photobleaching. An additional procedure is described to modify a gold surface to induce bilayer formation. © 2002 Elsevier Science

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INTRODUCTION

Supported lipid bilayers (SLBs) can be deposited on glass or other suitably modified surfaces by transfer of Langmuir-Blodgett films, spontaneous vesicle fusion, chemical attachment through tethered lipids, or a combination of the above (1–3). These bilayers are simple models of biological membranes and can be used in combination with various transducers for the study of membrane proteins and processes (4–8). In order for SLBs to be useful for the study of membrane proteins it is essential that they should be able to incorporate the membrane proteins to be studied; this has been shown, for some proteins, to necessitate an aqueous layer between the bilayer and the underlying substrate to accommodate extra-membrane protein domains. Formation of bilayers by vesicle fusion on hydrophilic supports has been shown to give an aqueous layer approximately 2 nm thick between the bilayer and the substrate (9), implying that bilayers formed by vesicle fusion are therefore suitable for the study of proteins with smaller extra-membrane domains.

A number of surface-sensitive techniques have been used to study the interaction of vesicle suspensions with solid surfaces, including electrochemical (10) and optical (5, 11–13) methods. Recently, acoustic wave devices have also been applied to these studies and have been shown to be able to monitor the mass and viscoelastic changes occurring during vesicle fusion on the device surface (14–16).

Different measurement techniques have individual requirements with regard to supported lipid bilayers. For example, electrochemical studies may require a bilayer with no holes; in contrast, occasional small holes can be an advantage for atomic force microscopy imaging studies, since they provide a means of determining the depth of the bilayer and thus provide an identifying characteristic on what would otherwise be a featureless surface. In order to be useful for analytical purposes supported lipid bilayers should be formed reproducibly; ideally, the preparation procedure should be simple, should not require specialized equipment, and should give a surface that can be regenerated.

In the experiments described here, a high-frequency surface acoustic wave device is used for studying the deposition of vesicles. This device is based on a waveguide geometry incorporating a layer of poly(methylmethacrylate) (PMMA) to enhance surface sensitivity (17). Since the polymer waveguide layer forms the surface that is exposed to solution it is this surface that must be modified to induce bilayer formation. Surface modifications to induce bilayer formation should be done in a manner that maintains the integrity of the PMMA layer. Exposure of the PMMA to solvents can cause it to swell, changing its waveguiding properties and decreasing the efficiency of transmission of the acoustic signal; such exposure should therefore be minimized.

Since liposomes are known to form bilayers when deposited on glass (3, 9, 12, 18), initial modifications of the acoustic devices were intended to produce a surface that was as similar as possible to glass. Initially, this was done by addition of a silica layer via chemical vapor deposition (CVD) on top of the PMMA waveguide. Although this produced surfaces that induced bilayer formation, cracks in the silica layer led to a considerable decay in the acoustic signal after rinsing with detergent.

The silicate gel modification described in this paper was developed to provide a stable surface that would have the silanol groups that are present on the surface of glass and that did not require specialized equipment to produce. Smooth and rough

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silicate-modified PMMA surfaces were formed by varying the preparation procedure. These surfaces were used in combination with an acoustic device to monitor liposome adsorption; for devices that induced bilayer formation, the time required to obtain a bilayer was determined. Although PMMA is used here as an integral part of the acoustic device, it can be added to the surface of planar electrodes or to gold-coated slides in a layer thin enough to be compatible with surface plasmon resonance measurements so that the modifications described here could be applied to other measurement techniques. A gold-coated acoustic device was also modified with a silicate layer to demonstrate the more general applicability of silicate gels for inducing bilayer formation.

MATERIALS AND METHODS

Chemicals. The POPC was purchased from Fluka; NBD-C₁₂-HPC from Molecular Probes Europe; TEOS, PMMA, 2-ethoxyethylacetate, (3-mercaptopropyl)trimethoxysilane, and chloroform from Aldrich; PBS tablets, Triton X-100, cholesterol, and stearylamine from Sigma and HCl from Fisher.

Acoustic device. The acoustic devices were prepared by photolithography at the Southampton Electronics Centre (Southampton, UK) using single crystal Y-cut, z-propagating 0.5-mm-thick quartz with a 200-nm gold overlayer held onto the quartz by a 10-nm chromium adhesion layer. The input and output interdigitated transducers (IDTs) produced by the photolithographic patterning consisted of 80 pairs of split fingers with a periodicity of 45 μm . The PMMA waveguide was deposited from a 22% w/w solution of medium molecular weight PMMA in 2-ethoxyethylacetate by spin-coating at 4000 rpm for 40 s (17). The PMMA-covered devices were heated to 195°C for 2 h in order to facilitate solvent evaporation and improve film adhesion. The devices were reused after cleaning in acetone.

Instrumentation and experimental setup. A Hewlett-Packard 4195A network analyzer was used to measure the amplitude and phase of the output electrical signal with respect to a reference signal. Data were collected on a PC using Lab View software. A perspex flow cell and silicone rubber gasket were used to hold the solution in place over the region of the device between the IDTs, exposing an area of approximately 36 mm². During experiments in which data were collected as a function of time, a 3 MHz region of the frequency spectrum near the maximum operating frequency was scanned every 43 s to monitor the signal. Data were collected at a fixed frequency so that one data point was collected every 43 s.

Preparation of lipid vesicles. Vesicles of 2-oleoyl-1-palmitoyl-*sn*-glycero-3-phosphocholine (POPC) with 1% fluorescent label (2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino) dodecanoyl-1-hexadecanoyl-*sn*-glycero-3-phosphocholine or NBD-C₁₂-HPC) were prepared by extrusion in PBS (0.01 M phosphate, 0.0027 M KCl, and 0.137 M NaCl, pH 7.4). Stock solutions of lipids were made up in chloroform and mixed to give a 1% fluorescent label; chloroform was then removed

by evaporation under nitrogen for 1 h. The lipids were then resuspended in PBS at a total lipid concentration of 2 mg/ml. Vesicles were prepared by extruding the suspensions 21 times through 100- or 50-nm-pore membranes (Avestin) using an Avestin Liposofast Basic extrusion apparatus. Liposomes used in experiments were prepared with the 100-nm-pore membranes unless otherwise specified. Liposomes were also prepared with POPC, cholesterol, and stearylamine at a 4 : 3 : 1 or 4 : 3 : 0 mole ratio. Stock solutions of lipids were mixed in the appropriate mole ratio and then dried, and then vesicles were prepared as described above. If stearylamine was present, care was taken to maintain the solution at room temperature during the evaporation of the chloroform in order to prevent precipitation.

Preparation of the rougher silicate surface. The TEOS and HCl were first mixed to form a gel by placing 0.25 g of TEOS in a 1.5-ml microcentrifuge tube and adding 5.8 M HCl to give a final volume of 1 ml. The tube was vortexed for 30 s or until only one liquid phase was visible. The mixture was left for 30 min at room temperature until macroscopic gelation had started to become apparent and then it was placed over the surface of the polymer-coated acoustic device and left for 2 min before being rinsed off with water.

Preparation of a smooth silicate surface that induced bilayer formation. The TEOS and HCl were mixed as above but with 0.05 g of TEOS added before being made up to 1 ml with 5.8 M HCl. The PMMA-coated acoustic device was covered with TEOS by spin coating; 0.05 g of TEOS was added to the device surface and left for 5 s before spinning at 4000 rpm for 40 s. The silicate mix was added to the device surface between 2.5 and 3 min after the end of the mixing procedure and left on the device for 30 s before spin coating as before for 40 s at 4000 rpm. The device surface was rinsed with water approximately 2 min after the end of the spin coating. Since the silicate mix is corrosive, care must be taken to use an appropriate vacuum chuck on the spin coater and to clean out of the bowl of the spin coater immediately after use. Modified devices were used for adsorption studies within 2 weeks of preparation.

Preparation of silicate layer on gold-coated device. A 20-nm gold layer was deposited on a PMMA-coated device in the region between the IDTs by thermal evaporation using an Edwards Auto 306 evaporator. The gold layer was modified with (3-mercaptopropyl)trimethoxysilane in ethanol for 10 min at a concentration of 1 mg/ml to promote adherence of the silicate layer. A silicate solution was prepared by mixing 0.25 g TEOS with 0.035 g 5.8 M HCl to give a solution with one phase; this was made up to 1 ml with ethanol and then diluted again in ethanol at a ratio of 1 : 20. The diluted solution was left in place over the modified gold for 10 min before being rinsed with ethanol and then with water.

Vesicle adsorption measurements. A continuous flow of PBS was pumped over the surface of the modified acoustic device at a rate of 0.083 ml/min. The vesicle suspensions were introduced and run over the surface of the acoustic device until

the acoustic signal had reached a steady state. The device was then rinsed for 5 min with PBS before being regenerated by the addition of 0.1% w/v Triton X-100 for 10 min followed by 20 min of PBS rinse.

Atomic force microscopy images. Glass microscope slides (BDH) were coated with PMMA and silicate in the same manner as the acoustic devices. Images were acquired with a Nanoscope III Multimode AFM (Digital Instruments, Santa Barbara, CA) operating in tapping mode under ambient conditions using silicon probes with a spring constant of 42 N/m (NCH Pointprobes, Nanosensors, Germany). The scan speed was typically 1–2 Hz with an rms amplitude set at 0.7 V.

Fluorescence microscopy. Devices used for the acoustic measurements were examined by fluorescence microscopy after the deposition of the lipid vesicles. A Nikon microscope was used with a B-2A filter block (λ_{ex} 450–490 nm; $\lambda_{\text{em}} > 505$ nm). A small spot on the sample was illuminated with the UV source and left to bleach; after this it was left in the dark for 10–15 min.

CVD of silica layer. A 100 nm thick silica layer was deposited directly on the polymer surface by rf plasma enhanced chemical vapor deposition using a capacitively coupled system (Oxford Instruments DP80) operating at a frequency of 13.56 MHz. A source gas mixture of SiH_4 , N_2O , and He was used at a pressure of 1000 m Torr. The substrate was heated to 350°C during the deposition.

THEORETICAL BACKGROUND

A brief description of the acoustic wave device and principles of the acoustic measurements are presented below for those readers not familiar with this technique.

Acoustic waves can be generated and detected on the surface of a piezoelectric solid by using a set of interdigitated transducers. An alternating current is applied to transducers at one end of an acoustic device, producing an acoustic shear wave on the underlying quartz surface via the piezoelectric effect. The acoustic signal moves across the device surface before being converted back to an electric signal by a matching set of transducers at the other end of the device. The wavelength of the acoustic wave is determined by the repeat distance of the interdigitated transducers which is in this case 45 μm . The frequency of operation is determined by the wavelength and by the acoustic shear velocity of the medium through which the wave is transmitted.

In the case where the acoustic wave consists predominantly of a shear horizontal component, i.e., the wave is polarized in the plane of the surface, it is possible to apply a liquid sample on the surface of the device without significantly damping the wave. Instead, the liquid sample in contact with the piezoelectric surface will oscillate with the surface, giving rise to an evanescent acoustic field at the solid/liquid interface. Oscillation inside the liquid will be carried out into the solution to a distance determined by the frequency (f) of the wave and the viscosity (η) and

density (ρ) of the solution according to $\delta = (2\eta/\rho\omega)^{1/2}$, where ω is the angular frequency and is equal to $2\pi f$. Any changes in the liquid entrained within δ which will affect the mass and viscosity at the interface will change the propagation characteristics of the wave (19). In addition, due to the piezoelectric nature of the solid, an evanescent electric field also exists at the solid/liquid interface, which is affected by the ions and dipoles in the solution.

In practice, changes in the properties at the interface will be detected by measuring the efficiency and velocity of the shear wave transmitted on the device surface where efficiency is given by amplitude in dB and velocity is related to the phase. Amplitude changes are indicative of viscoelastic changes at the solid/liquid interface; phase changes are mainly related to changes in the mass at the interface, although they will also be affected by bulk viscoelasticity changes.

In this work we are using the Love wave device which is the most sensitive shear acoustic wave device reported so far (20). It is based on a waveguide structure consisting of a solid substrate overlaid by a material of a lower shear acoustic velocity than that of the substrate. Waveguide geometries are particularly sensitive to surface effects since they concentrate the acoustic energy preferentially in the overlayer, thus, circumventing the problem of energy scattering inside the bulk of the piezoelectric crystal. In practice, a polymer-coated piezoelectric device was used to generate the waveguide geometry. The operating frequency of the PMMA-coated device was 103 MHz; for that frequency and a pure water sample δ is calculated to be 55 nm. The acoustic parameter monitored in the experiments described here is the insertion loss or amplitude of the wave measured in dB. Figure 1 gives a schematic representation of the surface acoustic wave device and the waveguide geometry.

RESULTS AND DISCUSSION

Surface Topography

Acoustic devices were coated with an amorphous silanol-bearing silicate gel either by direct application followed by a water rinse to give a rough surface or by spin coating after an initial spin coating with TEOS to give a smoother surface. The two application methods produced surfaces with topography illustrated in Fig. 2. Both surfaces were stable. The silicate gel would be expected to adhere well, as the PMMA adheres well to the silanol-bearing quartz substrate and the silicate gel would have the additional advantage of residual organic groups to aid interaction with the PMMA. The initial coating with TEOS can aid the formation of the smooth silicate layer since the TEOS has a contact angle on the PMMA of less than 5° and therefore spreads readily over the PMMA-coated device. The TEOS can then hydrolyze when in contact with the acidic gel mixture and condense with the silicate gel in solution.

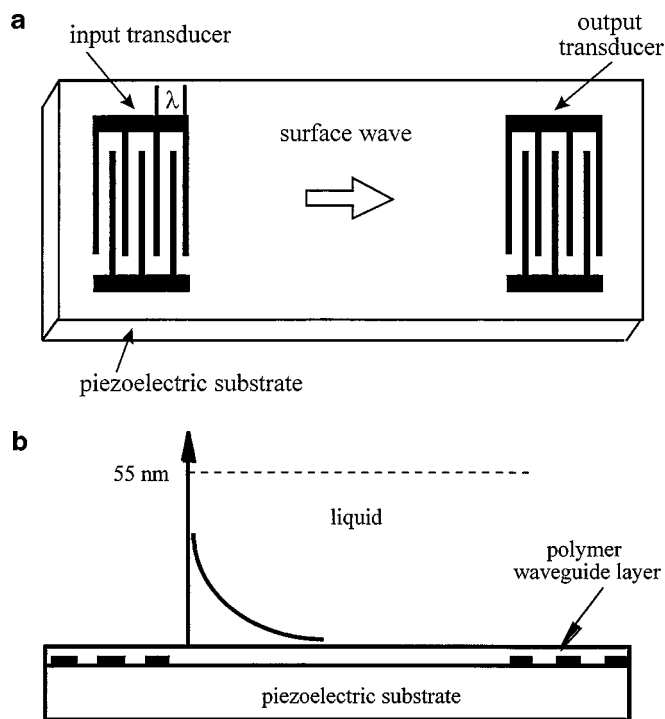


FIG. 1. Schematic representation of the (a) surface acoustic wave device; the surface wave is generated by the input and detected by the output interdigital transducer (IDT) and the wavelength λ is $45 \mu\text{m}$; (b) acoustic waveguide liquid sensor; a polymer layer ($\sim 1 \mu\text{m}$) is deposited on the surface of an acoustic device which supports shear horizontal waves. Loading the polymer layer with a liquid sample results in an acoustic evanescent field which extends $\delta = 55 \text{ nm}$ into the aqueous solution.

Vesicle Deposition and the Acoustic Response

The acoustic response as two successive rounds of 0.03 mg/ml POPC vesicles are deposited on and washed off a smooth silicate layer is shown in Fig. 3. Amplitude is shown as original data rather than as net change in order to show the strength of signals

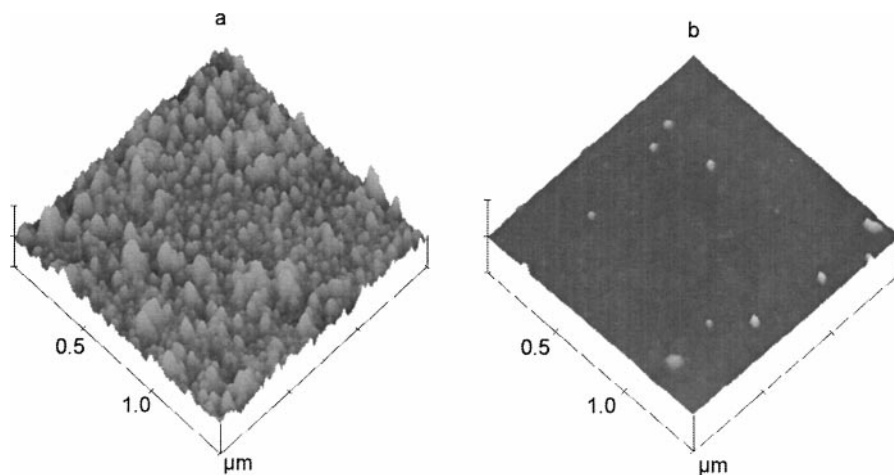


FIG. 2. Surface of silicate deposited on PMMA imaged by tapping mode AFM in air showing (a) a sample prepared by direct application of silicate gel to PMMA-coated glass and (b) a sample prepared by spin coating with silicate mixture following a spin-coating step with TEOS. Vertical scale is 25 nm .

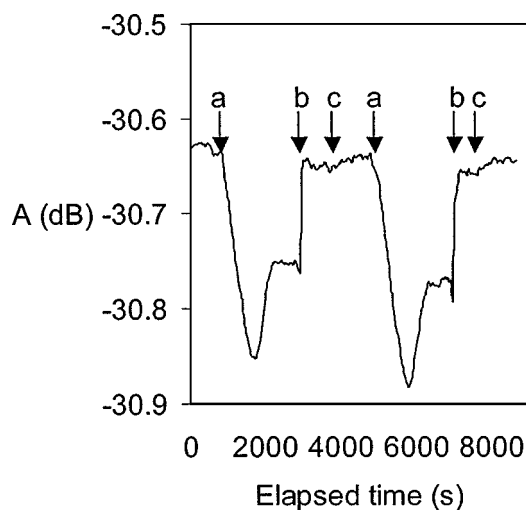


FIG. 3. Acoustic response as two successive rounds of 0.03 mg/ml POPC vesicles are deposited on and washed off smooth silicate. The start of the POPC vesicle deposition is indicated by a, the start of the detergent rinse by b, and the start of the buffer rinse by c.

acquired during a typical analysis. The frequency spectrum for the device used to collect data shown in Fig. 8 is shown in Fig. 4. Data shown in Fig. 8 were collected at 103.950 MHz .

The initial feature of the amplitude response is the decrease observed as the liposomes are deposited on the silicate surface, indicating a decrease in the efficiency of transmission of the acoustic signal. This is followed by a return to a higher amplitude signal as the deposition of vesicles continues, a pattern that has been previously associated with the transition to the more elastic bilayer (15). As the deposition of liposomes continues, the signal eventually reaches a steady state, which, in this work, is taken to be indicative of the formation of a complete lipid layer. Since the amplitude response gives a clearer indication of the processes occurring on the surface, it was used to determine the conditions required to form a complete bilayer. Addition of detergent causes

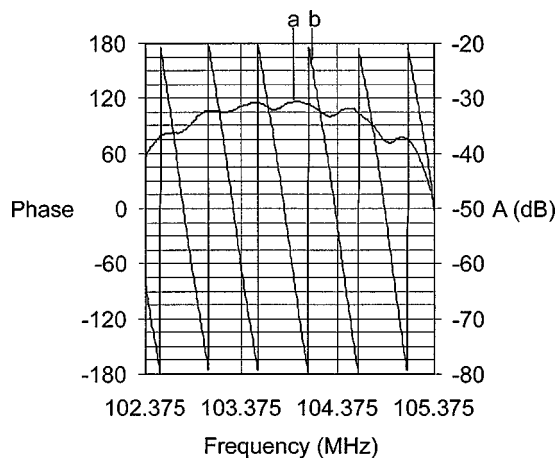


FIG. 4. Frequency response showing (a) amplitude and (b) phase as a function of frequency for a silicate gel-modified device in buffer.

an initial small drop in the signal as the detergent disrupts the adsorbed lipid layer and then an increase in the signal as the lipid layer is rinsed off the device surface. Some additional time is required for the detergent to be rinsed off.

The two rounds of deposition and rinsing show the degree of reproducibility of the signal. The surface is not in fact completely regenerated by the detergent and buffer rinses: successive rounds of deposition of a 0.03 mg/ml POPC vesicle suspension lead to an increase in the time required to reach a steady state in the signal after the start of vesicle deposition. During five rounds of vesicle deposition at 0.03 mg/ml on one device, the time required varied by 7%. The variation for measurements made on different devices was slightly less than 20%; this increased

variability may reflect some differences in surface roughness on the smooth silicate layer. Several devices were used during the course of these experiments but for precise comparisons measurements were made on one device. The observed patterns of data were then confirmed using additional devices.

Fluorescence Images

Recovery of fluorescence after the photobleaching indicates lateral diffusion such as would occur with a bilayer; lack of recovery indicates a vesicle layer. The presence of a bilayer on the smooth silicate was confirmed by demonstrating fluorescence recovery after photobleaching (FRAP) using a fluorescence microscope; images acquired immediately after bleaching and then after a recovery period are shown in Fig. 5. The line visible on the image acquired after recovery does not appear to be due to a scratch on the underlying surface; such a scratch would be expected to impede lateral diffusion (2a), which does not seem to have been a problem in this case.

Comparison of Vesicle Deposition on Rough and Smooth Silicate

The acoustic response associated with vesicle deposition on the rough and smooth silicate surfaces is shown in Fig. 6. Deposition on the rougher surface resulted in a monotonic decrease in signal amplitude with a relatively large maximum signal change; this is as expected for the formation of a viscoelastic vesicle layer (15, 16). On the smoother silicate surface, the initial signal change follows the same pattern as for deposition on the rougher surface. The minimum of the acoustic response when POPC is deposited on the smoother silicate

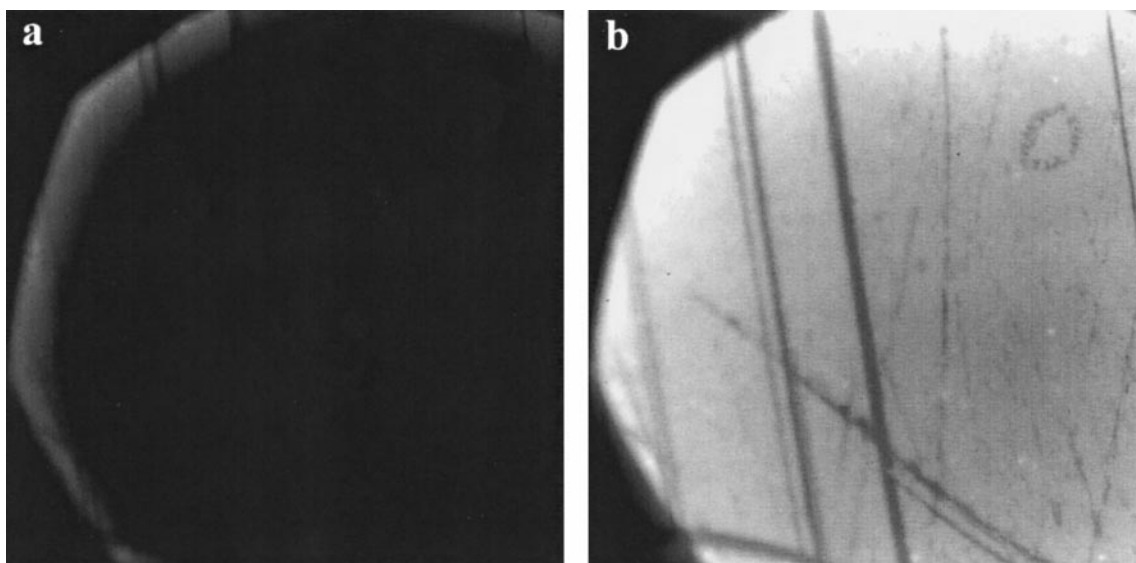


FIG. 5. Fluorescence microscopy images showing recovery after photobleaching for POPC deposited on an acoustic device with a smooth silicate layer. Photograph (a) acquired immediately after the bleaching step shows a very low fluorescence intensity except at the edges of the diaphragm where there has already been some recovery. Photograph (b) acquired approximately 10 min later shows recovery of fluorescence to the middle of the bleached area.

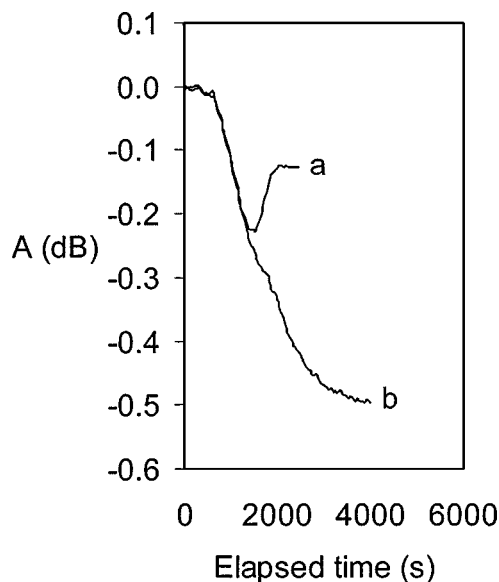


FIG. 6. Change in amplitude A (insertion loss) of the acoustic response in dB as a function of time as POPC vesicles at 0.03 mg/ml are applied to the smooth silicate (a) and the rough silicate (b).

is associated with the start of bilayer formation. The time required to reach this point is related to amount of liposomes on the surface rather than being solely a function of surface dwell time for an initial population of adsorbed vesicles. This is illustrated in Fig. 7, which shows no sign of decomposition of deposited vesicles when the deposition is halted near the minimum of the acoustic response. These results are in agreement with simulation studies that have implied that vesicle decomposition is not likely to be a spontaneous event but is instead more probable near islands of bilayer (21). In order to obtain a

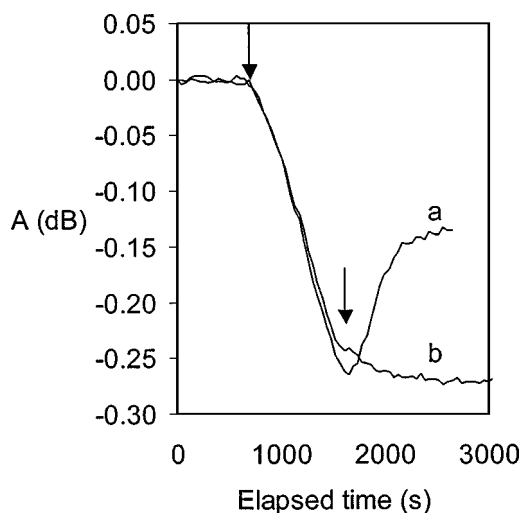


FIG. 7. Acoustic response as 0.03 mg/ml POPC vesicles are deposited on smooth silicate to form a complete bilayer (a) and with deposition halted before bilayer formation (b; vesicles deposited between times indicated by arrows).

bilayer, sufficient liposomes must therefore be applied to the surface: halting the deposition too soon may result in discrete adsorbed vesicles.

When comparing the ability of the rough and smooth silicate layers to induce bilayer formation, there are therefore two considerations. The roughness may affect the deformation of the liposomes after adsorption; it could also affect lateral interaction between vesicles if adsorption occurs at specific sites such as the peaks or troughs of the silicate layer, so that vesicles deposit with defined separation. The first consideration is likely to be more important: increasing the strength of the interaction between the liposomes and the negatively charged silanol groups on the silicate surface by incorporating stearylamine into liposomes to give them a positive charge can result in liposomes depositing as a bilayer on the rough silicate surface. Experiments carried out with vesicles containing POPC, cholesterol, and stearylamine at a 4 : 3 : 0 or 4 : 3 : 1 mole ratio resulted in the POPC and cholesterol liposomes being deposited as a vesicle layer and in the positively charged liposomes being deposited as a bilayer, as shown by fluorescence recovery after photobleaching. Typical net changes in amplitude of the acoustic signal as the 4 : 3 : 0 and 4 : 3 : 1 mole ratio liposomes were deposited were 1.2 and 0.3 dB, respectively. The smaller net change observed for the positively charged liposomes is as expected for formation of a bilayer although these numbers should not be compared directly since the charge and different lipid composition may also have some effect on the signal.

Although the results described above indicate that the rough silicate layer can be used to induce bilayer formation with some lipid compositions, the smooth silicate layer would be more generally useful.

Deposition of POPC Vesicles on Silicate Added over Gold Layer

These experiments were carried out to show that a suitable silicate layer could be deposited on a surface other than PMMA. Gold was modified with (3-mercaptopropyl)trimethoxysilane to act as an adhesive layer, with the sulfhydryl group expected to react with the gold, and the silanol groups produced after hydrolysis being able to react with the polymerized silicate. A solution of hydrolyzed TEOS, HCl, and ethanol was left in place over the modified gold to hydrolyze the (3-mercaptopropyl)-trimethoxysilane and then to leave time for the silicate in solution to condense and react with the surface.

The acoustic response as a 0.2 mg/ml suspension of 50-nm POPC vesicles was deposited on the surface was characteristic of bilayer formation: there was an initial drop of 0.45 dB followed by a rise in signal amplitude to give a net drop of 0.15 dB after the signal had reached a steady state. The presence of a bilayer could not be confirmed by FRAP due to problems associated with fluorescence quenching near a metal surface.

Coating the acoustic device with gold and exposing the waveguide to solvent decreased the efficiency of signal transmission so that the initial signal amplitude was -39 dB. This surface

modification was, therefore, not used for the other liposome deposition experiments described here but might be suitable for surface plasmon resonance measurements or other experiments where a gold layer is used. Since the thiol is used here only to promote adhesion of the silicate layer, this modification should still be effective for inducing bilayer formation if the quality of the thiol layer is poor. This is in contrast to mercaptoundecanol modifications of gold, where a good quality self-assembled layer is required to avoid deposition of liposomes as a vesicle layer (16). The silicate procedure might therefore be suitable for modifying gold surfaces that are not freshly prepared.

Time Required to Form a Complete Bilayer

The formation of a complete bilayer occupying all available space on the smooth silicate surface of the acoustic device was taken to be the point at which the acoustic signal reached a steady state. At a lipid concentration of 0.12 mg/ml, this occurred within 15 min. Figure 8 shows the time (t) required for a complete bilayer to be formed as a function of the POPC concentration for vesicles of 50 and 100 nm diameter. The time varied with vesicle concentration, occurring after a fixed amount of liposomes had flowed over the surface so that the relationship between concentration c of the liposome suspension and time t to form a complete bilayer took the form $ct = a$, where a is a constant.

This is consistent with a model (21) of a diffusion-limited adsorption rate W_{ad} given by

$$W_{ad} = [(3v_0D^2)/(xy)]^{1/3}c_0, \quad [1]$$

where v_0 is velocity of flow in the channel above the deposition surface, D is the diffusion constant for the liposomes, x is the distance across the channel through which the liposomes are flowing, y is the distance along the deposition surface parallel to the direction of flow, and c_0 is the concentration of liposomes

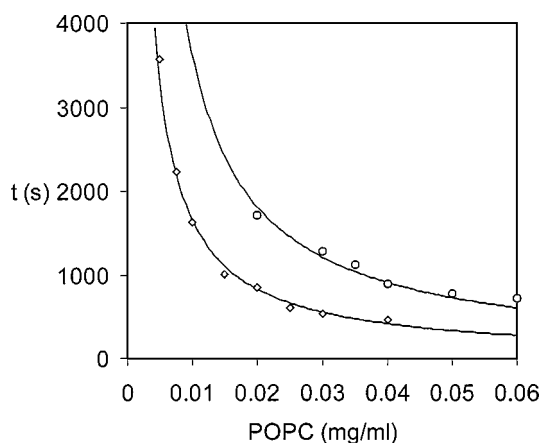


FIG. 8. Time required to form a complete bilayer as a function of vesicle concentration. Data for deposition of 100-nm vesicles is shown by open circles and the line is generated using the formula $ct = 16.57$, where $c =$ POPC concentration in mg/ml and $t =$ time in s. Data for 50-nm vesicles are indicated by open diamonds with a line generated using $ct = 36.33$.

in the bulk of the suspension. If the adsorption of liposomes continues at a constant rate throughout the formation of the bilayer then the amount adsorbed A will be given by $A = W_{ad}t$ where t is the elapsed time from the start of the deposition process. The amount of adsorbed liposomes in a complete bilayer will be fixed at a value A_1 by the available surface area; if W_{ad} is proportional to the concentration of liposomes in the bulk of the suspension then the relationship demonstrated in Fig. 8 where $ct = a$ is obtained, with the constant a being a function of v_0 , D , x , and the average y and of the total amount of liposomes in a complete bilayer. If [1] applies, then the expected relationship is $ct \propto (1/D)^{2/3}$. For uncharged spheres, the diffusion constant D is related to the radius by the Einstein-Stokes formula

$$r = \frac{kT}{6\pi\eta D} \quad [2]$$

where k is Boltzmann's constant, T is the temperature, and η is the viscosity of the medium. To a first approximation, the diameter of the liposomes will be the same as the diameter of the membrane pores used for the extrusion process so that the ratio of $(1/D)^{2/3}$ for 100 and 50 nm liposomes will be 1.6. Values obtained for ct (with c in mg/ml and t in s) are 36.3 and 16.6, respectively, for the 100 and 50 nm liposomes (see Fig. 8), giving a ratio of 2.2. If error limits of twice the standard deviation are used in the calculations then a value of 2.2 ± 0.7 is obtained, encompassing the ratio calculated above for the diffusion constants. Although the extent of the assumptions and the size of the errors mean that this comparison is of limited value it is given here to contrast with the ratio for ct values obtained when c is calculated in particles per milliliter rather than in total lipid mass. The number of particles in suspension at a given total lipid concentration can be calculated knowing the lipid density in a bilayer (4.2 ng/mm^2) (9) and by assuming that the area occupied by the lipids in liposomes is equivalent to the surface area of spheres with diameters of 50 or 100 nm. If c is given in particles per milliliter then the ct values for the 100 and 50 nm liposomes are 2.74×10^{14} and 5.03×10^{14} , respectively, giving a ratio of 0.55. The adsorption rate as measured here is therefore closer to being a function of total lipid concentration than a function of particle concentration. This is reasonable since the parameter measured by the acoustic device relates to the area covered by bilayer on the device surface; this is proportional to lipid mass irrespective of liposome diameter.

The results shown in Fig. 8 have two practical implications with regard to the efficiency of forming bilayers using the minimum required amount of lipid. Changing the total concentration of lipid in suspension will have no effect, but decreasing the diameter of the liposomes will improve the efficiency as measured in terms of the total amount of lipid in suspension flowing over a surface before a complete bilayer is formed. For the flow velocity and flow cell proportions used here, changing the liposome diameter from 100 to 50 nm changed the required total amount of lipid from 50 to 23 μg .

SUMMARY

The silicate gel layers described here provide a simple means of modifying PMMA to induce adsorption of either a POPC vesicle layer or a bilayer; the bilayers will be used in future studies of protein incorporation. The surface topography of the silicate layer and thus the structure of the adsorbed lipid layer relates to the method used to apply the silicate gel but is not dependent on the presence of specific functional groups in the underlying polymer. The procedures could therefore be extended to other substrates using an initial coating with TEOS or another silane to obtain a smooth silicate layer. Additionally, PMMA can be spin-coated onto different substrates and a silicate layer inducing bilayer formation can be added to gold, so that silicate gels should be useful for inducing bilayer formation on a variety of surfaces.

Production of a suitable surface does not by itself guarantee formation of a bilayer: liposomes must also be deposited for a long enough time at sufficiently high concentrations. Decreasing the diameter of the liposomes of the liposomes increases the efficiency of this process.

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