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Study of the Sensitivity of the Acoustic Waveguide Sensor

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The sensitivity of the acoustic waveguide sensor to mass deposition in the presence of liquid was optimized as a function of the over-layer thickness. The waveguide geometry consisted of a 0.2−2.2-μm poly(methyl)methacrylate (PMMA) over-layer deposited on the surface of a shear acoustic wave device and supported a Love wave. The response of each polymer-coated waveguide was initially assessed by monitoring the frequency and insertion loss of the device in the presence of air. Sensitivity to viscous and mass loading was studied by recording the amplitude and phase of the wave during the application of water and of a supported lipid bilayer, respectively, on the device surface. Supported bilayers are a versatile system for mass calibration in the presence of liquid because they can be formed spontaneously on a hydrophilic surface, resulting in a layer of reproducible mass density. Results clearly showed that the response of both amplitude and phase depends on the over-layer thickness and increases with the thickness of the polymer layer. Phase was generally found to be more sensitive than amplitude to both viscous water and mass loading. The maximum sensitivity to vesicles deposition was measured at 250 cm^2 g^{-1} and was detected when 1.3 μm of PMMA was used as a waveguide layer. Results showed that the sensitivity of the acoustic wave sensor can be improved by simply increasing the thickness of the PMMA and that supported phospholipid layers can form an ideal system for both mass calibration and interfacial modification.

During the last two decades, there has been an increase interest in developing surface-sensitive sensors to study biological interactions in liquid samples. Optical devices based on commercially available or in-house built systems are now used on a routine basis in most bioanalytical laboratories in order to measure the optical thickness of a thin mass layer deposited on the device surface. An emerging technique which can provide a powerful alternative to optical systems is based on acoustic devices. Acoustic wave devices have been proven to be as sensitive as optical ones and, most importantly, have been shown to simultaneously provide additional information which cannot be obtained with the existing sensors.[1,2]

Acoustic devices have traditionally been used in a gas environment, and their application in bio-sensing has just started receiving proper attention.[3] The only necessary requirement for using them in the presence of liquid is that the acoustic wave device supports a shear wave, which will ensure propagation in a solid/liquid interface without significant acoustic losses. The quartz crystal microbalance (QCM) is the most widely used device, which combines shear wave operation with simple instrumentation. Unfortunately, its low operating frequency considerably limits the sensitivity and, thus, potential applications of the system. High frequency, shear-operating acoustic wave devices can provide an attractive alternative for developing biosensors that are able to detect a wide range of analytes. Love wave (LW) and surface transverse wave (STW) devices support surface-guiding waves by utilizing a dielectric over-layer on the device surface and a metal grating between the transducers, respectively. Both systems have been shown to have high sensitivity to surface perturbation effects and have been used successfully for a number of applications, including the detection of biological analytes in direct or competitive immunosensors.[1,4−7]

The LW device exhibits the additional advantage of design flexibility because the surface-guiding effect is purely dictated by the properties and thickness of the over-layer. LW devices incorporating polymer, silica, or a combination of the above as guiding layers have been reported.[8−11] In these studies, the optimum over-layer thickness was determined after calibrating the device to the deposition of Langmuir−Blodgett and gold films in air. For biosensing applications, though, it is desirable to assess the optimum performance during the deposition of biomass in the presence of liquid. Such a study would provide a more realistic picture of the device's performance in a liquid sample because the response of the wave will also incorporate the contribution of viscous coupling as a result of the presence of the liquid layer.

The focus of this work was to investigate the sensitivity of the LW device as a function of the over-layer thickness, especially during mass deposition in the presence of liquid. The waveguide

(1) Gizeli, E.; Lowe, C. R.; Liley, M.; Vogel, H. *Anal. Chem.* **1997**, *69*, 9, 4808−13.
(2) Keller, C. A.; Kasemo, B. *Biophys. J.* **1998**, *75*, 1397−1402
(3) Ballantine, D. S.; White, R. M.; Martin, S. J.; Ricco, A. J.; Zellers, E. T.; Frye, G. C.; Wohltjen, H. *Acoustic Wave Sensors*; Academic Press: New York, 1997.
(4) Gizeli, E.; Stevenson, A. C.; Goddard, N. J.; Lowe, C. R. *Sens. Actuators* **1992**, *6*, 131−137.
(5) Tom-Moy, M.; Baer, R. L.; Spira-Solomon, D.; Doherty, T. P. *Anal. Chem.* **1995**, *67*, 1510−1516.
(6) Gizeli, E.; Lowe, C. R.; Liley, M.; Vogel, H. *Sens. Actuators B* **1996**, *34*, 295−300.
(7) Harding, G. L.; Du, J.; Dencher, P. R.; Barnett, D.; Howe, E.; *Sens. Actuators A* **1997**, *61*, 279−286.
(8) Gizeli, E.; Ph. D. Thesis, **1992**, University of Cambridge.
(9) Gizeli, E. *Smart Mater. Struct.* **1997**, *6*, 700−706.
(10) Du, J.; Harding, G. L.; Ogilvy, A.; Dencher, P. R.; Lake, M. *Sens. Actuators A* **1996**, *56*, 211−219.
(11) Du, J.; Harding, G. L. *Sens. Actuators A* **1998**, *65*, 152−159.

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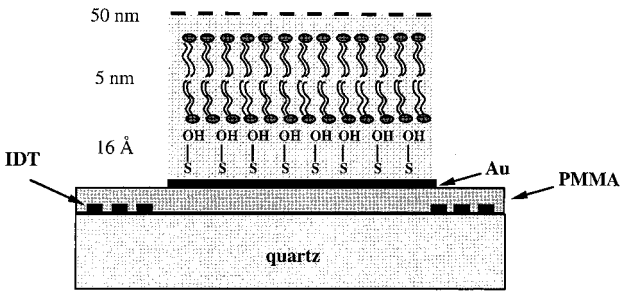

Figure 1. Schematic representation of the acoustic waveguide and the biological interface. The waveguide geometry incorporates a shear surface acoustic wave device over-layed with a polymer (PMMA) layer. The gold layer deposited on the polymer surface was modified by self-assembling a thiol layer (16 Å), followed by the spontaneous formation of a supported lipid bilayer (5 nm). For the ~107 MHz device, the acoustic evanescent field in the liquid sample is approximately 50 nm. The dimensions of the device and the interface layer are not to scale.

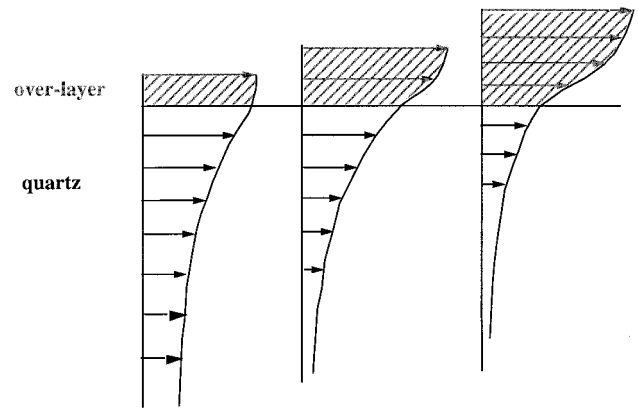

Figure 2. Schematic representation of the power flow in the polymer over-layer and the quartz substrate as a function of the over-layer thickness.

geometry used in this study incorporates poly(methyl)methacrylate (PMMA) over-layers. Polymer guiding layers were preferentially used because their very low shear acoustic velocity results in a high energy confinement on the device surface. In addition, polymer layers have several practical advantages because they can be formed on the device surface and subsequently removed by using relatively simple techniques. The formation of a supported phospholipid bilayer on the interface was used as a model system for measuring mass deposition in the presence of liquid. Supported lipid bilayers possess a number of desirable properties for this application. They can be formed spontaneously on a hydrophilic surface on application of a vesicle suspension. They result in a thin, ordered biofilm of reproducible thickness and surface density. Finally, their surface mass density can be easily measured using radiolabeled lipids and applied to calibrate the sensors' response as a function of the polymer thickness. In general, phospholipid layers are very attractive systems for biosensing applications because they can be used to provide a membrane-like environment that is suitable for the incorporation or attachment of receptors or proteins[12] and can be readily removed using a detergent solution, thereby regenerating the surface.[1]

In practice, supported vesicles of 2-oleoyl-palmitoyl-sn-glycero-3-phosphocholine (POPC) were formed on a thin gold layer, which was deposited on the surface of PMMA and modified by self-assembling a hydroxy-terminated thiol. The deposition of vesicles was studied by monitoring both the amplitude and the phase of the wave in real time. Figure 1 gives a schematic representation of the waveguide geometry and the piezoelectric crystal/liquid interface.

THEORETICAL BACKGROUND

The acoustic waveguide geometry consists of a quartz acoustic wave device, which supports a shear surface-skimming bulk wave (SSBW), over-layed by a dielectric material. If the shear acoustic velocity in the deposited layer (V_{layer}) is lower than that in the quartz (V_{quartz}) device, then the effect of the dielectric material is to convert the SSBW into a guided shear-horizontal wave, known as the Love wave; increase the coupling coefficient; and reduce the insertion loss of the device.[13−15] Polymers, such as PMMA, have been shown to be very effective wave-guiding layers due to their low shear acoustic velocity (V_{pmma} = 1100 m s^{-1}), as compared to that of quartz (V_{quartz} = 4950 m s^{-1}).[8] Another critical parameter in designing a waveguide sensor is the thickness of the guiding layer because it affects the distribution of the power flow between the quartz and the polymer. Figure 2 gives a schematic representation of the variation in the power flow as a function of the over-layer thickness for the first mode of operation. When the thickness of the over-layer is much smaller than the wavelength, λ, most of the energy is located in the quartz substrate, and the Love wave propagates with a velocity, V_{Love}, that is very close to V_{quartz}. When thicker layers are applied, but are still of a thickness smaller than λ, the energy is concentrated in the over-layer and the velocity of the Love wave tends toward V_{layer}. Between these two limits, the energy progressively transits from the quartz substrate into the layer and the velocity V_{Love} varies between V_{quartz} and V_{layer}, that is, $V_{layer} < V_{Love} < V_{quartz}$. Finally, in addition to the energy distribution, the particle displacement on the surface of the polymer layer increases with increasing the over-layer thickness, which results in structures which are more sensitive to surface perturbations.

The effect of a thin-mass layer deposited on the surface of a Love wave device is the changing of the frequency or velocity of the wave. The most useful way to describe quantitatively the effect of mass loading is by using the sensitivity formula S_m, which is defined as the fractional change of the frequency of the acoustic wave, Δ_f, as a result of mass loading, divided by the operating frequency, f_o, and the deposited mass per unit area ($h\rho$).

$$S_m = \frac{1}{f_o} \lim_{h \to 0} \frac{\Delta f}{[h\rho]} \tag{1}$$

By definition, mass deposition refers to a layer with a thickness that is negligible as compared to λ_{Love} and, thus, cannot act as a waveguide layer.

(12) Heuse, S.; Stora, T.; Schmid, E.; Lakey, J.; Vogel, H. *Biochim. Biophys. Acta* **1998**, 85507, 319−338.

(13) Tournois, P.; Lardat, C. *IEEE Trans. Sonics Ultrason.* **1969**, *SU-16* (3), 107−17.

(14) Tuan, H. S.; Ponamgi, S. R. *IEEE Trans. Sonics Ultrason.* **1972**, *SU-19* (1), 9−14.

(15) Josse, F.; Lee, L. *IEEE Trans. Sonics Ultrason.* **1982**, *SU-29* (5), 261−273.

Because the Love wave is predominantly a shear wave, 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 waveguide will oscillate with respect to the surface oscillation, giving rise to an acoustic evanescent field at the solid/liquid interface.[3] The effective liquid thickness, δ, coupled to the acoustic wave is defined as the solid/liquid interface and is given by

$$\delta = \sqrt{\frac{2\eta}{\rho\omega}} \tag{2}$$

where η and ρ are the liquid's viscosity and density and ω, the angular frequency. Liquid loading of the device surface will, thus, provide an additional wave/matter interaction mechanism which will be through viscous coupling. Equation 2 can be used to calculate an effective liquid thickness of 53 nm for a pure water sample at an operating frequency of 110 MHz. Surface perturbations that will change the mass of the interface will affect only the frequency, but perturbations that will change both the mass and viscoelasticity of the interface will affect both the frequency and the insertion loss of the wave. The modeling of the perturbation of a shear acoustic wave as a result of interfacial mass and viscosity changes has been described theoretically by a number of investigators.[16−22] However, the in situ calibration of the acoustic waveguide sensor to interfacial changes in the presence of a liquid sample has not been shown yet.

In this application, the frequency and insertion loss of the wave are monitored by following the phase and amplitude of the wave, respectively, using a network analyzer. The corresponding frequency change, Δf, can be calculated from the detected phase change, $\Delta\phi$, by using the following equation

$$\Delta f = \Delta\phi \frac{\lambda f_o}{360L} \tag{3}$$

where λ is the acoustic wavelength and L, the propagation path of the wave. Sensitivity is calculated using eq 1; but in this case, it is defined as S^{l}_{m} because mass loading takes place in the presence of liquid. In absolute values, S_m and S^{l}_{m} are expected to be different due to the effect of the viscous liquid on the device sensitivity.

EXPERIMENTAL SECTION

Device and Instrumentation. Single-crystal Y-cut (42.5°), *z*-propagating quartz was used as the piezoelectric substrate. The input and output interdigital transducers comprised a 100-Å chromium flash, followed by a 2000-Å gold over-layer. The interdigitated transducers (IDTs) consisted of 80 pairs of split fingers with a periodicity of 45 μm. Devices were patterned by conventional photolithographic techniques in the Southampton Microelectronics Centre. A Hewlett-Packard 4195A network analyzer was used to monitor the phase and amplitude of the wave, which are directly related to frequency and insertion loss, respectively. The uncoated device supports a surface-skimming bulk wave (SSBW) at 110 MHz. The temperature was held constant at 22.3 ± 0.1 °C by a peltier plate and a temperature controller. A Perspex flow-cell, a peristaltic pump, and poly(vinyl)-chloride (PVC) tubing were used to pump through liquid samples. A rubber sleeve exposing an area of approximately 23 mm^2 was used to seal the cell to the acoustic device.

Materials. 2-oleoyl-palmitoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Fluka, medium-molecular-weight poly(methyl)methacrylate (PMMA), 2-ethoxy-ethyl acetate and cyclohexane were purchased from Sigma, and mercaptoundecanol was synthesized as described in reference 23 in cyclohexane.

Polymer Coating-Gold Deposition. The device surface was cleaned in an ultrasonic bath for 10 min in each of the following solvents: 4 N NaOH, water, and ethanol. PMMA of medium molecular weight was diluted in 2-ethoxy-ethyl-acetate (Sigma). The polymer layer was applied over the entire clean surface of the device by spin-coating the appropriate polymer solution at 4000 rpm for 40 s. The film was further cured by heating the devices in a conventional oven for 1 h at 180 °C. The thickness of each film was measured by using a surface profilometer device (Dektak). After the polymer layer was prepared, the acoustic waveguide devices were placed in the vacuum evaporator chamber where a 50-nm gold layer was deposited on top of the polymer layer in the area between the IDTs by thermal evaporation at a pressure of 2×10^{-6} mbar and at a rate of 1 nm s^{-1}.

Thiol Self-Assembly. Self-assembled monolayers were formed on the freshly gold-coated devices by immersing them overnight in a 5 mM solution of mercaptoundecanol ($HS(CH_2)_{11}OH$) in cyclohexane. The devices were then washed several times with cyclohexane and air-dried under nitrogen for a few minutes. The above modification resulted in a surface with an advancing contact angle of water of less than 10°.

Deposition of Lipid Vesicle Solution. Unilamellar vesicles were prepared by extrusion. Initially, 1 mg of POPC in chloroform was put in a glass tube, and the solvent was removed by evaporation under nitrogen. The dried lipid film was redissolved in water and further extruded approximately 17 times through a 50-nm pore-size polycarbonate membrane using an Avanti extruder. The vesicle dispersion at a final concentration of 0.2 mg/mL (0.26 mM) was applied on the thiol-modified gold surface of the acoustic waveguide, followed by water solution using a flow-through cell at a flow rate of 1.4 μL/s.

RESULTS

(1) Frequency and Amplitude Response of the Waveguide Device in Air. The effect of the thickness of the polymer layer on the acoustic response of the device was initially studied in the presence of air. Figure 3 gives the change of the frequency as a function of the over-layer thickness for PMMA layers in the range of 0.2−2.2 μm. Frequency change is defined as the difference

(16) Kovacs, G.; Vellekoop, M. J.; Haueis, R.; Lubking, G. W.; Venema, A. *Sens. Actuators A* **1994**, *43*, 38−43.
(17) Kondoh, J.; Matsui, Y.; Shiokawa, S. *Jpn. J. Appl. Phys.* **1996**, *79* (3), 42−50.
(18) Wang, Z.; David, J.; Cheeke, N.; Jen, C. K. *IEEE Trans. Ultrason. Ferroelectro. Freq. Control* **1996**, *43*, 844−51.
(19) Kondoh, J.; Saito, K.; Shiokawa, S.; Suzuki, H. *Jpn. J. Appl. Phys.* **1996**, *35*, 3039−3096.
(20) Josse, F.; Shanaz, Z.; *J. Acoust. Soc. Am.* **1988**, *85* (4), 1556−1559.
(21) Kovacs, G.; Venema, A. *Appl. Phys. Lett.* **1992**, *61* (6), 639−641.
(22) Ogilvy, J. A. *J. Phys. D: Appl. Phys.* **1997**, *30*, 2497−2501.
(23) Bain, C. D.; Troughton, E. B.; Tao, Y. T.; Ewall, J.; Whitesides, G.; Nuzzo, R. G. *J. Am. Chem. Soc.* **1989**, *111*, 321−335.

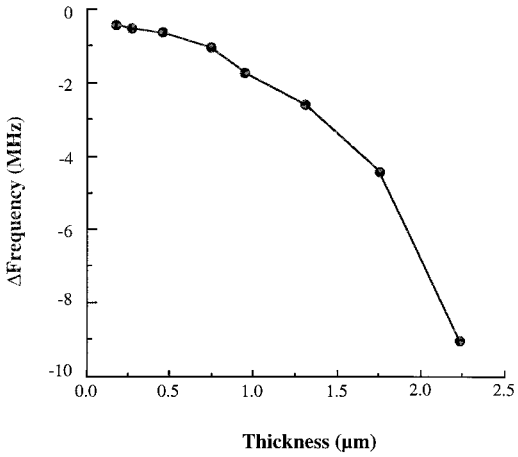

Figure 3. Frequency change of the PMMA-coated waveguide device as a function of the polymer thickness. ΔFrequency denotes the frequency difference between the coated and uncoated devices.

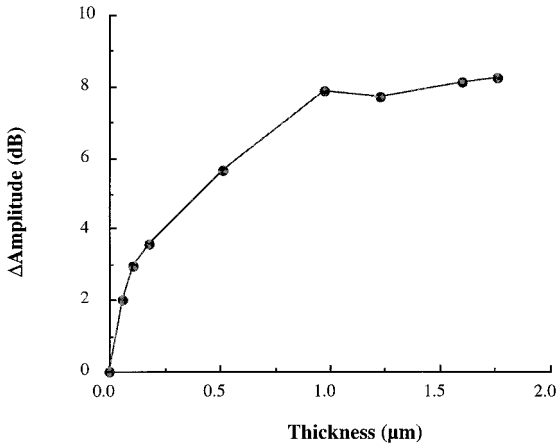

Figure 4. Change of the amplitude of the PMMA-coated device as a function of the polymer thickness. ΔAmplitude denotes the amplitude difference between the coated and uncoated devices.

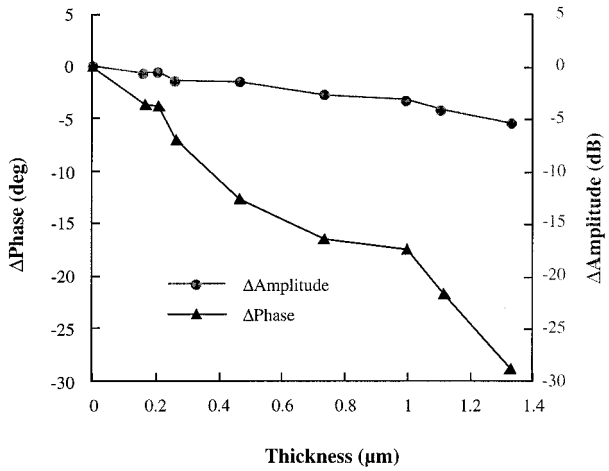

Figure 5. Phase and amplitude change upon addition of water onto the surface of the waveguide device, as a function of the over-layer thickness.

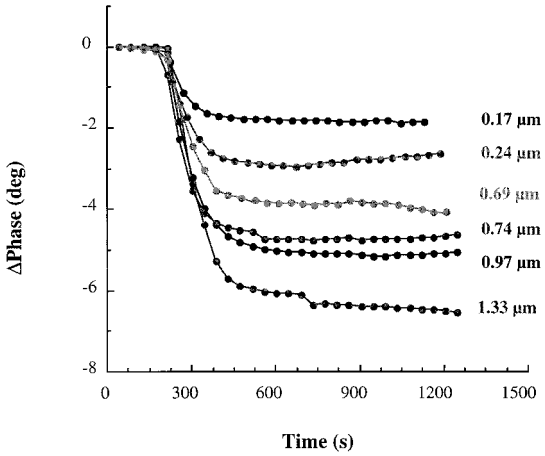

Figure 6. Kinetics of the formation of a supported lipid layer on the hydrophilic surface of five waveguide devices incorporating PMMA layers of varying thicknesses.

between the frequency of the Love wave and the uncoated device. The change of the insertion loss is detected by following the amplitude of the wave. Figure 4 gives the amplitude change for PMMA waveguide layers in the range of 0.2−1.8 μm. In a manner similar to frequency, amplitude change is defined as the amplitude of the waveguide device minus the amplitude of the uncoated device. The insertion loss of the uncoated device was typically around −26 dB. Both frequency and amplitude measurements were performed by using the network analyzer.

(2) Sensitivity to Liquid and Mass Loading; Kinetics of Mass Deposition. For those experiments in which the device was in contact with liquid, the device surface was modified by depositing a 50-nm gold layer which was made hydrophilic by self-assembling a hydroxy-terminated thiol layer. Figure 5 gives the phase and amplitude change of the LW during the application of water solution on the hydrophilic waveguide surface as a function of the polymer thickness. Each point corresponds to equilibrium which, in all cases, was reached almost instantaneously. Following water loading, a 0.2 mg/mL vesicle suspension of POPC in PBS was applied on each waveguide surface. Figure 6 shows the phase response in real time during the application of the vesicle suspension on the surface of six waveguide devices incorporating PMMA layers of differing thickness. The stable baseline in the beginning of each experiment corresponds to the acoustic signal when the device is in contact with water. In all cases, equilibrium was reached after 5 min of the vesicle injection followed by water rinse. The overall phase and amplitude change during POPC deposition was measured and is given in Figure 7 as a function of the over-layer thickness. Each point in Figure 7 was calculated after equilibrium took place, which was after approximately 15 min.

DISCUSSION

The construction of a surface-sensitive acoustic waveguide device requires optimization of the waveguide structure. Polymer over-layers have been shown to be a very effective waveguide layer due to the polymer's low shear acoustic velocity.[9] In this study, the effect of the polymer thickness is assessed under various conditions.

In the beginning, the performance of each polymer-coated waveguide device was assessed by recording the frequency and amplitude of each device in the presence of air. According to the mass-to-frequency sensitivity of acoustic wave devices, polymer-loading of the SSBW device is expected to affect the operating frequency of the wave. This is clearly shown in Figure 3, in which

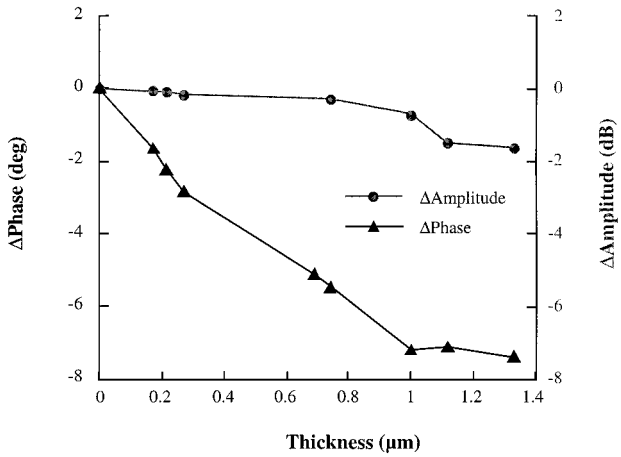

Figure 7. Phase and amplitude changes during the formation of a supported lipid bilayer on the hydrophilic surface, as a function of the over-layer thickness.

frequency decreases as the thickness of the polymer increases. In practice, the SSBW frequency peak at 110 MHz on the uncoated device shifts to lower frequencies when PMMA layers are deposited on the surface. The peak corresponding to the Love wave is detected between 109 and 101 MHz for polymer layers with a thickness between 0.2 and 2.2 μm, respectively. The decrease of the frequency is direct evidence of the decrease of the shear velocity of the Love wave when thicker polymer layers are applied, which is in accordance with theoretical predictions

In addition to the frequency decrease, the mode conversion and surface guiding of the LW can be monitored directly by following the amplitude of the wave. The positive amplitude change that is shown in Figure 4 indicates that the waveguide devices are less lossy than the SSBW device because more acoustic energy is detected by the transducers as the wave is guided to the over-layer and the upper part of the piezoelectric substrate. Furthermore, Figure 4 shows that amplitude change increases sharply with increasing the PMMA over-layer thickness for a thickness up to 1 μm and then reaches a plateau for thicknesses between 1 and 1.8 μm. For polymer layers between 1.8 and 2.2 μm thick, amplitude becomes considerably larger than that of the uncoated device until the wave disappears from the spectrum when the polymer becomes thicker than 2.5 μm (data not shown). This can be explained by taking into account acoustic losses inside the polymer layer, which become significant when thicker layers are applied on the device surface. Generally, Figures 3 and 4 can be used as a guideline for assessing the efficiency of other overlay materials to act as a waveguide layer and support a Love wave.

To fabricate sensitive acoustic waveguide devices for detecting biomolecular interactions, it is important to assess the effect of the over-layer thickness on mass deposition in the presence of liquid. For this reason, waveguide devices incorporating PMMA layers of varying thicknesses but with identical surface properties have been fabricated by depositing a thin gold layer on top of each PMMA waveguide. Gold was further modified using a self-assembled layer of a hydroxy-terminated thiol. The gold layer also acts as a shield for eliminating the unwanted acousto-electric interaction between the piezoelectric substrate and the liquid sample.[24] The sensitivity of each acoustic waveguide device to viscous loading was evaluated when water was applied on the gold-coated surface. According to Figure 5, the change in both signals depends on the PMMA thickness and becomes larger as the latter increases. Figure 5 also shows that phase is more sensitive than amplitude to water loading. It is worth mentioning that dielectric or conducting liquids such as water or buffer, respectively, will still interact with the electric field that is generated by the IDTs, which will not be eliminated in the above structure.[25] However, in contrast to what other investigators have reported,[10] in this case this interaction was not significant because the liquid was placed on top of the gold layer and between the IDTs. The above geometry allows stable operation in liquid with an average insertion loss of −35 dB.

The application of a vesicle suspension on the thiolated gold surface was used as a model system for the in situ deposition of a mass layer on the solid/liquid interface. It has been shown that the interaction of a vesicle suspension with a solid surface may result in the formation of a supported vesicle bi- or monolayer. The end result depends on a number of parameters, such as the roughness, charge, and hydrophilicity of the solid surface and the chemical properties of the lipids.[2,26−28] It has been shown that the interaction of a vesicle suspension with a hydrophilic surface results in the spontaneous self-assembly of a lipid bilayer on the hydrophilic surface.[1,2,6,29] Furthermore, the surface density of the bilayer was measured using ^{14}C-labeled lipids and found to be 4.2 ng mm^{-2}.[26] The above mass density can be used for calibrating the acoustic signal response to mass deposition in the presence of liquid.

The ability to follow the kinetics of interaction of a biological solution, such as a vesicle suspension, with a solid surface is very important for studying biomolecular interactions in real time. In this case, the kinetics of the formation of a lipid bilayer on the hydrophilic surface can be followed by monitoring both the phase and the amplitude of the wave. Figure 6 gives the phase as a function of time when a POPC vesicle suspension was applied on the surface of the hydrophilic gold-coated waveguide devices that incorporated PMMA layers of differing thicknesses. Because all PMMA-coated devices have the same surface properties, the differences in the devices' responses can only be attributed to the PMMA thickness. According to Figure 6, as the polymer thickness increases, the overall phase change due to lipid adsorption increases, too. Regardless of the over-layer thickness, the kinetics of the binding is very similar and consists of two phases, that is, an initial fast phase followed by a smooth transition to a slower second phase. The amplitude also varied with the polymer thickness, indicating that amplitude can be used for the real-time monitoring of the viscoelastic properties of the interface (data not shown).

(24) Ricco, A. J.; Martin, S. *J. Thin Solid Films* **1991**, *94*, 206.
(25) Leidl, J.; Oberlack, I.; Schaber, U.; Mader, B.; Droist, S. *Smart Mater. Struct.* **1997**, *6*, 680−688.
(26) Nollert, P.; Kiefer, H.; Jahning, F. *Biophys. J.* **1995**, *69*, 1447−1455.
(27) Tjarnhage, T.; Puu, G. *Colloids Surf., B* **1996**, *8*, 39−50.
(28) Melzak, K.; Ralph, E.; Gizeli, E. To be published.
(29) Liley, M.; Bouvier, J.; Vogel, H. *J. Colloid Interface Sci.* **1997**, *194*, 53−58.
(30) Rickert, J.; Brecht, A.; Gopel, W. *Anal.Chem.* **1997**, *69*, 1441−1448.
(31) MacMullen, C.; Mehta, H.; Gizeli, E.; Lowe, C. *J Phys. D: Appl. Phys.* In press.

The overall phase and amplitude change corresponding to the formation of a supported lipid bilayer is shown in Figure 7 as a function of the waveguide structure. The increase of the change of both signals with the PMMA thickness is consistent with what was observed during water loading (Figure 6). The largest phase change detected was with the 1.3-μm PMMA-coated device. Given that the surface density of the deposited POPC vesicles is 4.2 ng mm^2, S^1_m is calculated to be 250 cm^2 g^{-1}. This figure is more than the S_m observed during mass loading of a similar waveguide device in the presence of air.[8] The enhanced mass sensitivity in the presence of liquid has been both experimentally observed[10,27] and theoretically predicted.[28] An interesting observation, though, is that the change of the ratio (Δampl/Δphase) during water and vesicle loading is not stable but increases with increasing PMMA thickness. The above implies that there may not be an equal contribution from viscous and mass loading to the phase signal, which is the only parameter that depends on both.

Given a phase stability of 0.01 deg, the detection limit (3 $\times$ noise/sensitivity) of the 1.3-μm PMMA-coated biosensor is calculated to be 1.7 ng cm^{-2}. Further improvement of the device response could be achieved by using hybrid waveguide devices consisting of silica/PMMA over-layers, which have been shown to exhibit the highest sensitivity in the presence of air.[11] Finally, it is worth mentioning that the above analysis and conclusions are true for all PMMA waveguide biosensors. However, the absolute values can only apply to this particular system because the magnitude of the phase and amplitude changes depend on the operating frequency, initial loss, and history of the acoustic wave device.

CONCLUSIONS

The response of the acoustic waveguide sensor to viscous water and mass loading in the presence of liquid was assessed as a function of the waveguide structure. Mass loading involved the spontaneous formation of a lipid bilayer on the hydrophilic surface. Supported lipid bilayers provide an excellent model system for calibrating the Love-wave biosensor, because a mass layer of well-defined structure and surface density is spontaneously formed on a hydrophilic surface. The sensitivity of both amplitude and phase to water loading and lipid deposition was shown to depend strongly on the over-layer thickness. In addition, phase change, which depends on both mass and viscous loading was found to be larger than amplitude change, which is only sensitive to viscous loading. It was shown that the sensitivity of the Love biosensor can be enhanced without having to increase the operating frequency of the device or add any labels. The maximum sensitivity observed during mass deposition in the presence of liquid was with the 1.3-μm PMMA-coated device and was 250 cm^2 g^{-1}.

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