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Vesicle Adsorption on Mesoporous Silica and Titania

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Lipid bilayer formation via vesicle fusion on mesoporous silica and mesoporous titania was investigated using quartz crystal microbalance with dissipation monitoring (QCM-D) and fluorescent recovery after photobleaching (FRAP). Results showed that lipid bilayers were formed on mesoporous silica and that intact vesicle adsorption was obtained on mesoporous titania. From the FRAP results, it could be concluded that the lipid bilayer was fluid; however, it had a smaller diffusivity constant compared to bilayers supported on a nonporous silica.

Introduction

Phospholipid bilayers have properties that are similar to those of the cell membrane. They are of interest in the development of sensing, drug delivery, and catalysis devices.[1,2] One promising application, that has been explored by our research group, is to use lipid bilayers as hosts for transmembrane proteins that can function as sensing elements in biosensing devices.[3−5] Traditionally, such biosensors are constructed using either supported lipid bilayers (SLBs)[6] or they rely on an aperture spanning membrane.[7] Different advantages exist with the two designs; the SLBs provide high mechanical stability to the membrane, while the aperture spanning membrane results in an environment that is more similar to that of the cell membrane, that is, having a high lateral lipid fluidity and available space both above and below the membrane. A successful biosensor design would be a combination of these two concepts, where fluidity, space, and stability are provided to the membrane.

One promising proposal is to use mesoporous materials as lipid bilayer supports, where the pore walls provide stability to the membrane and the pores would result in a desired lipid environment. The properties of mesoporous materials, such as pore size, pore geometry, and surface chemistry, can be tailored, which allows us to precisely design the support for the desired application.[8] Previous studies have shown that it is possible to obtain pore-spanning lipid bilayers on mesoporous particles, using, for example, cryo transmission electron microscopy (cryo-TEM) and small-angle X-ray scattering (SAXS).[9−11] However, the mechanisms behind the lipid bilayer formation and the lateral lipid fluidity, which is a very important property of the bilayer, are still unexplored. The aim of the present work was to study the vesicle adsorption, followed by vesicle rupture, to form lipid bilayers on mesoporous silica and mesoporous titania using quartz crystal microbalance with dissipation monitoring, QCM-D,[12] and to investigate the fluidity of the lipids using fluorescent recovery after photobleaching, FRAP.[1,13] The results were compared to those obtained on nonporous substrates.

Experimental Details

Small unilamellar vesicles were prepared from purchased chloroform solutions of POPC (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (25 mg/mL), Avanti Polar Lipids, Alabaster, AL). The chloroform was removed under vacuum using a rotary evaporator resulting in a thin lipid film. The lipid film was hydrated with PBS buffer (0.010 M PBS buffer with 0.140 M NaCl and 0.0027 M KCl, Aldrich, pH 7.4) to a concentration of 5 mg/mL and sonicated using a bath sonicator for 10 min. The lipid mixture was extruded using a mini extruder (Avanti Mini-Extruder, Avanti polar lipids, Alabaster, AL) utilizing polycarbonate membranes having pore sizes of 100, 50, and 30 nm, 21 times for each membrane. The obtained vesicles were ∼30 nm in diameter as measured by dynamic light scattering (DLS) (Zetasizer nano-ZS Malvern Instruments, Worcestershire, U.K.); results not shown. Vesicles used for the FRAP measurements were prepared by the addition of 1 wt % of the fluorescent labeled lipid probe, rhodamine-DHPE (1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, Invitrogen, Carlsbad, CA). Prior to the QCM-D and FRAP investigations, the vesicles were diluted using PBS buffer to a final concentration of 0.05 mg/mL.

Cubic mesoporous thin films were prepared according to the method described by Alberius et al.[8,14] Mesoporous silica was prepared by dissolving 10.4 g of tetraethyl orthosilicate (TEOS, 98%, Aldrich) in a solution containing 5.4 g of diluted hydrochloric acid (0.01 M) and 12 g of ethanol (200 proof) at room

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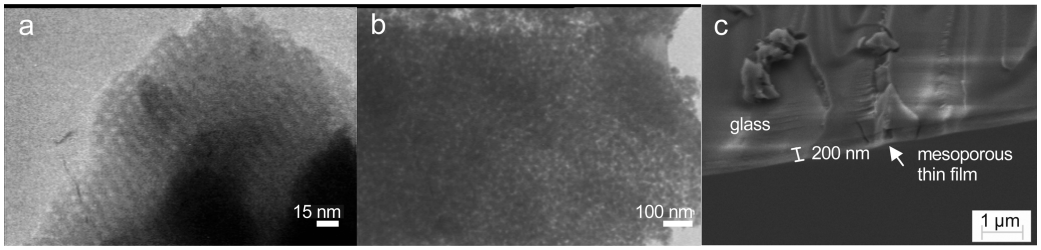

Figure 1. TEM micrographs of cubic mesoporous silica (a) and titania (b); SEM micrograph cross section of a silica thin film (film thickness ~200 nm) deposited on glass (c). In the TEM micrographs, the pores are visible as bright spots in the darker areas. From their arrangements, it could be concluded that the structure was cubic.[14]

temperature under vigorous stirring for 20 min. The solution was mixed with a solution containing 1.70 g of the triblock copolymer P123 (Aldrich) dissolved in 8 g of ethanol. In the case of preparing mesoporous titania, 4.2 g of titanium(VI)tetraethoxide (TOT, 95%, Aldrich) was added to 3.2 g of concentrated hydrochloric acid (12.1 M) under vigorous stirring for 5 min at room temperature. The solution was mixed with a solution containing 1.00 g of P123 dissolved in 8 g of ethanol (200 proof). Thin films were prepared by spin coating, 4000 rpm, onto glass substrates (microscopy glass slides) and AT-cut QCM-D crystals (purchased from Q-Sense AB, Gothenburg, Sweden) using a photoresist spinner (model 4000, Electronic Micro Systems, Salisbury, U.K.). The films were aged for 24 h in a relative humidity of 90%[15] followed by a heat treatment step, where the temperature was increased from room temperature at a rate of 1 °C/min to 400 °C, where it was left to dwell for 4 h. The formed mesoporous thin films were characterized using scanning electron microscopy, SEM (Leo Ultra 55 FEG SEM, Leo Electron Microscopy, Cambridge, U.K.), transmission electron microscopy, TEM (JEM-1200 EX II TEM operated at 120 kV, JEOL, Tokyo, Japan), and nitrogen adsorption (Micromeritics Tristar, Norcross, GA).

Using QCM-D, it is possible to measure the interactions between vesicles and the surface of a sensor crystal in real time. The vesicle−surface interaction is observed as a change in resonance frequency (Δf) and in dissipation (ΔD) of the coated sensor crystal, where Δf is related to the adsorbed mass (including coupled water) and ΔD is related to the frictional (viscous) losses in the adlayer.[16] The resonance frequency and dissipation were measured at the 5, 15, 25, and 35 MHz harmonics simultaneously. The frequency change is calculated according to $\Delta f_{\text{norm}} = \Delta f_n/n$, with n being the overtone (n = 1, 3, etc.). The adsorbed mass, Δm, of a rigid film can be calculated using the Sauerbrey relation, $\Delta m = -C\Delta f_n$ with the mass-sensitive constant, C = 17.7 ng·cm^{-2}·Hz^{-1} for 5 MHz.[17] The QCM-D measurements were performed using Q-sense D300 and E4 (Q-sense AB, Gothenburg, Sweden) instruments. The E4 was equipped with a QAFC 301 axial flow chamber. QCM-D crystals (14 mm) coated with silica and titania (Q-sense AB, Gothenburg, Sweden) were used. Mesoporous titania films were deposited on the titania coated crystals, and mesoporous silica films were deposited on the silica coated discs.

Using FRAP, the diffusion coefficient (D) of the lateral lipid diffusion can be obtained by quickly focusing an intense laser on a small region of a sample, which causes local irreversible photochemical bleaching of the fluorophores. If the lipids in the bilayer diffuse, the bleached fluorophores will mix with the unbleached fluorophores. The time it takes for the bleached area to be recovered is used to determine D of the fluorophores, which is referred to as the fluidity of the lipids. The diffusion coefficient is calculated according to $D = 0.224(w^2/\tau_{1/2})$, where w is the radius of the bleached area and $\tau_{1/2}$ describes half of the recovery time.[13] In this study, an inverted Nikon Eclipse Ti-E microscope (Nikon Corporation, Tokyo, Japan) equipped with an EMCCD Andor iXon camera (Andor Technology, Belfast, Northen Ireland) was used.

Results and Discussion

The formed silica and titania films had an ordered mesoporous cubic structure, as can be seen in the TEM micrographs in Figure 1a and b. The pores had a narrow size distribution with an average pore diameter of 39 Å for mesoporous silica and 37 Å for mesoporous titania, according to nitrogen adsorption analysis (see Supporting Information for details).[18,19] The thickness of the films was ~200 nm, as shown in the SEM micrograph in Figure 1c.

QCM-D crystals were successfully coated with mesoporous material without any loss of functionality, which was evidenced by the QCM-D results; see below. The QCM-D results are presented in Figure 2 as measured frequency and dissipation as a function of time obtained for the different investigated surfaces. The results clearly show that lipid bilayers are formed on both the mesoporous and nonporous silica (Figure 2a). This adsorption behavior is typical for spontaneous vesicle adsorption followed by vesicle rupture, where vesicles first adsorb on the surface until reaching a critical concentration and then rupture to form a solid-supported lipid bilayer.[20] When an adsorbed vesicle ruptures, hydrodynamically coupled solvent in the vesicle's interior is released, resulting in an increased frequency (decreased mass) and a decreased dissipation (lower degree of viscoelasticity). The total change in frequency was 26 Hz for the nonporous silica and 24 Hz for the mesoporous silica. Previous studies of lipid bilayer formation on nonporous silica have shown Δf values of 26 Hz, which according to Sauerbrey's relation equals the mass of a lipid bilayer.[20,21] The difference in observed final frequency shift of 2 Hz is believed to be an effect of the water that is bound to the surface.[22] When the bilayer forms on a substrate, water will be entrapped between the substrate and the lower leaflet of the bilayer, contributing to the observed frequency shift. When water-filled pores are present, it might be that less water is entrapped between the bilayer and the surface, due to less available solid support, and hence a smaller contribution to the frequency shift might be observed. Furthermore, if the bilayer is deflected down into the pores, the contribution from the entrapped water would

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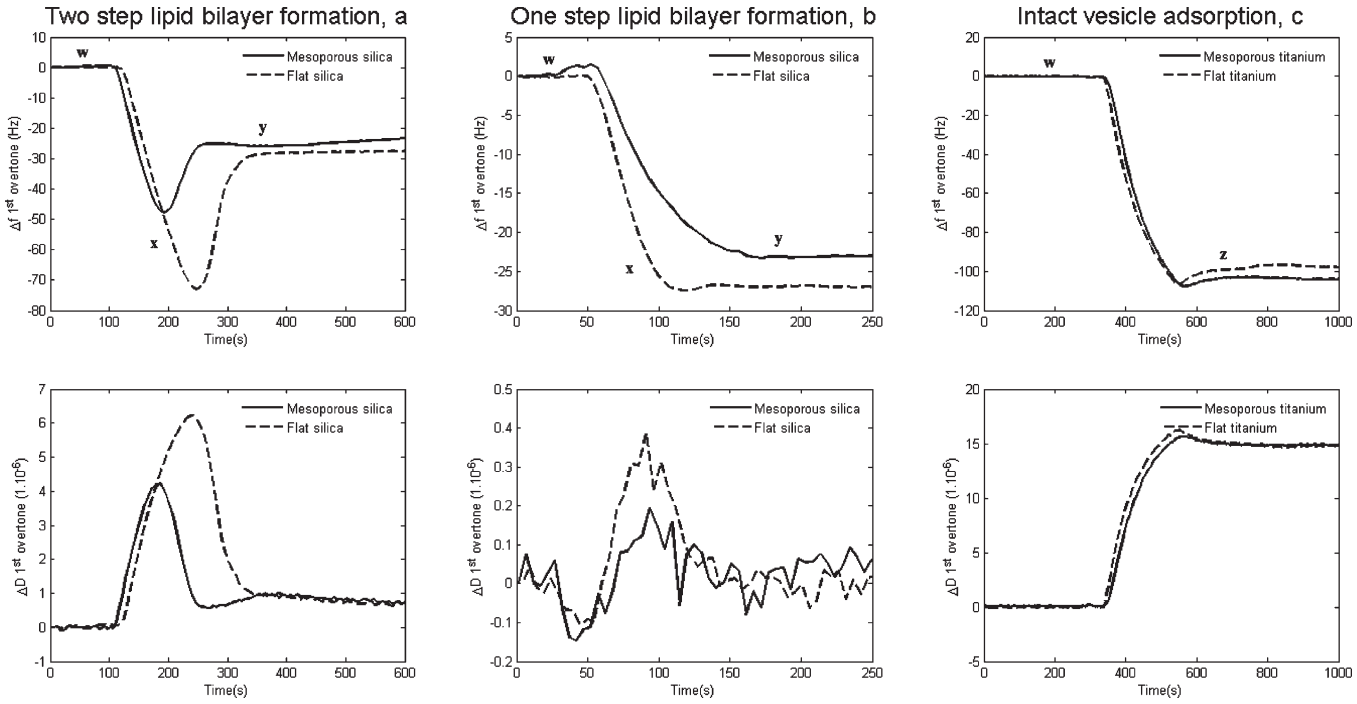

Figure 2. Results obtained using QCM-D which illustrate the formation of lipid bilayers on mesoporous and nonporous silica surfaces (a,b) and intact vesicle adsorption on titanium surfaces (c). Lipid bilayers are formed more rapidly on mesoporous silica than on nonporous silica according to (a) and (b). In (a), vesicles were prepared and dispersed in PBS buffer, which resulted in lipid bilayer formation via a two-step process, and in (b) vesicles were prepared in pure water and dispersed in PBS buffer just before injection in the QCM-D instrument, which resulted in lipid bilayer formation via a one-step process. Vesicle injection, *w*, vesicle rupture, *x*, lipid bilayer formation, *y*, and intact vesicles adsorption, *z*, are indicated in the figures.

be even less. Also the kinetics of the bilayer formation differed between the porous and the nonporous. As can be seen in Figure 2a, the bilayer formation is more rapid on the mesoporous silica compared to on the nonporous one. QCM-D measurements were also performed using vesicles prepared in pure water followed by mixing with PBS buffer immediately before injection in the QCM-D chamber (Figure 2b). As can be seen, the lipid bilayers are formed via immediate rupture of the vesicles. This behavior is an effect of the increased osmotic pressure caused by the different ionic strengths between the inside and outside of the vesicles.[23] Here the kinetics was more rapid on nonporous silica compared with porous silica. The reasons for this difference are still unknown, but the fact that no buildup of adsorbed vesicles is needed for lipid bilayer formation on the substrates, in the case when there is an osmotic pressure present, tells us that the pores play an important role in the vesicle−vesicle-surface interactions. The observed changes in dissipation, both for the vesicle rupture after reaching critical vesicle concentration and for the case with immediate rupture, indicate that the formed bilayers are rigid and have low viscoelasticity, and that no difference exists between the porous and nonporous substrates. In Figure 2c, QCM-D results obtained from the mesoporous and nonporous titania are presented. As can be seen, the frequency decreases down to ∼100 Hz and remains constant, and the dissipation is increased followed by a constant positive value (15×10^{-6}). These results are typical for intact vesicle adsorption, which often is observed on titania.[24,25] However, formation of lipid bilayers on titania has been obtained by other groups when, for example, Ca^{2+} has been present.[26,27] Silica has a lower isoelectric point (IEP ≈ 2[28]) compared to titania (IEP ≈ 4−5[28]), which means that it has a higher density of charges on the surfaces. As a consequence, the vesicles are exposed to higher stress/strain forces on silica, which leads to lipid bilayer formation.[23,29] The difference between silica and titania may also be explained by the thickness of the water layer that is formed on hydrophilic surfaces.[23] Water molecules are more tightly bonded to surfaces that are highly charged, which give rise to a thinner layer of water close to the surface. Accordingly, vesicles undergo a larger deformation on silica than on titania since they can get closer to the surface.

In Figure 3, typical FRAP results are presented. The figure shows the recovery of a photobleached area of rhodamine-DHPE on nonporous silica (Figure 3a) and on mesoporous silica (Figure 3b). Using the image analysis software developed by Hook's group,[1] the diffusion coefficients (D) were calculated, $\langle D \rangle = 2.78\ \mu m^2/s \pm 0.06$ ($n = 6$) on nonporous silica and $\langle D \rangle = 2.01\ \mu m^2/s \pm 0.04$ ($n = 6$) on the mesoporous silica. Total recovery was obtained on both silica surfaces. Jonsson et al. also obtained a decreased diffusivity of lipids on porous silica compared with nonporous silica, and they believed that this was an effect caused by the walls of the pores.[30] However, the same conclusion cannot be made from our results because the pore diameter is ∼4 nm, which is less than the thickness of a lipid bilayer (∼5 nm). As a consequence, it is physically impossible for the lipid bilayer to follow the silica wall into the pores. The

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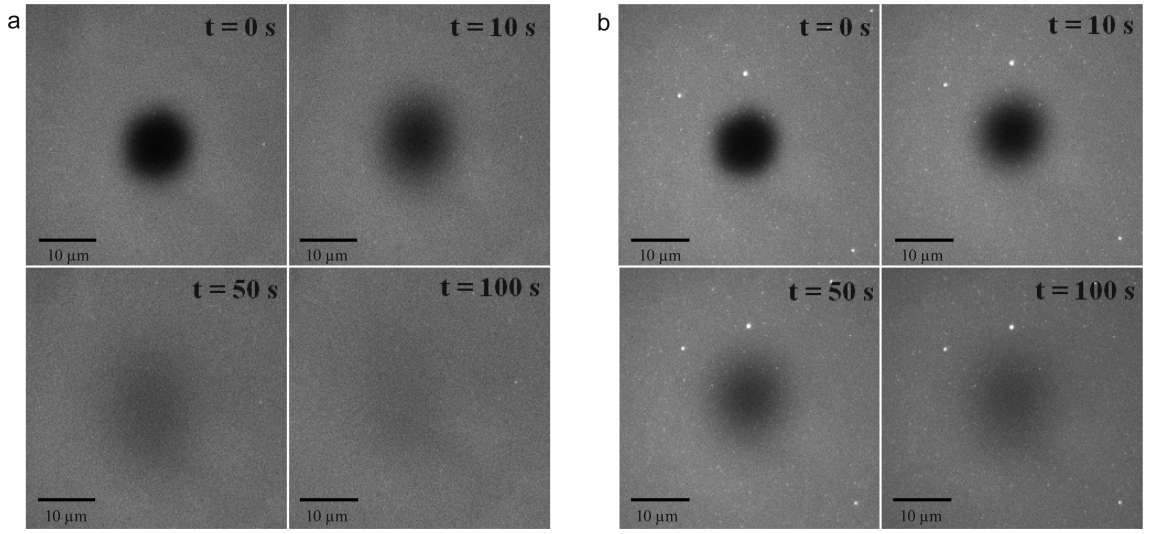

Figure 3. Results obtained using FRAP illustrating the recovery of a photobleached area of rhodamine-DHPE on nonporous silica (a) and mesoporous silica (b). Total recovery was observed on both silica surfaces; however, the diffusivity was lower on mesoporous silica than on nonporous silica.

decreased diffusivity is more likely an effect of the bilayer being slightly deflected into the pores; however, this has not been confirmed. The FRAP results also correlate well with the QCM-D findings. The more rapid bilayer formation on the mesoporous substrates indicates a stronger interaction between the substrate and the bilayer when mesopores are present.

Conclusion

QCM-D and FRAP results showed lipid bilayer formation on silica and intact vesicle adsorption on titania, regardless of porosity. According to the QCM-D results, bilayer formation was more rapid on the mesoporous films compared to that on the nonporous films, when no salt concentration difference across the membrane was present. FRAP analysis showed that the bilayer was fluid both on the mesoporous and on the nonporous materials; however, the diffusion constant was lower on the porous materials. Mesoporous silica is considered to be a promising support for lipid bilayers in biosensing devices.

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Supporting Information Available: Nitrogen adsorption isotherms and calculated pore diameters for mesoporous titania and mesoporous silica. This material is available free of charge via the Internet at http://pubs.acs.org.