The effect of temperature on supported dipalmitoylphosphatidylcholine (DPPC) bilayers: Structure and lubrication performance

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Abstract

Phospholipids fulfill an important role in joint lubrication. They, together with hyaluronan and glycoproteins, are the biolubricants that sustain low friction between cartilage surfaces bathed in synovial fluid. In this work we have investigated how the friction force and load bearing capacity of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) bilayers on silica surfaces are affected by temperature, covering the temperature range 25–52°C. Friction forces have been determined utilizing the AFM colloidal probe technique, which showed that DPPC bilayers are able to provide low friction forces over the whole temperature interval. However, the load bearing capacity is improved at higher temperatures. We interpret this finding as being a consequence of lower rigidity and higher self-healing capacity of the DPPC bilayer in the liquid disordered state compared to the gel state. The corresponding structure of solid supported DPPC bilayers at the silica–liquid interface has been followed using X-ray reflectivity measurements, which suggested that the DPPC bilayer is in the gel phase at 25°C and 39°C and in the liquid disordered state at 55°C. Well-defined bilayer structures were observed for both phases. The deposited DPPC bilayers were also imaged using AFM PeakForce Tapping mode, and these measurements indicated a less homogeneous layer at temperatures below 37°C.

Keywords:
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1. Introduction

Phospholipids have been argued to be main lubricating molecules in joints and in other organs, as e.g. pleura or pericardium, where lubrication is necessary for vital functions [1]. Indeed, phospholipids are found at relatively high concentrations, 0.1–0.2 mg/ml in synovial fluid [2], and they have been reported to form multilayer structures on cartilage surfaces [3]. Among the phospholipids found in joints, DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) is the most abundant saturated phospholipid [4] and therefore it is a natural choice to be investigated for elucidating the role of phospholipids in biological surface lubrication. Lipid bilayers have been extensively investigated using various...
techniques [5,6]. We know that the phase in which we find supported phospholipid bilayers depends on many factors among them type of lipid and temperature are important ones [7,8]. For freely floating fully hydrated DPPC four distinct phases – subgel (Ls), gel (Lg), ripple (P) and fluid (Ld) – have been described [9]. The acyl chains in the DPPC gel phase are characterized by ~32° tilt with respect to the bilayer normal [10]. The molecular arrangement in the ripple phase is more complex. It consists of two types of domains, described as splayed gel and gel-like, respectively, with disordered lipids found in the concave part of the kink between the domains [11]. In the fluid phase the DPPC chains are disordered. The properties of supported phospholipid bilayers are somewhat different since they are affected by the presence of the surface. For instance, it has been shown that in supported DPPC bilayers the transition temperature can be shifted by a few degrees compared to what is found for non-supported DPPC bilayers [12–14]. The properties of supported phospholipid bilayers, such as resilience to mechanical damage [15], have also been studied. Trunfio-Sfargiu et al. have shown that DPPC lipid bilayers in the solid phase generate friction coefficients as low as 0.002 up to a pressure of 1 MPa (which is comparable to the friction coefficient in synovial joints ranging from 0.002 to 0.006) [16]. These supported lipid bilayers, and the associated friction coefficient, were reported to be stable through extended time periods. The authors drew the conclusion that lower friction coefficients were correlated with larger resilience to AFM tip penetration [15]. In their later study of supported phospholipid bilayers in fluid state composed of dioleoyl phosphatidylcholine (DOPC) Dekkiche et al. found that while unbuffered NaCl solution improves the mechanical resistance of the DOPC bilayer to normal breakthrough by the AFM tip as compared to pure water solution, the lubrication is not improved. However, in Tris buffered NaCl solution DOPC bilayers show both enhanced resistance toward normal penetration as a result of improved bilayer cohesion and also low (0.035) friction coefficient due to increased bilayer–bilayer repulsion [17]. One important insight of this study is that the mechanical resilience of the phospholipid bilayer against normal load applied by a sharp AFM tip is not directly translated to better lubrication performance. Friction forces between surfaces coated with phospholipid liposomes have been investigated by Goldberg et al. who found very low friction forces, and explained this finding as being due to a hydration layer between the phospholipid headgroups [18,19].

In this paper we report how temperature affects the structure of bilayers formed at the silica–water interface as probed by X-ray reflectivity and AFM imaging. We observe a gel-to-liquid disordered transition, and explore how the lubrication performance is affected by the state of the bilayer. Our data demonstrate that low friction coefficients can be achieved both between DPPC layers in the gel state and in the liquid disordered state. Notably, the load bearing capacity is found to increase with increasing temperature, which we suggest is a consequence of the increased fluidity that facilitates self-healing of small defects in the bilayer.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Avanti polar lipids (catalogue No. 850355P) in powder form, and used as received. Sodium chloride (assay ≥ 99.8, catalogue No. 31434) and phosphate buffered saline tablets (catalogue No. P4417) were purchased from Sigma–Aldrich. The tablets were used for preparing 150 mM phosphate buffer saline solutions containing 2.7 mM potassium chloride, 137 mM sodium chloride, 10 mM Na2HPO4, and 1.8 mM KH2PO4. Chlorform (assay ≥ 99.5%, catalogue No. C2432) was purchased from Sigma–Aldrich, and used for dissolving DPPC powder. The water used in all experiments was purified by a Millipore system. The purified water had a resistivity of 18.2 MΩ cm at 25 °C, and the total organic carbon content was less than 3 ppb.

Silicon wafers with a 100 nm thick SiO2 layer (Wafernet, Germany) were used as the flat substrates in AFM imaging studies. They were cut into size, 13 × 13 mm², prior to experiments. For AFM force and friction measurements, the silica surfaces were slightly roughened by ultrasonication in 2% Hellmanex solution (Hellma, USA) for 30 min (Bandelin Sonorex Digitec, output power 640 W). This was done in order to increase the friction force between the bare silica surfaces, and thus more clearly visualize the lubricating effect of the DPPC bilayer. AFM images of the silica surface before and after roughening are provided in the Supporting Information. For the XRR measurements silicon wafers with a much thinner (10–15 Å as quantified by XRR) oxide layer and a size of 7.5 × 7.5 mm² were used as flat substrates. Both types of silicon wafers were cleaned by immersion into 2% Hellmanex solution (Hellma, USA) for 30 min. Next, they were rinsed with large amount of Milli-Q water, and dried with a gentle nitrogen flow. The surfaces employed in XRR measurements were always used directly after cleaning.

2.2. Preparation of solutions

Small DPPC vesicles were prepared by the sonication method [20]. First, the desired amount of DPPC powder was dissolved in a small amount of chloroform (~0.5 mL). The solvent was then evaporated under a gentle nitrogen flow by rotary evaporation in order to form an even, thin lipid film on the bottle walls. A water jet pump was used to reduce the pressure and facilitate removal of any residual chloroform. Next, phosphate buffer saline (PBS) or NaCl (150 mM) solution, was added and the mixture was vortexed for 2 min and then allowed to stand for an hour at 55 °C. This solution was placed into an ultrasonic bath (Bandelin Sonorex Digitec, output power 640 W) and sonicated for 60 min until the dispersion became almost clear. The solution was then diluted to 0.5 mg/mL and sonicated for another 15–30 min until totally transparent.

The average DPPC vesicle size was determined by dynamic light scattering and found to be around 110 nm in diameter [21]. The temperature was kept at 55 ± 2.5 °C during the whole preparation process. The vesicle solution was stored in a thermostated container at 55 °C, and used in the subsequent experiment within 4 h. A pH of 7.3–7.6 for the final vesicle stock solution in PBS was determined. PBS buffer solution without DPPC was first degassed for 2 min and then allowed to stand for an hour at 55 °C. The pH of this PBS solution was 7.5.

2.3. Quartz crystal microbalance with dissipation (QCM-D)

A quartz crystal microbalance with dissipation monitoring (Qsense Omega Auto, Biolin Scientific, Sweden) was employed to follow DPPC deposition onto AT-cut silica sensors (QSX303, fundamental frequency of 5 MHz, Biolin Scientific, Sweden). The sensors were cleaned by first immersing them into 2% Hellmanex (Hellma, USA) solution for 30 min, followed by rinsing with a large amount of Milli-Q water. The sensors were subsequently dried under gentle nitrogen flow. The solutions were injected into the measuring chamber by an integrated pump using a flow rate of 20 μL/min. The adsorption was monitored at a temperature of 47 °C by following changes in resonance frequency, Δf, of the sensor and dissipation factor, ΔD, which describes the coupling between the sensor and its environment. A large value of ΔD is found for thick and viscoelastic layers whereas thin and rigid layers
result in small $\Delta D$ values. The sensed mass, $\Delta m$, was calculated using the Sauerbrey equation: [22]

$$\Delta m = -C\frac{\Delta f}{n} \tag{1}$$

Here $C$ is a constant that depends on the density and thickness of the quartz crystal and equals 0.177 mg m$^{-2}$ Hz$^{-1}$ for our crystals, and $n$ is the overtone number. This relation is valid when adsorption leads to small changes in $\Delta D$ [23], as was the case in our study.

2.4. AFM forces and friction measurements

All forces acting between a DPPC coated roughened silica surface and a DPPC coated µm-sized silica particle were measured by employing a Multimode 8 Pico Force atomic force microscope (Bruker, USA). Rectangular tipless cantilevers (CSC12-F, Mikro-Mash, Estonia) with approximate dimensions of 250 µm in length and 35 µm in width were used in the experiments. The values of the normal ($k_n$) and torsional ($k_t$) spring constants were obtained by utilizing the AFM tune IT v2.5 software (Force IT, Sweden) and the thermal noise method [24,25]. After calibration of the spring constants, a silica particle with a diameter of about 7 µm was glued to the tipless cantilever using a two-component epoxy resin (Araldite from Huntsman, UK), an Ependorf Micromanipulator 5171 and a Nikon Optiphot 100S reflection microscope. This microscope was also used for determining the size of the colloidal probe. Prior to experiment, cantilevers with glued particles were cleaned by UV irradiation for 15 min (output 15 mW/cm², BioForce, US). For all experiments the cantilever deflection never exceeded the range where the detector response is close to linear [26]. The experiments were performed in liquid environment inside a fluid cell (MTFML, Bruker, USA, volume ≈ 100 µL). The desired temperature (25–52 °C) was achieved by a heating controller (Veeco, USA). The liquid cell and the heating controller were also used in AFM PeakForce experiments. We note that the surface temperature in the cell is slightly lower than the temperature set by the control unit. The real surface temperature was in a separate experiment determined for each set-point temperature, using a temperature sensor attached to the surface, and it is the surface temperature that is relevant and thus reported in this work.

All surface force measurements were performed at a constant approach and retraction velocity of 400 nm/s. At this velocity the hydrodynamic force can be considered negligible [27]. The zero position is defined as the hard wall contact (constant compliance region) and does not contain any information on the bilayer thickness. The friction measurements were performed by sliding the surfaces forward and backward 10 times at each load. The sliding distance was 1 µm in each direction and the scan rate was 0.2 Hz, giving a sliding speed of 400 nm/s. The slow scan axis was disabled during the experiments to ensure continuous sliding over the same line, and the data were analyzed by using the program AFM Force IT (Force IT, Sweden).

2.5. AFM PeakForce imaging

A Multimode Nanoscope V (Bruker, USA) instrument in PeakForce Tapping mode was utilized for recording topographical images of DPPC bilayers adsorbed on flat silica surfaces in contact with aqueous solutions at different temperatures. A silicon nitride cantilever (ScanAsyst-Fluid+, spring constant 0.7 N/m, tip radius 2 nm, Bruker) was used for all imaging experiments. The value of the cantilever spring constant was determined as described above. With PeakForce tapping it is possible to collect surface morphology and surface material property data simultaneously at a controlled (low) feedback force [28,29], which is important when imaging soft matter samples. In this work we focus only on topographical information, which was recorded using a peak force of 500 pN. The NanoScope Analysis (Version 1.20, Bruker) software was employed to analyze the recorded AFM PeakForce data. The height images were flattened to remove surface tilt. A scan rate of 1 Hz was used in all experiments.

2.6. X-ray reflectivity, XRR, measurements

The X-ray reflectivity measurements on the silica–liquid interface were performed at the beamline B19, Delta, Germany [30] and the X04SA, SLS, Switzerland [31]. For the measurements a reflectivity sample cell developed for applying hydrostatic pressure was used [32]. The experiments were performed with an X-ray wavelength of $\lambda = 0.452$ Å. The structure of DPPC bilayers at three different temperatures (25 ± 0.2) °C, (39 ± 0.2) °C and (55 ± 0.2) °C was investigated. For each measurement a fresh sample was used, and the pressure in the hydrostatic sample cell was 60 bar.

In X-ray reflectivity measurements the specular reflected intensity $I$ is measured as function of the incident angle $\theta$. The scattered intensity is modulated by the sample’s electron density perpendicular to the surface ($\rho_f$) by: [33]

$$I(q) = R_f \left| \frac{1}{\rho_f(z \to \infty)} \int_{-\infty}^{\infty} \frac{dp_e}{dz} e^{iqz} dz \right|^2 \tag{2}$$

Here $q$ denotes the wave vector transfer perpendicular to the surface which is given by:

$$q = \frac{4\pi}{\lambda} \sin(\theta) \tag{3}$$

$R_f$ denotes the Fresnel reflectivity (the reflectivity of a perfectly flat surface) and $z$ is the position perpendicular to the sample surface.

Lipid bilayers were prepared on silicon wafers with a thin oxide layer by vesicle fusion at 55 °C. The wafers were immersed into 150 mM NaCl solution containing 0.5 mg/ml DPPC vesicles and kept in this solution for 10 min, after which they were rinsed with 150 mM NaCl. Subsequently the DPPC covered wafers were transferred into the sample cell, ensuring that the wafers remained covered with liquid at all times.

The reflectivity data were modeled using the Parratt algorithm [34] in combination with the effective density [35] model to account for interfacial roughness. For this system a model consisting of 6 layers (Fig. S4 in Supporting Information) in total was constructed and then fitted to the data.

3. Results and discussion

We start this section by providing information on DPPC adsorption on silica surfaces. Next, the structure of the deposited DPPC bilayer at different temperatures as determined by X-ray reflectivity measurements is reported. These measurements provide detailed information on the bilayer structure and how the electron density varies normal to the surface plane. The lateral variation of the bilayer structure on the surface is then addressed using AFM PeakForce tapping. Next, we describe the surface forces acting between DPPC bilayers on silica surfaces at different temperatures, and then we address friction forces between such layers.

3.1. Adsorption of DPPC

The adsorption of DPPC vesicles on silica surfaces and their break-up into a bilayer structure has been reported in detail in our previous work [21,36], where DPPC vesicles were adsorbed from 155 mM NaCl solution. In this work we also consider adsorption from 150 mM PBS. Thus, it is of interest to compare adsorption from these two solutions, and in Table 1 we report the adsorption
2. The corresponding adsorbed mass, as recorded terminal group, tail-group with the terminal CH₃ group, headgroup, tailgroup, CH₃ parts of the sample in the following order starting from the silicon: silicon dioxide, headgroup, tailgroup, CH₃ headgroup, tailgroup. The parameters used for describing each of these layers and a sketch of the layer system are provided in the supporting information, see Table S1 and Fig. S4. The thickness of the silicon dioxide layer was measured before sample preparation, without a DPPC bilayer, in order to minimize the number of fitting parameter and ambiguities in the fitting process.

The density model shows that the DPPC bilayer at 25 °C has a total thickness of 5.6 nm. The electron density profile nicely reveals the bilayer structure with one headgroup oriented toward the water phase and the other one attached to the silicon wafer. The tailgroups with the terminal CH₃ region are located between these two headgroup layers. At a temperature of 39 °C some slight changes in the bilayer structure occur. The electron density of the CH₃ region and the roughness of the DPPC–water interface increases. This is a consequence of the increased thermal energy of the chains that increases the chain mobility as the temperature is increased. Most importantly the layer thickness is the same at 25 °C and 39 °C.

A further increase in temperature to 55 °C changes the structure of the DPPC bilayer more significantly. The thickness of the bilayer decreases to 4.8 nm and the roughness increases. The decrease of the layer thickness shows that the bilayer at 55 °C is in the liquid disordered state, whereas the bilayers held at 39 °C and 25 °C are in the gel phase. Similar results, showing a smaller bilayer thickness than at 25 °C and 39 °C, were obtained using neutron reflectivity [37]. Due to the higher temperature the bilayer starts to melt, which decreases the layer thickness as also observed by AFM imaging [38]. Furthermore, the single lipid molecules are much more mobile in the liquid disordered state and therefore the electron density profile of the bilayer structure becomes smeared out, which is observed as an increased roughness.

3.2. X-ray reflectivity

The Fresnel normalized reflectivity data for DPPC bilayers at the three different temperatures investigated is shown in Fig. 1 along with the fits. The curves show regular oscillations that suggest the formation of a well-defined layer system. The reflectivity profiles obtained at 25 °C and 39 °C show only minor differences, whereas a clear shift of the first minimum to higher q-values can be observed for the curve measured at 55 °C. This indicates that the DPPC bilayer at 55 °C has a smaller thickness than at 25 °C and 39 °C.

Fig. 2 shows the modeled electron density profiles. For a proper fit a model with 6 layers had to be used. The layers represent the parts of the sample in the following order starting from the silicon: silicon dioxide, headgroup, tailgroup, CH₃ terminal group, tailgroup, headgroup. The parameters used for describing each of these layers and a sketch of the layer system are provided in the supporting information, see Table S1 and Fig. S4. The thickness of the silicon dioxide layer was measured before sample preparation, without a DPPC bilayer, in order to minimize the number of fitting parameter and ambiguities in the fitting process.

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3.3. AFM imaging

The surface morphology of DPPC bilayers at different temperatures is illustrated in Fig. 3. We focus on images over areas of 1 × 1 μm² that provide information on the homogeneity and roughness of the layers rather than detailed molecular images of individual phospholipids. The DPPC bilayer was deposited on the silica surface at a temperature of 52 °C by exposing the surface to a 0.5 mg/mL DPPC vesicle solution in 150 mM PBS for 10 min. The DPPC vesicle solution was then replaced by pure 150 mM PBS solution and the images were recorded in this solution. The images recorded at 52 °C and on cooling to 47 °C and 37 °C are similar, showing gentle height variations. A further cooling to 32 °C renders the surface more coarse and small grainy structures are clearly seen in the image recorded at 25 °C. Heating the bilayer again to 32 °C does not change the morphology back to that observed at the same temperature as on cooling, suggesting a hysteresis during the time scale of the measurement (the time

<table>
<thead>
<tr>
<th>Deposition condition</th>
<th>∆f/n (Hz)</th>
<th>∆D × 10⁻⁶</th>
<th>Sensed mass (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150 mM PBS</td>
<td>24.4 ± 1.8</td>
<td>0.4 ± 0.3</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>155 mM NaCl</td>
<td>26 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>4.6 ± 0.1</td>
</tr>
</tbody>
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between recording images at the different temperatures is about 30 min). Heating to 37 °C and above changes the morphology toward that observed at these temperatures prior to cooling.

We note a striking difference between the structural transitions observed by XRR and by AFM imaging. In XRR we observe a gel to liquid disordered structural transition above 39 °C. In contrast, in the AFM images the samples at 52 °C and 37 °C are in the same morphological state whereas a structural transition occurs as the temperature is reduced even further, and a similar result has been reported for phospholipid bilayers on mica [39]. Thus, the transition observed by AFM imaging occurs at lower temperature than that observed by XRR. This suggests that the kinetic energy of the tapping AFM cantilever tip is partly transferred to the supported bilayer, and that this energy is sufficient to cause an observable shift in the phase transition to a lower temperature. Indeed, it has been observed that the values of onset and end of transition from gel $L_g$ to liquid disordered $L_d$ phase for mica supported DPPC bilayers shifted to slightly lower temperatures in effect of the force exerted by the AFM tip [12]. It has also been observed that aggregate structures of cationic surfactants on mica surfaces reported by AFM imaging are inconsistent with XRR data for the same systems [40], and this was suggested to be due to the perturbing effect of the tapping AFM tip. Based on these considerations we propose that the XRR data reflects the unperturbed bilayer structure, whereas the AFM images report structures that are affected by interactions with the tip, which in our case lowers the gel to liquid disordered transition temperature.

At sufficient low temperatures, at or below 32 °C, the bilayer imaged with AFM remains in the gel phase and small domains are visible. Since the area per molecule is smaller in the gel phase compared to the liquid disordered phase, formation of domains upon cooling is expected as no DPPC is present in bulk solution and thus no additional adsorption can occur. The height variation across the surface is somewhat larger when the grainy structure is observed, see Fig. 3.

One way to characterize the temperature dependence of the surface morphology is to quantify the roughness, e.g. using the root mean square, $R_q$, value defined as:

$$R_q = \sqrt{\frac{1}{n} \sum_{i=1}^{n} y_i^2}$$  \hspace{1cm} (4)

Here $y$ is the height, relative to the plane that defines the average height as zero, at each pixel in the image and $n$ is the number of pixels. The $R_q$ roughness of the bare silica surface was found to be around 0.2 nm, decreasing to about 0.15 nm after forming the DPPC
bilayer on the surface at 52 °C (see Fig. 4). This suggests that the DPPC bilayer spans some of the topographical features of the original silica surface.

The roughness remains approximately constant as the temperature is decreased from 52 °C to 37 °C. However, when the temperature is decreased further to 32 °C and 25 °C a significant increase in roughness is observed as the grain-like structure shown in Fig. 3 develops. The roughness decreases again when the temperature is raised to 37 °C or above. Again, we note a striking difference between the XRR data that demonstrate increased roughness at higher temperatures and the AFM images that report decreased roughness at higher temperatures. The clue to this difference is that the bilayer investigated by XRR is unperturbed, whereas the AFM images show a bilayer that is compressed by the tip. At low temperatures, in the gel phase, the bilayer is stiff and does not deform much under compression (see also Fig. 5). The bilayer is more easily deformed under compression in the liquid disordered state, which smears out topographical differences. In contrast, XRR measurements report the increased mobility of the molecules as an increase in roughness. In this context we note that the thickness of DPPC bilayers deposited on mica in the gel state as determined by AFM is similar to our XRR data on silica. However, the AFM thickness of DPPC bilayers on mica in the liquid disordered state is significantly lower than reported by XRR on silica in this study [39]. This observation can be rationalized by the notion that XRR report structures of unperturbed bilayers whereas AFM report structures of the bilayers under compression of the AFM tip.

A 3 × 3 µm² AFM image, recorded at a temperature of 32 °C, where some bilayer defects can be seen is reported in Fig. 5. Such defects could be noted at some areas where the temperature was low, ≤32 °C and this is in agreement with data showing hole formation in supported phospholipid bilayers in gel phase [41,42]. We could not find any similar defects at temperatures above 37 °C, suggesting that the defect density decreases with increasing temperature. Fig. 5 also shows two height lines crossing some of the defects. We note that the height difference is about 6 nm, which corresponds to the thickness of the DPPC bilayer including strongly bound water in the headgroup region, and this measure of the layer thickness is consistent with the value of 5.6 nm determined by XRR in this work.

Fig. 4. The Rq roughness of the silica surface, and of DPPC bilayers deposited on silica and in contact with 150 mM PBS at different temperatures. The Rq-values were calculated from 1 × 1 µm images. Filled and open circles represent the data obtained upon decrease and increase in temperature, respectively. The arrows indicate the direction of the temperature change during the experiment.

Fig. 5. AFM topographical image of a DPPC bilayer with defects in PBS buffer solution. Height lines over the regions marked with the corresponding colors on the topographical image are shown below the image. The temperature was 32 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Surface forces

The forces acting between DPPC bilayer covered silica surfaces were measured in 150 mM PBS solution at different temperatures between 25 °C and 52 °C. The force curves obtained at 25 °C and at 47 °C are displayed in Fig. 6, whereas the forces measured at 32, 37 and 52 °C are shown in the supporting information. We note that no long-range interactions, neither attractive nor repulsive, are observed at any temperature. Further, there is no hysteresis between forces measured on approach and on separation. The repulsion observed at short separations is due to a combination of hydration and protrusion forces [43,44]. The former is due to dehydration of the hydrophilic phosphatidylcholine headgroup as the two bilayers are forced together. The latter arises due to the thermal motion of the phospholipids normal to the surface, which increases with temperature as manifested as an increased roughness in XRR data, and this repulsive contribution increases with temperature. We notice this trend in the surface force curves (see Fig. 6), demonstrating more long-range repulsion at higher temperatures. This observation is also consistent with osmotic pressure measurements, using phospholipid multilayers, which demonstrate stronger repulsion in the lamellar phase than in the gel phase [45,46].

3.5. Friction forces

The friction forces measured between DPPC bilayers supported on silica surfaces are reported in Fig. 7 as a function of load at different temperatures. In all cases the friction force is very low, and we analyze the data using Amontons’ rule [47,48] that states that
the friction force, $F_f$, is proportional to the load, $F_n$, with the proportionality constant being known as the friction coefficient, $\mu$:

$$F_f = \mu F_n$$  \hspace{1cm} (5)

Good lubrication performance is achieved as long as the DPPC bilayers remain intact during the combined action of load and shear, with the friction coefficient being less than 0.03 when the temperature is at and below 47 °C. At 52 °C the data is less reliable due to temperature-induced instabilities in the AFM, and we therefore do not report a friction coefficient for this temperature even though the friction force remains low.

The maximum load, 20 nN, used in these experiments can be converted to average pressure by using the JKR model [49] as detailed in our previous work [36]. It is found that the maximum average pressure is about 42 MPa, which is close to 2 times higher than the axial load that the cartilage can sustain [50]. Thus, the load bearing capacity of the deposited bilayers under optimal conditions is large. However, in some cases we note that the bilayer structure is destroyed during shearing as illustrated in Fig. 8.

In this example the friction force determined at 25 °C remains low up to a load of around 18 nN, demonstrating that the DPPC bilayer is intact and carries the load. However, as the load increases further the friction force increases rapidly. This suggests that the load bearing capacity in this case is 18 nN and above this load the bilayer structure has been compromised by the combined action of load and shear. The failure of the bilayer activates new energy dissipative processes that increase the friction force. For instance, attractive hydrocarbon–hydrocarbon contacts may develop between the sliding surfaces and breaking and reforming of such contacts will contribute to the high friction force. When the load is decreased again, the friction force gradually decreases but it remains significantly above that observed on loading. This demonstrates that once destroyed, the bilayer does not heal again at 25 °C.

The load bearing capacity of the bilayer in a given experiment can be determined accurately from curves of the type shown in Fig. 8. However, the load bearing capacity found at a given temperature differs between different experiments and one has to use a statistical evaluation as illustrated in Fig. 9.

Despite the fact that the load bearing capacity does not have a well-defined value at a given temperature we can draw the interesting conclusion that the stability of the deposited bilayer under load and shear increases significantly as the temperature is increased. At 25 °C, when the bilayer is in the gel state, 50% of the experiments (4 out of 8) showed bilayer failure at a load of 20 nN or below. In contrast, no experiment performed at 52 °C (5 in total) and 1 of 7 experiments performed at 47 °C, where the bilayer is in the liquid disordered state, show bilayer failure at or below 20 nN. Thus, it appears that the fluidity of the bilayer above the chain melting temperature improves the load bearing capacity.

**Fig. 6.** Force normalized by radius as a function of surface separation at 25 °C and at 47 °C measured between silica surfaces covered by a DPPC bilayer across 150 mM PBS. Filled and unfilled symbols represent forces measured on approach and separation, respectively.

**Fig. 7.** Friction force between DPPC bilayer coated silica surfaces at different temperatures immersed in 150 mM PBS as a function of load. Inset: Comparison of friction forces between DPPC bilayer coated silica measured across 150 mM PBS (unfilled circles) and across 155 mM NaCl (filled circles) at 47 °C. For clarity error bars are shown only for data obtained at 25 and 47 °C.

**Fig. 8.** An example of a friction vs. load cycle measured between DPPC bilayers on silica surfaces across 150 mM PBS buffer solution. In this case the bilayer was compromised at a load of just below 20 nN. Friction forces measured on loading and unloading are shown with filled (●) and unfilled (○) symbols, respectively. The temperature was 25 °C.
by reducing the rigidity of the layer. The fluidity of the layer at these temperatures also explains the difficulty of finding imperfections in the bilayer during AFM imaging at these temperatures, and suggests a self-healing ability. Thus, if a small hole in the bilayer is caused by the action of load and shear at these high temperatures, DPPC molecules in the bilayer can diffuse, driven by surface pressure gradients, to fill the empty space. This process is less likely to occur when the bilayer is in the gel state. We note that this observation contrasts to the stability of the bilayers under compression with a sharp AFM tip. In this case a higher mobility significantly decreases the load needed to puncture the bilayer, and the resistance toward penetration by the tip is thus higher in the gel phase than in the liquid disordered state [51].

4. Conclusions

We have determined the temperature dependence of the structure of DPPC bilayers on silica surfaces and their stability under load and shear. Our data hint at that silica supported DPPC bilayers exhibit only two phases that contrast to DPPC in the bulk phase where three phases can be observed [5]. These findings are in line with DSC studies that concluded that the ripple phase is not present for solid supported bilayers, and instead a direct phase transition from the gel phase to the liquid disordered phase occurs [14,52]. AFM imaging shows a transition from a smooth surface to a grainy interface as the temperature is lowered to 32 °C. In contrast, the XRR measurements show a gel to liquid disordered transition above 39 °C where the layer thickness changes. We propose, in line with a recent report [40], that AFM imaging disturbs the layer and the kinetic energy of the tapping AFM tip is sufficient to lower the transition temperature by several degrees. The fact that XRR reports the structure of an unperturbed layer and AFM reports images under the compressive action of the tip rationalizes that the layer roughness is observed to increase with temperature due to increased thermal motion in the XRR measurements, whereas the roughness observed in AFM images is reduced due to the increased compressibility of the layers above the phase transition temperature.

The increased thermal motion perpendicular to the surface with increasing temperature, as observed as an increased surface roughness with XRR, also results in increased protrusion repulsion during surface force measurements. The friction force measurements show that DPPC bilayers exhibit good lubrication properties with a low friction coefficient (<0.03) that is close to independent of temperature, i.e. both when the deposited DPPC bilayer is in the gel state and when it is in the liquid disordered state. Our data demonstrate that the load bearing capacity of the DPPC bilayer increases with increasing temperature, i.e. with increasing fluidity of the bilayer. We suggest that the increased fluidity results in a certain self-healing ability as the lipids might diffuse into defects driven by surface pressure gradients. In contrast, the stability of phospholipid bilayers against puncturing by a sharp AFM tip is significantly smaller in the liquid disordered phase compared to in the gel phase [51].

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jcis.2014.12.042.

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