Supplementary Information

Hyaluronan and phospholipids in boundary lubrication

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Materials

1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids (Catalogue number 850355P) in form of lyophilized powder. Hyaluronan, was received as a gift from Novozymes and was HyaCare Medical device grade. The sample of hyaluronan was analysed by asymmetric flow flow-field fractionation (AFFFF) at Postnova Analytics GmbH, Germany using the Postnova Analytics AF2000 MT FFF system. Analysis was performed using 2 mg/mL hyaluronan solution in 100 mM NaCl.

Preparation of DPPC vesicles

Unilamellar vesicle dispersions of DPPC were prepared by firstly dissolving DPPC in small amount of chloroform (Sigma Aldrich, purity ≥99.5%). Next it was dried under gentle flow of

nitrogen. The DPPC was then dispersed in 155 mM NaCl solution to a concentration of 1 mg/mL by firstly vortexing at room temperature and then leaving it at least an hour at 55°C. Subsequently, the hydrated dispersion was vortexed for 2 minutes and then sonicated for 90 min at 55°C in a water bath using a Bandelin Sonorex Digitec ultrasonic bath (power 640 W) until the dispersion became almost clear. After dilution to 0.5 mg/mL the DPPC dispersion was further sonicated for 30 min, until totally clear. The pH in the dispersion was 5.6 – 6.1, and it was used within 4 hours of preparation. The size of the DPPC vesicles produced was characterised by dynamic light scattering (Brookheaven Instruments) and found to have a monomodal size distribution with a mean hydrodynamic diameter of 110 nm.

Ellipsometry

The ellipsometry measurements were performed with a thin film ellipsometer, type 43603-200E (Rudolph Research), using the technique of null ellipsometry. As a light source a xenon arc lamp was used with a filtered wavelength of 401.5 nm. The angle of incidence was set to 67.5°.

The substrates used were silicon wafers coated with a 34 nm silica layer. The substrate was characterized by determining the ellipsometric angles in air and in water. Adsorption was measured *in situ* in a cuvette with a solution volume of 5 mL, and using continuous stirring. The solution temperature was 55 °C. The ellipsometric angles Ψ and Δ were measured continuously and used to calculate the mean thickness (d_f) and refractive index (n_f) of the adsorbed layers.[1] While both the thickness and refractive index are rather sensitive to small changes in the ellipsometer angles, the variations in these parameters are coupled.[2] Thus, the adsorbed mass, which is proportional to the thickness and the difference in refractive index between the layer and the bulk, is obtained with a better accuracy than either of these two parameters.

The DPPC adsorption from 0.5 mg/mL vesicle solutions in 155 mM NaCl was allowed to proceed for 15 min, and then the system was rinsed with 155 mM NaCl for 20 min. The adsorbed amount and layer thickness was evaluated using an optical homogeneous 4-layer

model (silicon – silicon oxide – DPPC – solution) and the refractive index increment, *dn*/*dc*, of DPPC, 0.138 mL/g.[3]

Next, hyaluronan, 0.5 mg/mL in 155 mM NaCl, was added to the cuvette and adsorption was allowed to proceed for 50 minutes, after which the solution was rinsed with 155 mM NaCl for 30 minutes. The adsorbed amount of hyaluronan was evaluated using an optical homogeneous 5-layer model (silicon – silicon oxide – DPPC – hyaluronan – solution) and the refractive index increment of hyaluronan, 0.165 mL/mg, as determined using an Optilab DSP refractometer (Wyatt). In the analysis, the DPPC layer was assumed to be unaffected by the hyaluronan addition (thickness 4.5 nm, refractive index 1.4381).

Quartz Crystal Microbalance with Dissipation (QCM-D)

Quartz crystal microbalance with dissipation (QCM-D) experiments were carried out by using a Q-sense E4 instrument (Q-sense, Gothenburg, Sweden). The QCM-D measurements utilized quartz crystals QSX303 (AT cut with a fundamental frequency of 5 MHz) coated with a 50 nm thick silica layer. Prior to use, the crystals were cleaned by immersing into 2% Hellmanex for 30 min, and then rinsed by copious amount of Milli-Q water. Before use the sensors were dried under a gentle flow of filtered nitrogen gas.

The crystal was oscillated at its resonant frequency in water. The resonance frequency *(f)* and the energy dissipation *(D)* were determined as described by Rodahl *et al.*[4] The frequency and dissipation values for the bare QCM crystal at 55±0.02 °C were first determined in 155 mM NaCl solution. Next, the DPPC vesicle dispersion in 155 mM NaCl was injected and frequency and dissipation signals were monitored to follow vesicle adsorption and subsequent break-up to a supported DPPC bilayer. The adsorption step was terminated after 10 min, when the cell was subsequently rinsed with 155 mM NaCl solution to remove non-adsorbed phospholipids.

Next, the 0.5 mg/mL hyaluronan solution in 155 mM NaCl was injected. The QCM data

obtained during hyaluronan adsorption was analysed using the Voigt model,[5] using the frequency and dissipation values for the DPPC layer as the baseline.

Atomic force microscopy (AFM)

Imaging

Topographical and nanomechanical images of DPPC bilayers and bilayers with adsorbed layers of hyaluronan in aqueous solution were obtained with a Multimode Nanoscope V (Bruker) operating in Peak Force Tapping mode using a triangular silicon nitride cantilever (ScanAsyst-Fluid+, Bruker). Peak Force Tapping is a relatively new imaging mode designed for collecting simultaneous information about surface topography and surface material properties. [6-10] Briefly, the surface position is modulated by a sine wave where the sample during each period of oscillation is moved into contact with the AFM tip. Feedback electronics adjust the averaged surface position such that the maximum cantilever deflection (the peak force) equals a predetermine set point value. The information about cantilever deflection and piezo position can be converted to force vs. distance curves describing the tip-sample interaction during approach and separation as described for AFM based force measurements below. From such a force curve one can read the surface deformation due to the tip-sample interaction, the maximum adhesion force between the tip and the sample and the amount of energy that is dissipated during the interaction. In the present study we focus on surface topography and surface deformation. Due to the ability to scan the sample with a low and controlled feedback (peak) force – 500 pN in this study - and limited shear forces between the tip and the sample, this imaging mode is highly suitable for imaging soft delicate samples as the ones investigated in this study.[11, 12]

The experiments were conducted by using a fluid cell with inlet and outlet allowing exchange of solution in between measurement. First, an aqueous solution of DPPC small unilamellar vesicles was injected and a lipid bilayer was spontaneously formed on the silica substrate by vesicle

fusion. Next, additional DPPC were removed by rinsing with 155 mM NaCl. Hereafter aqueous hyaluronan solution was injected and after 10 min non-adsorbed hyaluronan was removed by rinsing with 155 mM NaCl. After each rinsing step, several images were obtained at different surface positions. The DPPC, NaCl and Hyaluronan solutions were before injection kept at 55 °C in a heat bath. The temperature in the fluid cell was fixed at 55 °C by a thermal application controller attached to a Bioheater element (Bruker) mounted under the sample. The temperature on the sample surface was calibrated by an external thermostat and controlled with an accuracy of ± 1 °C.

Force and friction measurements

The normal and frictional forces acting between a DPPC or DPPC/hyaluronan coated silica surface and a DPPC or DPPC/hyaluronan coated μm-sized silica particle, were measured by use of a Multimode Nanoscope III Pico Force atomic force microscope (Bruker) using the procedures detailed elsewhere.[13] Colloidal probe cantilevers were constructed by attaching a d = 7 μm silica particle to a tipless cantilever (CSC 12, F-lever, Mikromasch) with a two component epoxy glue (Araldite Rapid Epoxy) by use of an Ependorf Micromanipulator 5171 and a Nikon Optiphot 100S reflection microscope. This microscope was also used to measure the size of the silica particle. Before attachment of the silica particle the normal and torsional spring constants of the cantilever were determined by the Sader method as described elsewhere.[14, 15] All force curves in this study were performed at a constant approach and retraction velocity of 400 nm/s. At this velocity the effects of hydrodynamic forces can be considered negligible.[16] Raw data (photo detector signal vs. piezo extension) were transformed to force vs. distance data (force curves) by use of the cantilever spring constant and the optical lever sensitivity found from the constant compliance region between the bare silica surfaces in buffer solution. In these measurements the relative movement of the surfaces was measured, and the zero separation is

defined by the hard-wall contact (constant compliance region). For all measurements, the cantilever deflection was never exceeding the range where the detector signal is approximately linear.[17] The friction measurements were performed by sliding the surfaces backwards and forwards ten times at each normal load and registering the cantilever twist angle. The sliding distance was 1 µm in each direction and the scan rate was 1 Hz, giving a sliding speed of 2 µm/s. The angular twist of the cantilever was converted to friction force by use the lateral photo detector sensitivity, the torsional spring constant and the particle size as described elsewhere.[18] All force and friction measurement where conducted at the same condition as for the AFM imaging as described above.

Conversion of applied force to pressure

In order to convert the applied force to pressure the contact area was calculated from the JKR theory[19] as:

$$A = \pi\left[\frac{R}{E^*}\left(F_n + 6\pi R\gamma + \sqrt{12\pi R\gamma F_n + (6\pi R\gamma)^2}\right)\right]^{2/3} \quad (2)$$

where R is the colloidal particle radius, F_n the normal load and γ the interfacial tension calculated from the measured adhesion force, F_{adh}, as:

$$\gamma = \frac{F_{adh}}{3\pi R} \quad (3)$$

E* is the effective elastic modulus of the surface, calculated from the Young's modulus and the Poisson ratio of the silica surface and the silica probe as:

$$E^* = \left(2\left(\frac{1-\nu_{silica}^2}{E_{silica}}\right)\right)^{-1} \quad (4)$$

where E_{silica} = 72 GPa and ν_{silica} = 0.17.[20]

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