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Lubrication synergy: Mixture of hyaluronan and dipalmitoylphosphatidylcholine (DPPC) vesicles

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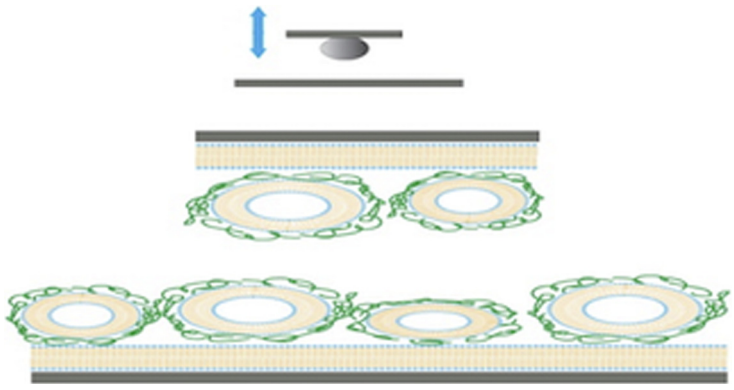
GRAPHICAL ABSTRACT



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ABSTRACT

Phospholipids and hyaluronan have been implied to fulfil important roles in synovial joint lubrication. Since both components are present in synovial fluid, self-assembly structures formed by them should also be present. We demonstrate by small angle X-ray scattering that hyaluronan associates with the outer shell of dipalmitoylphophatidylcholine (DPPC) vesicles in bulk solution. Further, we follow adsorption to silica from mixed hyaluronan/DPPC vesicle solution by Quartz Crystal Microbalance with Dissipation measurements. Atomic Force Microscope imaging visualises the adsorbed layer structure consisting of non-homogeneous phospholipid bilayer with hyaluronan/DPPC aggregates on top. The presence of these aggregates generates a long-range repulsive surface force as two such surfaces are brought together. However, the aggregates are easily deformed, partly rearranged into multilayer structures and partly removed from between the surfaces under high loads. These layers offer very low friction coefficient (<0.01), high load bearing capacity (≈23 MPa), and self-healing ability. Surface bound DPPC/

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hyaluronan aggregates provide a means for accumulation of lubricating DPPC molecules on sliding surfaces.

1. Introduction

The smooth motion of synovial joints has intrigued scientists for years. This has inspired work to understand the nanomechanical properties of the hierarchical and nanostructured cartilage surface [1], identify the important biolubricants [2], and develop biomimetic lubricants that can provide low friction and high load bearing capacity between sliding surfaces in aqueous solutions [3,4]. It is now well understood that key biolubricants include phospholipids, hyaluronan [5,6], and glycoproteins (like lubricin) with an overall bottle-brush structure [7]. We suggest that the outstanding performance of the synovial joint is not due to the properties of any single biolubricant, but the key is to understand synergism in mixtures containing the different components [2,8,9]. In this report we focus on two components, hyaluronan and dipalmitoylphosphatidylcholine, but emphasize the natural biolubrication system is significantly more complex and utilizes also other biolubricants.

Hyaluronan is a linear anionic polysaccharide present in the synovial fluid at a concentration of 1.4–3.1 g/L [10]. It consists of repeating D-glucuronic acid and *N*-acetyl-glucosamine units connected via alternating $\beta_{1,3}$ and $\beta_{1,4}$ glycosidic bonds. The pK_a of D-glucuronic acid is about 3.3 [11]. Hyaluronan alone does not provide any exceptionally low friction force between model surfaces [12]. However, in association with phospholipids, hyaluronan is a potent lubricant [13]. Dipalmitoylphosphatidylcholine, DPPC, is an abundant saturated phospholipid found in the synovial fluid [14,15]. The phase transition temperature for DPPC in contact with bulk water is 41 °C. Above this temperature DPPC is in the liquid crystalline phase and thus the acyl chains are fluid-like, whereas below this temperature they are solid-like in the gel phase. A ripple phase has also been reported to exist between the gel and liquid crystalline phases [16]. Recently, the structure of DPPC bilayers deposited at the silica-aqueous interface was characterised both in the fluid and gel state using X-ray reflectivity measurements [17]. We have previously shown that sequential adsorption of DPPC and hyaluronan can build a composite layer that allows a large amount of phospholipid to be present on the surface, and this layer provides low friction and high load bearing capacity on silica surfaces [6].

It is, however, difficult to see how sequential adsorption would occur in a biological environment. It is more likely that the components forming the adsorbed layer will be present in the solution and may form self-assembly aggregates that attach to the surface. In this work we explore the adsorption and lubrication performance of self-assembled aggregates, by studying adsorption from a 155 mM NaCl solution containing equal mass concentrations of hyaluronan and DPPC onto silica surfaces. The self-assembly structure formed in mixed hyaluronan and DPPC vesicle solutions was investigated by small angle X-ray scattering. The adsorption process was monitored by QCM-D, and the morphology of the layer was probed by AFM imaging. Surface and friction forces were determined with the AFM colloidal probe technique. We found that the layers formed by adsorption from mixed DPPC/hyaluronan solutions were less well ordered than the DPPC bilayers formed in absence of hyaluronan. However, the mixed layers were able to sustain high loads and sliding was characterized by a very low friction coefficient. It was also noted that low friction was recovered after structural rearrangements induced by the combined action of load and shear, suggesting a self-healing ability.

2. Materials and methods

2.1. Materials

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) was purchased from Avanti Polar Lipids (catalogue No. 850355P, Avanti, USA) in powder form and used as received. Hyaluronan with an average molecular weight M_w of 6.2×10^5 g/mol was a kind gift from Novozymes (Denmark), and the molecular weight distribution, $M_w/M_n = 1.9$, was characterized by means of asymmetric flow flow-field fractionation, AFFFF by Postnova analytics GmbH. Sodium chloride (ACS reagent, assay ⩾ 99.0%) and chloroform (ACS assay ⩾ 99.5%, catalogue No.C2432) were purchased from Sigma-Aldrich (USA). The water used in all experiments was purified by a Millipore system consisting of a Milli-Q Integral 15 unit, including a final 0.22 μm Millipak filter. 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 SiO_2 layer (Wafernet, Germany) were used as flat substrates in AFM studies. They were, prior to experiment, cut into size of 13×13 mm^2, and then 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 before being stored in a clean and sealed environment.

2.2. Preparation of solutions

For the SAXS and the surface sensitive measurements two different solution preparation protocols were used. The reason for this is that higher lipid concentration is needed for SAXS measurements to yield a good signal-to-noise ratio, and for such measurements the extrusion method was used. Here, DPPC powder was dissolved in chloroform in a small glass vial. Subsequently the solvent was evaporated under a gentle nitrogen flow in order to form a thin lipid film on the glass walls. In order to remove any residual chloroform the vial was placed in a vacuum oven over night (55 °C, 0.1 mbar). 155 mM NaCl solution was added to have a DPPC concentration of 8 mg/mL. The mixture was vortexed and placed in a thermostat shaker for 2 h (55 °C, 300 rpm). Afterwards the solution was passed 35 times through an Avanti mini extruder fitted with a membrane with a pore size of 50 μm. The whole preparation was done at 55 °C, which is well above the phase transition temperature. After this the vesicles were mixed with a hyaluronan solution to yield the final solution with equal hyaluronan and DPPC concentrations of 4 mg/mL.

For the surface sensitive measurements DPPC vesicles were prepared by the sonication method [18]. First the desired amount of DPPC powder was dissolved into 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 and thin lipid film on the bottle walls. A water jet pump was used to remove any residual chloroform. Next, a 155 mM NaCl solution was added to give a DPPC concentration of 1 mg/mL and the mixture was vortexed for 2 min and then allowed to stand for 1 h at 55 °C. This solution was placed into an ultrasonic bath (Bandelin Sonorex