How synergistic aqueous lubrication is mediated by natural and synthetic molecular aggregates

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Abstract. Nature lubricates in aqueous environment, and thus the example of a human synovial joint with its seamless function has been a fascination for scientists since the times of the birth of modern science. Here, inspired by nature, we investigate the mechanistic function of three different types of synergistic molecular aggregates. Firstly, we show how simple phospholipids lubricate hydrophilic model surfaces of silica and how this lubrication is facilitated further by the presence of an anionic polysaccharide, hyaluronan, due to the enhanced surface build-up of lubricant material. Next, we mimic natural polyelectrolyte-surfactant aggregation by employing a highly positively charged polyelectrolyte and anionic surfactant that strongly associate both in the bulk and at the surfaces by building structured aggregates that lubricate due to hydration lubrication. This occurs despite of the presence of strong attraction between the lubricated surfaces. This is an example of synergistic lubrication due to particular internal structural arrangement of the aggregates. Finally, we investigate the case of synergistic lubrication due to preferential surface ordering of two biological polyelectrolytes, cartilage oligomeric matrix protein and lubricin, that leads to favourable lubrication.

1. Introduction
Since the beginning of modern science inquisitive minds admired and wondered about the structure and function of the human synovial joint. The surgeon William Hunter wrote in Philosophical transactions of the Royal Society in 1743: “The fabric of the joints in the human body is a subject of so much the more entertaining, as it must strike every one that considers it attentively with an idea of fine mechanical composition. Wherever the motion of one bone upon another is requisite, there we find an excellent apparatus for rendering that motion safe and free”\cite{1}. In other words, the cartilage surfaces covering the bones are perfectly lubricated by the molecules and molecular aggregates of the synovial fluid. Today we start getting a grasp on what molecular constructs and their aggregates that are responsible for the lubrication of cartilage. However, despite the appreciable age of the considered topic, until recently we find a heated debate as to what is “the vital active ingredient in synovial fluid (SF) that imparts effective boundary lubrication”\cite{2} of synovial surfaces. To give a perspective, while Hills was convinced that it is simple phospholipids that lubricate the surfaces of the cartilage \cite{3, 4}, Jay et al. argued for the case of a glycoprotein, lubricin \cite{5, 6}. The ingenious machinery of Nature has blended multiple components, macromolecular and small molecular weight, in both cartilage and
synovial fluid [7]. We argue that it is for a reason that aggregates of molecules work far more efficiently as lubricants than the individual components. There are multiple modalities by which the lubrication synergies of the components may be achieved. This applies so much to biological as to synthetic molecular aggregates. In the following we will illustrate this by showing three such cases of synergistic action of the self-assembled molecular aggregates in lubrication. In the first case we employ phospholipids and hyaluronan for friction control on silica surfaces. In the second case we investigate cationic polyelectrolyte and anionic surfactant aggregates on silica surfaces. And in the third case we study associating biological glycoproteins at mildly hydrophobic polymeric surfaces.

2. Experimental

2.1 Materials

Hyaluronan, HA, is a linear polysaccharide consisting of a repeating disaccharide unit of β1-4-D-glucuronic acid and β1-3-N-acetyl-D-glucosamine [8], Figure 1a, left. The hyaluronan used was of relatively low molecular weight (average $M_w = 6.2 \times 10^5$ g mol$^{-1}$ as determined by asymmetric flow field fractionation). Dipalmitoylphosphatidylcholine, DPPC, is a molecule with a zwitterionic headgroup of phosphatidylcholine and a double C16 hydrocarbon tail group, Figure 1a, right. The solubility of DPPC in water is very low and the DPPC molecules aggregate in layered structures when in contact with water and assemble at interfaces [9]. The cationic polyelectrolytes used were poly[3-(2-methyl propionamido)-propyl]trimethylammonium chloride (polyMAPTAC) with a molecular weight of 480 000 g mol$^{-1}$ or poly[2-(propionyloxy)ethyl]trimethylammonium chloride (polyCMA) with a mean molecular weight of $1.5 \times 10^6$ g mol$^{-1}$. The monomeric segments of the polyelectrolytes are shown in Figure 1b, top. The surfactant used was anionic sodium dodecyl sulphate, SDS, Figure 1b, bottom. Lubricin was isolated from synovial fluid collected from a pool of patients diagnosed with rheumatoid arthritis (Figure 1c, left), and a monomeric form of recombinant human cartilage oligomeric matrix protein (COMP)/trombospondin-5 with predicted molecular mass of 81.8 kDa (Figure 1c, right) was used.
2.2 Surfaces and force measurements.
Silica or mica was employed as a smooth hydrophilic surface. The silica surface is suitable for phospholipid bilayer formation [10] and subsequent HA adsorption. Silica and mica are negatively charged. Poly(methylmethacrylate), polyMMA, was used as a surface for lubricin and COMP adsorption. This was motivated by the slightly hydrophobic character of polyMMA (contact angle of water on polyMMA is 68°) that promotes adsorption of molecules with hydrophobic chain regions.

Normal and friction forces were measured between the surfaces by the atomic forces microscope – colloidal probe technique that is described in detail before [11]. Measurements were performed in a fused silica liquid cell. Forces were measured between coated a silica surface and a silica sphere with diameter of ~7 μm, or between a coated polymethylmethacrylate (polyMMA) surface and a polyMMA sphere with a diameter of ~10 μm.

3. Results and discussion

3.1 DPPC and HA
Forces acting between pure DPPC bilayers in 155 mM NaCl solution (physiological ionic strength) at 47 °C (above chain melting temperature) have been reported [12] and are shown in the inset of Figure 2 left. They are characterized by a steep repulsion appearing at short separations upon approach and separation, which is understood as being due to hydration and protrusion forces characteristic for phospholipid bilayers [13]. Adsorbing HA on the DPPC bilayers results in a longer-range repulsive force and a weak pull-off force upon separation.

Friction forces between DPPC bilayers immersed in 155 mM NaCl solution are very low, with friction coefficient of 0.01, and of very high load bearing capacity, sustaining pressures of at least around 68 MPa (Figure 2, right). Friction forces between DPPC layers with HA adsorbed are comparable to these between pure DPPC bilayers until a load of about 27nN is reached (in our experimental set-up corresponding to a pressure of 56 MPa) with a friction coefficient of 0.03. Upon
further increase of the load, the friction force is rapidly increasing, thus we witness a disintegration of the DPPC – HA layer, and no recovery of the layer occurs on separation (Figure 2, right).

When the HA and DPPC are mixed in bulk solution at the ratio of 0.5 mg/mL DPPC and 0.5 mg/mL HA and are allowed to adsorb on silica surfaces for 40 minutes followed by rinsing, the force curves reflect the fact that the adsorbed layer is heterogeneous and there can be several adsorption layers or aggregates expelled from between the surfaces upon compression [14] (Figure 3, left.) Interestingly, the average friction force measured with such mixed layers adsorbed on the surfaces is low and the value of the friction coefficient is as low as 0.006, well comparable to that reported in synovial joints [15]. Thus, we suggest that the initially inhomogeneous layer re-arrange upon load and shear into phospholipid bilayers or multilayers separated by tenaciously attached though easily sheared water layers. The role of HA in this case is synergistic in that it facilitates the collection of lubricating phospholipids at the surfaces.

Figure 3. Left: Long-range repulsive forces measured upon first compression of the layers (●). Inset: the 10th approach (●) and separation (○). Right: Friction force as a function of load between two silica surfaces carrying a mixed DPPC-HA adsorption layer formed from solutions containing 0.5 mg/mL DPPC and 0.5 mg/mL HA in 155 mM NaCl and rinsed with 155 mM NaCl. The solid line has a slope of 0.006 and the dashed line of 0.01. The measurements were performed at 47 °C. Reproduced with permission from ref. [14] (doi.org/10.1016/j.jcis.2016.10.091) under terms of https://creativecommons.org/licenses/by-nc-nd/4.0/

3.2 polyCMA/polyMAPTAC and SDS
Cationic methacrylate-based polymers, such as polyCMA and polyMAPTAC, readily adsorb on negatively charged surfaces to compensate for their charge, see [16] and references therein. They also readily associate with anionic surfactants, as SDS. The association results in spontaneously organized structures both in bulk [16] and at interfaces [17] with the cylindrical SDS micelles in hexagonal arrangement that are stabilized by the polyelectrolyte wrapped around them [16]. The surface forces mediated by the polyCMA/polyMAPTAC and SDS aggregates are peculiar. They reflect the layering of cylindrical SDS aggregates at the surfaces and the forces oscillate from repulsive to attractive with the periodicity of that of an SDS micellar diameter of 40 Å [18] (Figure 4, left). Note, that very strong normal alternating repulsive and attractive forces operate between the surfaces. Similar normal forces can be detected also at silica surfaces pre-coated with polyMAPTAC and immersed in 1 cmc SDS solution [19]. The friction forces measured between polyMAPTAC coated surfaces in the presence of 1 cmc SDS solution in 1 x 10^{-4} M KBr are shown in Figure 4, right. These data are exciting as the friction force is very low up to a load of 9 mN m^{-1} (corresponding to a pressure of about 20 MPa), except for two transient peaks occurring at normalized forces of 3 - 4 mN m^{-1} and 8 mN m^{-1}. This friction peaks are due to restructuring of the aggregates under compression and shear. Thus, it is clear
that aggregation structures mediate good surface lubrication – low friction combined with high load bearing capacity along with self-healing despite the presence of strong normal attractive forces. Thus, we encounter a case of synergistic lubrication brought about due to the structural arrangement of the aggregates that enable free flow of interspacing water layers that surround the surfactant headgroups. Note, that the individual components by themselves in this case are not good lubricants for negatively charged surfaces: SDS, being negatively charged, does not adsorb on negatively charged surfaces at all [16, 17 and references therein], and polyCMA/polyMAPTAC that do adsorb on negatively charged surfaces to compensate for their charge, generate strong bridging between the surfaces and the friction between such surfaces coated with individual polyelectrolytes is high [19].

Figure 4. Left: Force normalized by radius as a function of surface separation determined using Surface Forces Apparatus. They were measured between mica surfaces pre-coated with polyCMA across a solution containing 1 cmc SDS and 10⁻⁴ M KBr. Reprinted with permission from ref. [18]. Copyright © 2000 American Chemical Society. Right: Friction force as a function of load between one mica and one silica surfaces pre-coated with polyMAPTAC and immersed in solution of 1 cmc SDS in 10⁻⁴ M KBr. Filled symbols designate compression and open symbols represent retraction of the surfaces. Reproduced from ref. [19] with permission from The Royal Society of Chemistry.

3.3 Lubricin and COMP

Lubricin is one of the biomacromolecules that has been suggested as being imperative to synovial joint lubrication. Indeed, lubricins molecular architecture with a dense highly hydrated polysaccharide brush in the core of the molecule and two anchoring naked protein endings (Figure 1e) appears to be conducive to good lubrication. Thus, lubricin’s ability to lubricate model surfaces has been investigated but the results have indicated poor lubrication performance of lubricin alone [20]. One reason for this is that lubricin does not adsorb strongly enough onto the model surfaces used, and other molecules that anchor and structure lubricin into an ordered layer are needed [21], [22-24].

COMP is one of the biomolecules of cartilage that promotes anchoring of lubricin in a fashion that facilitates lubrication. Flowers et al. identified COMP-lubricin complexes in arthritic synovial fluid and found evidence for non-covalent binding between the C-terminal of COMP and the N-terminal of lubricin [25]. This is important, as it seems that COMP is the protein in the cartilage that is able to induce attachment and orientation of lubricin such that its lubrication ability is expressed optimally. Some data obtained for the COMP-lubricin synergistic pair are presented in Figure 5 [26]. It is seen in the inset of Figure 5 that the friction force when lubricin is directly adsorbed on polyMMA is high, and lubricin is eroded from the surface during shearing, as can be understood from the hysteresis between the measured curves on loading and unloading. The attachment of COMP on polyMMA is robust and no hysteresis is observed in the friction curves, but the friction is rather high. However, when lubricin is adsorbed on COMP-coated polyMMA surfaces, a durable layer is formed and no erosion is observed up to a pressure of 7 MPa along with significant reduction in friction (friction coefficient is 0.06 at the highest load applied). Thus there is orientational attachment synergy between
COMP and lubricin that enhances lubricins frictional performance that is inherent in its molecular architecture.

![Friction measurements](https://example.com/friction measurement.png)

**Figure 5.** Main figure: Friction as function of load measured between two COMP-coated polyMMA surfaces (●), and COMP-lubricin coated surfaces (▲). Inset: Friction forces measured between two lubricin-coated polyMMA surfaces (loading ■, and unloading □). Reproduced with permission from ref. [27] (doi.org/10.1016/j.jcis.2017.02.007) under terms of https://creativecommons.org/licenses/by-nc-nd/4.0/

4. Conclusion

There are different ways to achieve surface lubrication in aqueous environment as good as that in mammalian joints. When using potent lubricants as phospholipids, it is imperative to supply an ample amount of the lubricant to the surface. Biopolymers, such as HA, can do this by operating as carriers for the lubricant and thus assuring the surface reservoir of the lubricant. To achieve good surface lubrication we do not even need to have a substance that is a good lubricant by itself. As long as the mixture of the components is self-assembled into aggregates that have a capacity to accommodate water layers that are tenaciously bound to the aggregates yet mobile along the surface, lubrication synergy is achieved. This is the mechanism of hydration lubrication. And finally, macromolecules with a blocky structure that provide hydration of the surface can also be used and here we find synergies in how one macromolecule (e.g. COMP) can aid adsorption of another (e.g. lubricin) that results in low friction.

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