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Friction Force Microscopy of Lubricin and Hyaluronic Acid between Hydrophobic and Hydrophilic Surfaces

Debby P. Chang^{1,2}, Nehal I. Abu-Lail³, Jeffrey M. Coles^{1,2,4}, Farshid Guilak^{4,5}, Gregory D. Jay⁶, and Stefan Zauscher^{1,2,4,*}

¹Department of Mechanical Engineering and Materials Science, Duke University, Durham NC 27708

²Center for Biologically Inspired Materials and Material Systems, Duke University, Durham NC 27708

³Chemical Engineering and Bioengineering, Washington State University, Pullman WA 99164

⁴Center for Biomolecular and Tissue Engineering, Duke University, Durham NC 27708

⁵Department of Surgery, Duke University, Durham NC 27710

⁶Department of Emergency Medicine, Rhode Island Hospital, Providence, RI 02903

Abstract

Lubricin and hyaluronic acid (HA), molecular constituents of synovial fluid, have long been theorized to play a role in joint lubrication and wear protection. While lubricin has been shown to function as a boundary lubricant, conflicting evidence exists as to the boundary lubricating ability of hyaluronic acid. Here, we use colloidal force microscopy to explore the friction behavior of these two molecules on the microscale between chemically uniform hydrophilic (hydroxyl-terminated) and hydrophobic (methyl-terminated) surfaces in physiological buffer solution. Behaviors on both surfaces are physiologically relevant since the heterogeneous articular cartilage surface contains both hydrophilic and hydrophobic elements. Friction between hydrophobic surfaces was initially high ($\mu=1.1$, at 100nN of applied normal load) and was significantly reduced by lubricin addition while friction between hydrophilic surfaces was initially low ($\mu=0.1$) and was slightly increased by lubricin addition. At lubricin concentrations above 200 $\mu\text{g/ml}$, friction behavior on the two surfaces was similar ($\mu=0.2$) indicating that nearly all interaction between the two surfaces was between adsorbed lubricin molecules rather than between the surfaces themselves. In contrast, addition of HA did not appreciably alter the frictional behavior between the model surfaces. No synergistic effect on friction behavior was seen in a physiological mixture of lubricin and HA. Lubricin can equally mediate the frictional response between both hydrophilic and hydrophobic surfaces, likely fully preventing direct surface-to-surface contact at sufficient concentrations, whereas HA provides considerably less boundary lubrication.

Keywords

Colloidal force microscopy; Lateral force microscopy; Atomic force microscope; Friction; Tribology; PRG4; Wear; Glycoproteins; Hyaluronic acid; Lubricin; Joint lubrication; Cartilage lubrication; Self-assembled monolayer; Synovial lubricants

*Corresponding Author, Professor Stefan Zauscher, Department of Mechanical Engineering and Materials Science, 144 Hudson Hall, P.O. Box 90271, Durham, NC 27708, zauscher@duke.edu, Phone: (919) 660-5360, Fax: (919) 660-8963.

Introduction

Articular cartilage lines the ends of diarthrodial joints and is subjected to large range of loading conditions while exhibiting extraordinarily low friction and wear.¹ These remarkable properties are attributed in part to the complex interactions between articular cartilage and synovial fluid. Synovial fluid is a dialysate of blood plasma that fills the joint cavity, where it provides nutrients to the cartilage and at the same time aids in joint lubrication.¹ It lubricates the articular cartilage to minimize friction and wear of this bearing surface and to prevent damage that may lead to degenerative diseases such as osteoarthritis.² Lubricin and hyaluronic acid (HA) are two constituents of synovial fluid thought to have a role in boundary lubrication.

Boundary lubrication,³ which is accomplished by lubricants adsorbed or bonded to the opposing bearing surfaces and is operative where surfaces come into contact,⁴ is one of multiple lubrication mechanisms protecting cartilage surfaces from friction and wear. Hydrodynamic lubrication,^{5, 6} which is most effective for smooth joint surfaces at very high sliding speeds, and squeeze-film lubrication,⁷ which delays cartilage-cartilage contact for hundreds of milliseconds,⁸ can allow separation between surfaces of loaded joints. Where cartilage surfaces come in contact, the biphasic/poroelastic properties of the tissue drastically reduce the load on solid tissue components (hydrostatic lubrication by interstitial fluid pressurization).^{9, 10} Interstitial fluid pressurization initially supports 90% or more of the total applied normal load on a loaded joint and subsides slowly,¹¹ transferring the load further to boundary lubricated solid-solid contacts. Since nearly all friction and wear occurs in the boundary lubrication regime, successful boundary lubrication is considered vital to joint health.^{12, 13}

Lubricin, a product of the gene proteoglycan 4 (*Prg4*), is a 1404 amino acid glycoprotein (Mw ~ 240 kDa) present in synovial fluid at a concentration of about 200 µg/ml.¹² Early studies in which synovial fluid was found to lubricate with similar effectiveness regardless of viscosity^{14, 15} led to the isolation of lubricin as the boundary lubricating component of synovial fluid.^{16, 17} Lubricin has been shown to lubricate different articulating surfaces under boundary lubrication conditions, including cartilage-cartilage,¹⁸ cartilage-glass,¹⁹ latex-glass,^{20, 21} and mica-mica⁴ interfaces. The ends of the molecule are globular and hydrophobic with somatomedin B N-terminal (SMB)-like domains and a C-terminal hemopexin (PEX)-like domain.²² The central region of the molecule contains a mucin-like domain which is extensively glycosylated with $\beta(1-3)\text{Gal-GalNAc}$ partially capped with sialic acid (NeuAc).^{12, 23} The abundant negatively charged sugars in this domain provide repulsive hydration forces which contribute to boundary lubrication.²³ Lubricin shares many structural similarities with members of the mucin family, such as mucous saliva, cervical mucus, gastric mucus and colonic mucin which lubricate and protect a large range of epithelial surfaces.^{20, 24, 25} This broad physiological function provides a further motivation to study the mechanism by which lubricin mediates friction.

Many questions remain regarding lubricin function. While recent work has shown that lubricin interacts with cartilage primarily via C-terminal domains,²⁶ it is not known with what molecular components of the cartilage surface it interacts. Although there is evidence that boundary lubrication by lubricin necessitates surface-attachment,^{14, 27} there is also evidence for lubricin free in solution aiding in lubrication.¹⁹ One study has also found that removal of the surface zone of cartilage (and thus the lubricin-containing layer) had little effect on friction properties, calling into question the necessity of an adsorbed surface layer for lubrication.²⁸ Phospholipids have been proposed as a boundary lubricant for cartilage and, although phospholipids are known to be present in synovial fluid²⁹ and on the cartilage surface,³⁰ evidence of their role in lubrication remains unclear.^{21, 31} Finally, some evidence suggests an interaction between lubricin and HA leading to more effective lubrication.

Hyaluronic acid, a high molecular weight ($M_w \sim 7 \text{ kDa}$ to 4 MDa),³² linear, negatively charged biopolymer, is composed of alternating units of glucuronic acid $\beta(1-3)$ and *N*-acetylglucosamine $\beta(1-4)$.³³ It is a major constituent of synovial fluid at a concentration of $1-4 \text{ mg/ml}$ in healthy individuals.^{18, 34} While HA is primarily responsible for the viscosity of synovial fluid,^{35, 36} conflicting evidence exists as to its boundary lubricating ability.^{15, 37, 38} For example, HA has poor boundary lubricating properties between both hydrophobic and hydrophilic model systems because it anchors poorly to each surface.^{35, 39} Other studies have suggested that lubricin may function as a surface anchor for HA, which then aids in joint lubrication.⁴⁰ Interaction between HA and lubricin has also been linked to strain energy dissipation in synovial fluid.⁴¹ Currently, HA is used as a viscosupplement, providing palliative pain relief when it is injected into the joint cavity of patients suffering from osteoarthritis.⁴²

Recently, we reported the steric repulsion, molecular adhesion and adsorption behavior of lubricin, HA and a mixture of the two on hydrophobic and hydrophilic model substrates, chosen to model the extremes of the cartilage surface.³⁹ We found that lubricin is amphiphilic and physisorbs strongly onto both methyl- and hydroxyl-terminated surfaces where it imparts strong large repulsive interaction between two surfaces pressed into contact. Considering lubricin's structure and the subtle differences in the steric interaction distance and adhesion energy found on the two model surfaces, we speculated that lubricin likely adsorbs onto the hydrophobic surface via hydrophobic N- and C-terminus forming a loop-like conformation, and adsorbs anywhere along the hydrophilic central mucin-like domain onto hydrophilic surfaces forming an extended tail-like conformation (Figure 1). This is in contrast to HA, which does not adsorb significantly or develop significant repulsive interactions. Here we report on friction force measurements using friction force microscopy (FFM) in the presence of human lubricin, HA and in a mixture the two to further understand the role of lubricin in boundary lubrication and to examine the hypotheses that lubricin and HA, alone or in combination, provide boundary lubrication properties that presumably reduce friction in diarthrodial joints.

Materials and Methods

Lubricant Solutions

Lubricin was purified from human synovial fluid as described previously.²³ A series of lubricin solutions ($25 - 400 \text{ } \mu\text{g/ml}$) was prepared from stock solution by dilution with phosphate buffered saline (Gibco, $1\times \text{ PBS}$, $\text{pH } 7.4$). The concentration range was chosen to bracket the physiological value of $\sim 200 \text{ } \mu\text{g/ml}$ found in synovial fluid.¹² Hyaluronic acid ($1.5-1.8 \text{ MDa}$, sodium salt from *Streptococcus Sp*, Biochemica) solutions ($0.5 - 3.34 \text{ mg/ml}$) were prepared by dilution with PBS. The concentration range was chosen to bracket the physiological value of $\sim 3 \text{ mg/ml}$ found in the synovial fluid.³⁴

Preparation of Colloidal Probes and Chemically Controlled Surfaces

Gold-coated colloidal probes functionalized with alkanethiolated self-assembled monolayers (SAMs) were prepared as described previously.³⁹ Briefly, borosilicate glass microspheres ($10 \pm 1.0 \text{ } \mu\text{m}$ diameter, Duke Scientific Co., Palo Alto, CA) were glued with a one part photo-curing epoxy (Norland Optical Adhesive #81, Norland, Cranbury, NJ) on commercially available AFM cantilevers (V-shaped Si_3N_4 , 0.58 N/m spring constant, Veeco™, Plainview, NY). The colloidal probes were then coated with a 5 nm chromium adhesion layer followed by a 45 nm gold layer using an electron beam metal evaporator (CHA Industries, Fremont, CA).⁴³ Glass slides or cover slips were also gold coated as described above. Before gold evaporation, the slides/cover slips were cleaned in a piranha solution at $80 \text{ } ^\circ\text{C}$ ($75\% \text{ H}_2\text{O}_2$, $25\% \text{ H}_2\text{SO}_4$ by volume) for 10 min and washed thoroughly with Milli-Q grade water.

Self-assembled monolayers of methyl or hydroxyl terminated thiol were obtained by immersing ozone cleaned, gold-coated glass slides/cover slips and colloidal cantilevers overnight in a 1 mM ethanol solution of 1-dodecanethiol or 11-mercapto-1-undecanol, respectively (Aldrich Chemicals Ltd., St. Louis, MO). After an overnight incubation, the substrates and the cantilevers were washed with copious amounts of ethanol, sonicated in ethanol (with the exception of colloidal probes), then dried in a stream of nitrogen.

Contact angle measurements with a Rame-Hart contact angle goniometer (Model 100, Rame-Hart instrument co., NJ) were used to determine the quality of the SAM monolayer. Static contact angles were taken in ambient condition at room temperature with Milli-Q grade water (18.2 MΩcm) on at least 4 different locations on the same sample and reported as an average value.

Friction Force Measurement

A MFP-3D AFM (Asylum Research, Santa Barbara, CA) was used to measure the friction and normal interaction forces. The cantilever normal spring constant (k_n nN/nm) was determined prior to any measurements from the power spectral density of the thermal noise fluctuations in air.⁴⁴ The normal photodiode sensitivity (S , nm/V) was determined from the constant compliance regime upon approach against glass.⁴⁵ The lateral calibration factor (α , nN/V) was obtained by the wedge calibration method^{46, 47} using equations adjusted for spherical probes.⁴⁸ A Si(100) calibration standard with a 30° sloped region fabricated by focused ion beam milling⁴⁹ was used for lateral calibration.

Friction between probes and functionalized substrates was measured by scanning perpendicular to the long axis of the cantilever.⁵⁰ To minimize crosstalk between normal and lateral signals, friction force was determined by taking half the difference between the lateral deflection signal obtained from the forward (trace) and backward (retrace) scanning directions. The effect of scan size on friction was measured at a sliding speed of 40 μm/s and an applied normal load of 100 nN. The effect of sliding speed on friction was measured with over a scan size of 20 μm and an applied normal load of 100nN. Friction was measured as a function of normal load by first increasing the applied load incrementally from 0 nN to ~115 nN and then decreasing the load incrementally until the tip became separated from the surface. Normal and lateral signals were monitored simultaneously over 256 scan lines, with measured force values averaged over 256 points per scan line. To obtain representative and consistent friction results, 51 friction versus load data were obtained on the same 20 μm line region over the entire load range and measurements were repeated at three different locations. We previously estimated pressures of up to ~85 kPa under these loading conditions, by applying the Alexander-de Gennes model to measure normal force interactions.³⁹ Since pressures in a human joint under normal weight bearing conditions are typically around 500 kPa, with peak pressures in excess of 5 MPa,⁵² and considering that the solid components of cartilage bear around 10% of the total pressure under normal weight bearing conditions (with 90% borne by fluid pressure),¹⁰ the pressures in our experiments are in the physiological range for moderate loading situations.

Lateral force measurements were performed in the presence of each solution after 15 minutes of equilibration in which the probe was kept far removed from the surface. Measurements of friction in the presence of both molecules were conducted using approximate physiological concentrations of each molecule (200 μg/ml lubricin, 3 mg/ml HA) in the following order: first PBS, second HA, third lubricin and fourth a mixture of HA and lubricin, again at physiological concentration of each molecule. HA was removed from surfaces between the second and third steps by rinsing with copious amounts of PBS. As shown previously, this treatment is sufficient to remove HA as it does not adsorb significantly on either hydrophobic or hydrophilic surfaces.^{35, 39} All measurements were repeated with at least 2 sets of probes and substrates.

Results

Characterization of substrate surfaces

We measured the water contact angle of the self-assembled monolayers (SAMs) to determine their quality. The measured contact angles of the 1-dodecanethiol (CH₃-terminated) and 11-mercaptoundecanol (OH-terminated) SAMs were $103^{\circ} \pm 9^{\circ}$ (hydrophobic) and $30^{\circ} \pm 5^{\circ}$ (hydrophilic), respectively, and agree well with the published literature values.⁵³

Effect of Scan Size, Sliding Speed, and Scan Location on Friction Forces

Friction was measured between a flat surface and a spherical probe, each modified with either a methyl- or hydroxyl-terminated SAM, in the presence of either lubricin or HA at their physiological concentrations. We assessed the effect of scan size on friction force at an applied normal load of 100 nN and at a sliding speed of 40 $\mu\text{m/s}$. In these experiments all friction forces became independent of scan size above $\sim 5 \mu\text{m}$ (Supplementary Figure S1a). We thus performed all subsequent friction measurements at a scan size of 20 μm . The effect of sliding speed on friction was determined at an applied normal load of 100 nN and a scan size of 20 μm (Figure S1b). These experiments showed that friction depended only weakly on sliding speed for speeds greater than 10 $\mu\text{m/s}$ in both lubricin and HA solutions. Based on these results, we conducted all subsequent friction measurements at a constant sliding speed of 40 $\mu\text{m/s}$.

To assess the reproducibility of the measurements, we performed repeated measurements of friction force as a function of normal load in the presence of a lubricin solution (i) at three varied locations (Figure S2a), and (ii) at one constant location (Figure S2b). The results from these measurements suggest that measurements are repeatable and do not depend on the location on the substrate surface.

Effect of Lubricants on Friction Forces

Lubricin

Friction forces measured as a function of applied normal load in the presence of a range of lubricin concentrations between hydrophobic (CH₃-terminated SAM) or hydrophilic (OH-terminated SAM) surfaces are shown in Figures 2, 3 and 4. Figure 2a and 2b show the differences between friction forces measured in lubricin solutions and in PBS control for the hydrophobic and hydrophilic surfaces, respectively. Large friction forces were observed on hydrophobic surfaces in absence of lubricin at all positive applied loads, and at negative loads decreasing to approximately -30 nN , when the tip finally pulled free from the opposing surface (Figure 2a). Friction at negative normal loads arises from the large adhesive interactions between the hydrophobic surfaces. On hydrophilic surfaces, the friction forces are less than those observed in the presence of lubricin solution (Figure 2b).

Typical sets of friction force curves between methyl- and hydroxyl-terminated SAM surfaces for a range of lubricin concentrations are shown in Figures 3 and 4, respectively. On methyl-terminated SAM surfaces, friction hysteresis was observed at the lowest lubricin concentration (25 $\mu\text{g/ml}$), showing general lower friction force upon unloading the contact at high normal load. At higher lubricin concentrations, friction measurements from the loading and unloading segments overlapped completely. On the hydroxyl-terminated SAM surfaces, friction force curves exhibited hysteretic behavior even at higher lubricin concentrations, where the friction forces upon unloading the contact were slightly lower than upon loading the contact.

Figure 5 shows the measured friction force at 100 nN of applied normal load and the corresponding instantaneous coefficient of friction (μ) at different lubricin concentrations. In PBS solution, large friction coefficients ($\mu = 1.1$) occurred between hydrophobic surfaces,

however, upon addition of even a small amount of lubricin, the friction force dropped dramatically ($\mu = 0.1$) and then increased slightly with increasing lubricin concentration, plateauing at 200 $\mu\text{g/ml}$ at $\mu = 0.2$. Friction between hydrophilic surfaces was initially small ($\mu = 0.1$), and increased with increasing lubricin concentration to plateau around 100–200 $\mu\text{g/ml}$ also at $\mu = 0.2$.

Hyaluronic Acid (HA)

Friction forces measured between methyl- and hydroxyl-terminated SAM surfaces in the presence of HA are shown in Figure 6. Between the methyl-terminated SAM surfaces, large attractive forces were present such that friction occurred even at negative applied loads, suggesting the presence of adhesive forces between probe and substrate (Figure 6a). Although the addition of HA decreased the observed pull-off load, it did not alter the magnitude of the measured friction force. On the hydroxyl surfaces, similar friction-load behaviors were observed for both PBS and HA solutions (Figure 6b). Importantly, the presence of HA did not significantly change the overall frictional behavior between either methyl- or hydroxyl surfaces.

Lubricant Mixtures

Friction forces between methyl- and hydroxyl-terminated SAM surfaces in the presence of three lubricant solutions (3.3 mg/ml HA, 200 $\mu\text{g/ml}$ lubricin and mixture of 3.3mg/ml HA + 200 $\mu\text{g/ml}$ lubricin) and in PBS solution are plotted in Figures 7a and 7b as a function of normal force. Again, large friction forces were observed in PBS even in the adhesive regime between methyl-terminated SAM surfaces,. In the presence of lubricin or the mixture solution, a significant reduction of friction and adhesion occurred (compared to little or no reduction of friction by HA). Between hydroxyl-terminated SAM surfaces, addition of lubricin or the lubricant mixture brought a significant increase in the overall friction force (again compared to little to no change in friction by HA).

Friction Force Fit

Our microscopic friction measurements in the presence of lubricin show non-linear dependence on the applied normal load (e.g., Figure 2). This behavior is in contrast to the macroscopic laws of friction (Amontons' law), where friction, F_f , is directly proportional to the applied normal load, L ,

$$F_f = \mu L, \quad (1)$$

where μ is the coefficient of friction. The linear friction vs. load behavior is due to multiasperity contacts between macroscopic bodies such that the true contact area increases with increasing normal load.⁵⁴ In a microscopic contact, the true contact area can often be estimated from the single-asperity contact theories, such as embodied by the Hertz model for non-adhesive contact, and the Johnson-Kendall-Roberts (JKR) model or the Derjaguin-Müller-Toporov (DMT) model when attractive forces are present.⁵¹ Because of the presence of adhesion in our measurement, the experimental friction data (F_f vs. L) were fitted to the general transition approximation proposed by Carpick, Ogletree and Salmeron (COS)^{51, 55} to describe the frictional behavior for the intermediate cases between JKR and DMT models

$$F_f = F_{f0} \left(\frac{\alpha + \sqrt{1 - L/L_c}}{1 + \alpha} \right)^{4/3} \quad (2)$$

by fitting the friction at zero load F_{f0} , pull-off force L_c and transition parameters α . The transition parameter, $\alpha = 1$ corresponds exactly to the JKR model exhibiting only short-range adhesion, $\alpha = 0$ corresponds to the DMT model exhibiting long-range surface force and $0 < \alpha < 1$ corresponds to the intermediate cases.⁵¹ (See Supporting Information for further details) The COS model captures the observed nonlinearity in the friction force curves (solid line fits in Figures 3 and 4), and implies that friction is likely proportional to the true area of contact.

Discussion

In our previous work,³⁹ we showed that lubricin is amphiphilic, adsorbs onto both hydrophobic and hydrophilic surfaces, and mediates normal force interactions by imparting strong steric repulsive interactions to the contacting surfaces. On hydrophobic surfaces, our data indicated that lubricin likely forms loop-like conformations, anchored with its hydrophobic N- and C-termini. Meanwhile, it likely adsorbs to hydrophilic surfaces anywhere along its central domain, adopting a tail-like conformation. This was found to be in contrast to HA, which did not adsorb well to either hydrophobic or hydrophilic surfaces. Here, we studied the friction force interactions of these molecules and showed that adsorbed lubricin mediates friction between microscale contacts on both hydrophobic and hydrophilic surfaces while HA has relatively little effect on friction.

Large and highly variable friction forces were measured between hydrophobic surfaces in the absence of lubricin or HA, but addition of lubricin, even at low concentrations (25 $\mu\text{g/ml}$, a concentration well below that necessary to yield monolayer coverage³⁹), was sufficient to significantly reduce friction. As expected, friction between smooth, hydrophilic surfaces was low in the absence of lubricin, and addition of lubricin increased friction to values similar to those seen on hydrophobic surfaces in the presence of lubricin.. The similar friction values seen here on hydrophobic and hydrophilic surfaces suggest that lubricin is able to coat the surfaces sufficiently to prevent contact of the underlying substrates. This points to a dual mechanism of wear prevention by lubricin. First, lubricin coats the surfaces thoroughly, allowing any surface wear to occur in the lubricin layer rather than the cartilage. Second, lubricin reduces the coefficient of friction by dissipating shear energy.

The microscopic COF measured here is in general agreement with the only other microscopic lubricin friction study with a surface forces apparatus, showing COF of lubricin adsorbed onto hydrophobic surfaces to be around 0.39 and 0.27 under high and low loads, respectively.⁴ On charged surfaces, lubricin undergoes an irreversible shear-induced rearrangement at pressures of several hundred kPa (approximately ten times the pressure exerted in this study) that significantly affects friction between adsorbed lubricin layers.⁴ Thus, some conformations of lubricin have different frictional properties than others. However, we find that lubricin mediates friction independent of differences in surface conformation of lubricin that we previously observed on these surfaces³⁹, at least over the range of forces studies here

The presence of HA did not significantly change friction properties between the surfaces (Figure 6). This lack of lubricating ability of HA in solution has also been observed in microscopic friction experiments with a surface force apparatus (SFA),³⁵ and in macroscopic friction measurements in a latex-on-glass interface.⁴⁰ The poor adsorption behavior of HA has been suggested to be responsible for this behavior. Without some mechanism to adhere to surfaces, HA will be readily squeezed out of the contact interface and thus contribute little to mediate either friction or wear. To function as an effective boundary lubricant, HA needs to be anchored onto the bearing surface. On the native cartilage tissue, HA may have specific binding affinity to components of the cartilage surface which is not present on the model surfaces used in this study. To the extent to which HA contributes to cartilage lubrication, it

must do so either through mechanisms other than molecular level boundary lubrication, such as fluid film or gel film formation, or through interaction with other cartilage components.

Some studies have found HA and lubricin together to lubricate more effectively than either molecule on its own. This was seen in a latex-glass interface under high contact pressure (180 kPa)⁴⁰ and in a cartilage-cartilage interface under similar pressure¹⁸ and may be due to either specific or non-specific interactions. By studying the tribological behavior of lubricin and HA with colloidal probe microscopy, we were able to focus on the local molecular interactions between the two lubricants and avoid the complex interaction with the cartilage surface. In our current study, the mixture of lubricin and HA at physiological concentrations up to 85kPa³⁹ contact pressure did not synergistically lower friction on both hydrophobic and hydrophilic surfaces. Friction in presence of the mixture was similar to that observed in lubricin solution alone. This friction result agrees well with our previous adsorption and normal force interaction study,³⁹ showing that HA, alone or in combination with lubricin, does not adsorb or exert significant repulsion on surfaces. We conclude that there is likely no specific molecular interaction between lubricin and HA, or at least that any interaction has a limited effect on adsorption and friction mechanics under the conditions measured here. The lack of synergistic interaction may be due to the difference in HA interaction with the test surfaces and also on the contact pressure tested.

The large friction forces seen between hydrophobic surfaces in PBS are likely due to the strong hydrophobic attraction between the methyl-terminated SAM surfaces. Many mechanisms have been proposed to explain hydrophobic attractive forces ranging from solvent structuring, electrostatic interaction, cavitations to nanobubble bridging; however, no single model can fully explain the observed attraction.⁵⁶ The large variation in the friction forces between substrates in PBS may arise from the nanoscale roughness of the probes, which causes variability in the adhesive forces⁵⁷ and leads to a range of friction forces. The variability in the friction force decreased significantly in the presence of lubricin.

Friction hysteresis between loading and unloading the contact was seen in several cases: 1) between surfaces in the presence of HA, which binds poorly to the surfaces (Figures 6 and 7), 2) between hydrophobic surfaces at low lubricin concentration (25 $\mu\text{g/ml}$, Figure 4), i.e., a concentration at which lubricin covers the surfaces incompletely, and 3) between hydrophilic surfaces in the presence of lubricin at several concentrations (Figure 4). Hysteresis may be attributed to the time and load dependent conformational changes in the interfacial molecular layer that occur upon sliding. As suggested previously,^{4, 39} lubricin likely adsorbs onto the hydrophobic surface via hydrophobic N- and C-terminus forming a loop-like conformation, and adsorbs anywhere along the hydrophilic central mucin-like domain onto hydrophilic surfaces forming an extended tail-like conformation. Therefore, lubricin molecules attached to the hydrophobic surfaces are less likely to be sheared away than those on hydrophilic surfaces where dangling ends of lubricin molecules on one surface may interact with those emanating from the opposing surface giving rise to hysteric behavior. Moreover, the hysteresis may also come from the time dependent conformation changes of lubricin, where compressed lubricin on hydrophilic surfaces may need more time to return to the extended quasi-equilibrium state.

The model surfaces used in the present work do not mimic all the features of the articular surface; however, they provide a starting point to investigate the influence of surface chemistry on biolubrication. In contrast to the model surfaces, the cartilage surface is topographically and chemically heterogeneous. A second limitation of this study is that, since adsorbed material is significantly softer than the underlying surfaces, a thicker adsorbed layer results in a larger contact area which has the effect of increasing the coefficient of friction. This may be the cause for the friction increase seen with increasing concentration of lubricin on both model surfaces. This effect is not expected to occur to the same extent on cartilage asperities since mechanical

properties the underlying material are more similar to those of the molecules at the surface. In areas of conformal contact between cartilage surfaces, this effect will not be a factor at all.

The coefficients of friction measured here are in good general agreement with both microscale and macroscale measurements on articular cartilage friction in the boundary regime.^{4, 58–60} Macroscopic COF measured on cartilage surface, after allowing the fluid pressurization to dissipate, reaches an equilibrium value of about 0.15–0.2.⁵⁹ Microscopic measurement showed that the COF on murine and porcine cartilage is around 0.25.⁶⁰ These intrinsic frictional coefficients of solid cartilage surface are comparable to the 0.2 instantaneous COF measured on the lubricin coated model surfaces found in this study (Fig. 6b). A recent study has also shown the concentration-dependant effect of lubricin on boundary lubrication of articular cartilage to become effective over the range of 10–20 µg/ml, with the lowest coefficient of friction seen at 50 µg/ml,⁶¹ a very similar result to what we find for the methyl-terminated model surfaces. Contact angles of the normal articular surface can approach 100 degrees⁶² which is close to the contact angles we measure on our methyl functionalized surfaces. Hydrophobic interaction between cartilage and lubricin molecules may play a key role in cartilage lubrication and should be studied further.

Conclusions

We have shown that lubricin mediates the friction behavior between chemically modified model surfaces even at relatively low concentrations. The use of model surfaces simplified the complex cartilage surface which has been shown to be hydrophobic and presents a uniform surface chemistry to the lubricants. It focuses on the molecular interaction by which lubricants function to mediate normal and friction forces in absence of fluid pressurization.

Lubricin adsorbed strongly onto both methyl and hydroxyl surfaces, where at sufficient surface concentration, gave rise to similar magnitudes of friction forces. High friction forces between hydrophobic surfaces were significantly reduced by adsorbed lubricin while the low friction forces between hydrophilic surfaces were somewhat increased. Lubricin may adsorb differently on the hydrophobic and hydrophilic surfaces, however, regardless of its adsorbed conformation, lubricin alters the frictional behavior on both surfaces almost equally. We surmise that lubricin shields the surfaces from direct contact and thus reduces wear. This behavior is in direct contrast to HA, which does not adsorb and does not appreciably alter friction between the model surfaces. The frictional behavior in a physiological mixture of lubricin and HA is similar to that of lubricin alone. This observation is consistent with our previous results on adsorption and steric interaction, which also showed no significant synergistic effect between lubricin and HA. Our result is consistent with the recognition that lubricin coats the cartilage surface where it mediates the frictional behaviors. The role of lubricin may not be to provide ultra low coefficient of friction, but to function as a chondroprotectant to maintain joint health.

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Figure 1. Schematic representation of the shearing interface of lubricin on a) hydrophobic, methyl-terminated, and b) hydrophilic, hydroxyl-terminated SAM surfaces.

Figure 2.

Friction forces plotted as a function of the applied normal load between two a) methyl-terminated and b) hydroxyl-terminated SAM surfaces in PBS solution (cross) and in a range of lubricin concentrations (25–400 $\mu\text{g/ml}$). The lines represent the fit to the COS contact mechanic model (equation 2) from the accenting normal load data.

Figure 3.

Typical friction vs. normal load curves measured between methyl- terminated SAM surfaces for a range of lubricin concentrations. The friction data is obtained from ramping the normal load from low to high (open circles) and then back to low load (filled circles). The solid lines represent the fit to the COS contact mechanic model (equation 2) from the accented normal load data.

Figure 4.

Typical friction vs. normal load curves measured between two hydroxyl-terminated SAM surfaces in a range of lubricin concentrations. The friction data is obtained from ramping the normal load from low to high (open circles) and then back to low load (filled circles). The solid lines represent the fit to the COS contact mechanic model (equation 2) from the accenting normal load data.

Figure 5. Measured friction force at 100 nN of applied normal load as a function of lubricin concentration. The right axis shows the instantaneous coefficient of friction, COF, at each concentration. The error bars represent the standard deviation obtained from three different locations.

Figure 6. Friction forces plotted as a function of the applied normal load between a) methyl-terminated and b) hydroxyl-terminated SAM surfaces for a range of HA concentrations.

Figure 7.

Friction forces plotted as a function of the applied normal load between a) methyl-terminated and b) hydroxyl-terminated SAM surfaces in the presence of 200 $\mu\text{g/ml}$ lubricin, 3.3 mg/ml HA and a mixture lubricin and HA.