The role of lubricant entrapment at biological interfaces: Reduction of friction and adhesion in articular cartilage

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A R T I C L E  I N F O

Article history:
Accepted 6 April 2011

Keywords:
Adhesion
Articular cartilage
Boundary film
Friction
Lubrication

A B S T R A C T

Friction and adhesion of articular cartilage from high- and low-load-bearing regions of bovine knee joints were examined with a tribometer under various loads and equilibration times. The effect of trapped lubricants was investigated by briefly unloading the cartilage sample before friction testing, to allow fluid to reflow into the contact interface and boundary lubricants to rearrange. Friction and adhesion of high-load-bearing joint regions were consistently lower than those of low-load-bearing regions. This investigation is the first to demonstrate the regional variation in the friction and adhesion properties of articular cartilage. Friction coefficient decreased with increasing contact pressure and decreasing equilibration time. Briefly unloading cartilage before the onset of sliding resulted in significantly lower friction and adhesion and a loss of the friction dependence on contact pressure, suggesting an enhancement of the cartilage tribological properties by trapped lubricants. The results of this study reveal significant differences in the friction and adhesion properties between high- and low-load-bearing joint regions and elucidate the role of trapped lubricants in cartilage tribology.

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1. Introduction

Lubrication plays a critical role in preventing solid–solid interactions between contacting surfaces in relative motion. In biological systems, water and macromolecules such as lipids, mucins, and other glycoproteins provide lubrication to a variety of organs and tissues, including gastrointestinal tract, vagina, ocular surface and tear ducts, pericardium, pleura, mouth, and synovial joints (Hills, 2000; Neu et al., 2008). Articular cartilage in synovial joints is a model system exhibiting complex surface characteristics intimately related to disease of the tissue such as osteoarthritis (Neu et al., 2008). The tribological properties of articular cartilage depend on the material properties of the composite tissue and the biophysical properties of surface molecules as well as their interaction with interarticular fluid. Articular cartilage is a multiphase material consisting of a fluid phase comprising 60–85\% of the tissue wet weight (ww) (Mow et al., 1992; Forster and Fisher, 1996) and a solid phase mainly composed of collagen (\textasciitilde 15–30\% ww) and proteoglycans (\textasciitilde 4–7\% ww) (Mow et al., 1992). Water and lubricant pools form between asperity microcontacts of loaded articular cartilage (Soltz et al., 2003). Various mechanisms responsible for the development of trapped lubricants have been proposed, including weeping (Lewis and McCutchen, 1959), boosted (Walker et al., 1968), and squeeze film mechanisms (Hou et al., 1992). Regardless of its origins, entrapped fluid plays a critical role in maintaining low friction during initial shear loading. Depletion of the lubricant pools by spreading and diffusion in conjunction with tissue relaxation increase the real contact area at the cartilage–cartilage interface. Under these circumstances, lubrication for maintaining low friction and protecting the cartilage against mechanical wear solely depends on the formation and timely replenishment of boundary lubricant films at the tissue surface.

The dominant mechanism of lubrication in articular cartilage depends on various factors such as contact pressure, duration of loading, and molecular constituents at the tissue surface. The pressure distribution of articular cartilage within the joint varies by location depending on physiological functional loading (Neu et al., 2007). In addition, the distributions of proteins such as superficial zone protein (Young et al., 2006; Neu et al., 2007), type I and II collagen (Lorenz et al., 2005), and glycosaminoglycans (Rogers et al., 2006) at the cartilage surface also vary by location depending on functional loading. Therefore, in this study, the friction coefficient of cartilage from high- and low-load-bearing regions of articular joints was first measured under various loads and preload durations to determine a baseline of the friction response of cartilage. The effect of trapped lubricants on the...
friction behavior of high-/low-load-bearing cartilage regions was then examined under different contact pressures by briefly unloading the samples for a few seconds to allow external fluid to flow between the depressurized cartilage and the glass countersurface and molecules adsorbed onto the cartilage surface to rearrange. To isolate lubrication provided by trapped lubricants, the duration of unloading was limited in order to minimize the amount of interstitial fluid repressurized into the tissue. Results are presented to illustrate the relative importance of trapped lubricants in articular cartilage tribology under various loading conditions affecting the intensity of asperity contact interactions and fluid exudation from the tissue.

2. Experimental procedure

2.1. Tissue acquisition

Bovine osteochondral explants were obtained from tibiofemoral joints of 1–3 week old calves, acquired from a local abattoir within 6 h of sacrifice. Explants were harvested from medial anterior (M1) and posterior (M4) locations of the distal femoral condyles (Fig. 1A), corresponding to regions of relatively high (M1) and low (M4) contact pressure in vivo (Neu et al., 2007; Nugent-Derfus et al., 2007), hereafter referred to as high- and low-load-bearing cartilage, respectively. After extracting the explants with a 5-mm-diameter coring reamer, the uppermost 4 mm of the cartilage was trimmed with a custom jig. The explants were then stored in a culture medium consisting of Dulbecco’s modified Eagle’s medium (DMEM)/F12 at 37°C and 5% CO2 for 24 h before friction testing to preserve cell viability. The DMEM/F12 culture medium contained 0.2% bovine serum albumin (Sigma-Aldrich, St Louis, MO), 1% penicillin and streptomycin (Invitrogen, Carlsbad, CA), and 50 μg/mL ascorbic acid (Sigma-Aldrich).

2.2. Friction and adhesion measurements

Cartilage friction was examined with a pin-on-disk tribometer (Fig. 1B) operated in reciprocating sliding (Neu et al., 2007; DuRaine et al., 2009). Pin specimens consisted of cartilage explants affixed to acrylic pins by ethyl cyanoacrylate, while the disk specimen was a polished glass substrate. Before friction testing, the glass disk was sonicated in phosphate buffered saline (PBS) (Sigma-Aldrich). To maintain tissue hydration, cartilage explants were fully immersed in 10 mL of PBS through the duration of testing. To examine the effect of the static preload on cartilage friction (Forster and Fisher, 1996; Basalo et al., 2006), before friction testing, the cartilage explant was allowed to equilibrate for 2, 10, or 30 min (Neu et al., 2007; DuRaine et al., 2009) under the applied normal load (n=6 for each load and preload time combination). The normal load was varied between 0.9 and 24.3 N, corresponding to a mean contact pressure in the range of 0.32–0.96 MPa, determined from the Hertz theory for an elastic modulus of glass and cartilage equal to 70 GPa and 1.5 MPa, respectively. Minimum values of contact pressure and equilibration time were similar to those of previous studies (Neu et al., 2007; DuRaine et al., 2009), allowing for friction testing in the boundary lubrication mode without inducing excessive suction pressures on the tissue, which could lead to the depletion of the boundary film. Since the elastic modulus of high- and low-load-bearing cartilage regions is typically equal to 1 MPa (Athanasiou et al., 1991; Froimson et al., 1997) and 2 MPa (Hayes and Mockros, 1971; Boschetti et al., 2004), respectively, an average elastic modulus of cartilage equal to 1.5 MPa was used in the calculation of the mean contact pressure. In all of the friction tests, the sliding speed was set equal to 0.5 mm/s and the wear track radius was fixed at 5 mm, resulting in a sliding distance of 7.85 mm per oscillation direction. The friction force was measured in 0.1 s intervals over a time period of 60 s using Labview (National Instruments, Austin, TX).

A separate set of cartilage explants were used to examine the effect of trapped lubricants formed by briefly unloading the specimens to allow fluid to reflow into the cartilage–glass contact interface. Explants affixed to the pin were preloaded as described above, briefly unloaded for ~3 s (by lifting the pin with the affixed cartilage, while maintaining the explant submerged into the PBS bath), and then brought into contact with the glass disk under the applied normal load. Sliding was initiated immediately after the specimens were brought again into contact under the static load, and friction force data were acquired in 0.1 s intervals for a time period of 1 min using Labview. The friction coefficient μ was obtained as the ratio of the measured friction force F and the total normal load, which is equal to the sum of the applied normal load L and the adhesion force L_ad, which acts as an additional normal load at the contact interface; thus, $F = \mu (L + L_{ad}) = F_{ad} + \mu L$, where $F_{ad}$ (≡ L_ad) is referred to as the adhesion force during shearing under zero
applied normal load. Friction coefficients of explants from high- and low-load-bearing cartilage regions measured for different equilibration times were plotted against the mean contact pressure. The friction force was also plotted against the applied normal load to determine the friction coefficient and the adhesion force during shearing at the cartilage–glass interface from the slope and the intercept, respectively, of a linear fit through the friction force data with the zero-load axis.

2.3. Statistical analysis

Significant differences between the effects of joint location, equilibration time, and applied normal load (mean contact pressure) were determined by a three-way analysis of variance (ANOVA). A significance level of $\alpha = 0.05$ and corresponding Bonferroni-adjusted $p$-values of $p < 0.05$ for joint location effects, $p < 0.0167$ for equilibration time effects, and $p < 0.0024$ for load effects, were used to identify statistically different friction results. To determine whether differences between M1 and M4 friction coefficients were statistically different under all loading conditions, equivalence testing using a null hypothesis $H_0: \mu_{M1} \leq 0.9\mu_{M4}$ with a significance level $\alpha = 0.05$ was performed for various combinations of equilibration time and applied normal load. The null hypothesis was rejected for $p > 0.05$, indicating that M1 and M4 friction coefficients were within 10% of each other. Standard errors from the mean adhesion forces were calculated from error propagation of the uncertainty associated with the linear fits through the friction force data.

To analyze the effect of trapped lubricants on articular cartilage friction, a paired $t$-test was conducted for each combination of joint location, equilibration time, and normal load. A significance level of $\alpha = 0.05$ and $p < 0.05$ was used to determine significant differences between friction coefficients of cartilage–glass interfaces loaded continuously under a given static load and those of contact interfaces that were briefly unloaded before the initiation of sliding under the same static load.

3. Results

3.1. Friction dependence on lubricant entrapment and replenishment

Immediate replenishment of fluid at the articular surface after prolonged static loading resulted in a dramatic decrease (relative to baseline values) in friction coefficient (Fig. 2). Temporary removal of the applied load yielded the most significant effect on friction for relatively low loads (0.9 and 3.6 N) and intermediate (10 min) or long (30 min) equilibration times. For all equilibration times and loads equal to 0.9 and 3.6 N, low-load-bearing joint locations (M4) demonstrated higher sensitivity to lubrication by fluid entrapped at the contact interface, caused by briefly unloading the specimens before testing, whereas friction of high-load-bearing joint locations (M1) was more sensitive to the preload removal for the longer equilibration time (30 min) through the entire load range (0.9–24.3 N). For a 30 min equilibration time (Fig. 2A), M1 friction coefficient demonstrated a significant decrease upon the reentry of fluid at the contact interface for all normal loads tested, while M4 friction coefficient exhibited a pronounced decrease subsequent to the fluid reentry only for relatively light loads (0.9 and 3.6 N). For a 10 min equilibration time (Fig. 2B), M1 friction coefficient decreased after brief unloading for relatively low loads of 0.9 and 3.6 N (0.32 and 0.51 MPa, respectively) compared to continuous loading, while M4 friction coefficient decreased significantly after brief unloading for loads in the range of 0.9–12.6 N. For a 2 min equilibration time (Fig. 2C), M1 friction coefficient did not change significantly following brief unloading, whereas M4 friction coefficient decreased significantly in the load range 0.9–12.6 N (0.32–0.77 MPa) ($p \leq 0.0377$).

3.2. Friction dependence on joint contact location, equilibration time, and contact pressure

The friction coefficient of cartilage from high-load-bearing articular regions (M1) was lower than that of low-load-bearing articular regions (M4) for all combinations of contact pressure and equilibration time ($p < 0.0001$) (Fig. 3). However, this difference decreased at relatively high contact pressures, resulting in similar steady-state friction coefficients for both joint locations at all equilibration times and contact pressures above 0.70 MPa. Equivalence testing (performed for each combination of load and equilibration time to determine for which, if any, condition this significance was lost) showed that M1 friction coefficient was significantly less than M4 friction coefficient for loads (pressures) less than 12.6 N (0.77 MPa), 3.6 N (0.51 MPa), and 5.4 N (0.58 MPa) and corresponding equilibration times equal to 2 min ($p \leq 0.035$), 10 min ($p \leq 0.008$), and 30 min...
Friction coefficients of both M1 and M4 cartilage increased significantly with equilibration time \((p < 0.0001)\). However, differences between M1 and M4 friction coefficients decreased with equilibration time. Friction coefficients in the load range 0.9–1.8 N (0.32–0.41 MPa) differed significantly from those for higher loads \((p < 0.0069)\), whereas those in the load range of 3.6–5.4 N (0.51–0.58 MPa) were significantly different from those in the load range of 6.3–24.3 N (0.61–0.96 MPa) \((p < 0.0073)\).

### 3.3. Adhesion dependence on joint contact location and equilibration time

For a given equilibration time, the adhesion force during shearing under zero normal load was determined from the intercept of a linear fit through the friction force versus normal load data with the \(y\)-axis, as shown in Figs. 4A and 5A, for example. In the absence of unloading before the onset of sliding, low-load-bearing (M4) cartilage exhibited higher adhesion than high-load-bearing (M1) cartilage for all combinations of load and equilibration time (Fig. 4B). Adhesion tended to increase with decreasing equilibration time for M4 cartilage, and to decrease after 10 min of equilibration or to increase after 30 min of equilibration for M1 cartilage. When the contact was briefly unloaded before sliding, pools of trapped lubricants decreased the adhesion of both M1 and M4 cartilage, except for M1 cartilage after 30 min of equilibration time (Fig. 5B). Similar to continuously loaded contacts (Fig. 4B), briefly unloaded M1 cartilage exhibited significantly lower adhesion than briefly unloaded M4 cartilage in all cases, except for the longest equilibration time (30 min) (Fig. 5B).

### 4. Discussion

The results presented above elucidate the effects of joint location, mean contact pressure, and preload duration before the onset of sliding on the efficacy of trapped lubricants (either trapped fluid pools or molecular films adsorbed at the tissue surface) to reduce cartilage friction. An important result is the decrease in friction after unloading cartilage for a few seconds before the initiation of sliding, suggesting a key role of trapped lubricants in the tribological properties of articular cartilage.
The effect was most significant under lighter loads and longer equilibration times (Fig. 2). A similar decrease in friction due to unloading for 1 to 45 min under static loading was observed in a previous study (Forster and Fisher, 1996). Friction of high-load-bearing (M1) joint regions did not change significantly for a short (2 min) equilibration time (Fig. 2C), most likely because M1 friction coefficient was fairly low over the entire load range for 2 min equilibration time. For a relatively short duration under static loading, the water within the tissue did not completely escape from the stiffer and less permeable M1 cartilage, continuing to provide lubrication during subsequent sliding. Alternatively, lubricant entrapment at the contact interface due to brief unloading greatly reduced the friction coefficient of low-load-bearing (M4) cartilage (Fig. 2C). In particular, briefly unloading the contact before sliding resulted in significantly lower M4 friction coefficient for normal loads less than 12.6 N at all equilibration times (Fig. 2). M1 joint regions exhibited typically much lower friction coefficients after brief unloading over the entire load range, especially for equilibration time equal to 30 min (Fig. 2A) and 10 min (Fig. 2B). This finding indicates better lubrication conditions at high-load-bearing cartilage regions than low-load-bearing cartilage regions, suggesting a higher ability to uptake fluid from the surrounding lubricant after long periods under compressive loading before the commencement of shear loading. This result may also be related to the higher contents of mucins and surface-bound glycosaminoglycans (GAGs) in M1 than M4 joint regions that allowed these surface molecules to quickly trap the surrounding water between the asperity microcontacts. Importantly, unloading for this brief time is considered insufficient to repressurize the cartilage interstitial fluid (Eckstein et al., 1999).

Articular cartilage from high-load-bearing regions of synovial joints was found to exhibit better lubrication characteristics than cartilage from low-load-bearing regions. This was observed under almost all combinations of contact pressure and equilibration time. The authors have previously demonstrated the location dependence of cartilage friction (Neu et al., 2007; DuRaine et al., 2009) for contact pressures in the lower end of the physiological range (Brown and Shaw, 1984; Lee et al., 2006). The present study is the first to clearly demonstrate this result regardless of load magnitude and equilibration time, and to define the conditions under which the difference diminishes in significance. In the absence of sufficient amounts of trapped lubricants, the friction coefficient of high-load-bearing regions was appreciably lower than that of low-load-bearing regions up to contact pressures of 0.77 MPa for 2 min under static loading (Fig. 3C), which is the most physiologically relevant condition in the present study. Even in the most extreme condition of 30 min under static loading, high-load-bearing cartilage still showed lower friction than low-load-bearing cartilage for contact pressures up to 0.58 MPa (Fig. 3A). Although previous studies have demonstrated regional variations in other mechanical properties, the present investigation is the first to report the regional variation in friction response of articular cartilage.

The friction dependence on functional loading can be interpreted in the context of other mechanical and biochemical properties of cartilage. Cartilage from high-load-bearing sites of the joint possesses higher compressive modulus (Athanasou et al., 1991; Froimson et al., 1997) and lower tensile modulus (Akizuki et al., 1986) than cartilage from low-load-bearing joint sites. In addition, the permeability of high-load-bearing cartilage is less than that of low-load-bearing cartilage (Froimson et al., 1997; Williamson et al., 2001). Higher deformation resistance under compressive loading would result in smaller real contact area, and, in turn, higher local pressures at high-load-bearing sites. Thus, the lower friction of high-load-bearing cartilage may be attributed to its higher compressive stiffness and lower permeability than those of low-load-bearing cartilage. With the increase of the load (contact pressure) and the equilibration time, interstitial water should be depleted from the tissue, and the friction coefficients of high- and low-load-bearing cartilage should approach similar values, as demonstrated by the results of this study (Fig. 3).

Superficial zone protein (SZP), also known as lubricin and PRG4, is a mucinous proteoglycan localized in the superficial zone of cartilage (Schumacher et al., 1994; Jay et al., 2001) that has been identified as a key boundary lubricant (Schmidt et al., 2007; Chan et al., 2010). The distribution of SZP has also been shown to be higher in high-load-bearing regions of the joint compared to low-load-bearing regions (Young et al., 2006; Neu et al., 2007; Nugent-Derfus et al., 2007). A similar regional dependence of GAG and proteoglycan contents has been reported previously (Rogers et al., 2006; Neu et al., 2007). The higher content of SZP and other surface molecules, including aggrecan and hyaluronan, which may act synergistically to improve cartilage compressive and tribological properties (Schmidt et al., 2002; Schmidt et al., 2007; Chan et al., 2010), should provide better boundary lubrication to highly compressed cartilage. Furthermore, GAGs tend to attract water through their negative charges, thereby enhancing the lubricating ability of SZP. This function of GAGs and mucins is also important in both the formation and maintenance of trapped lubricant pools.

Adhesion of high-load-bearing cartilage was also typically lower than that of low-load-bearing cartilage (Figs. 4B and 5B). The positive correlation of friction and adhesion agrees with previous reports of
friction increasing with adhesion (Israelachvili et al., 1994). The friction coefficient of articular cartilage appears to be largely dominated by adhesion mechanisms, especially after long equilibration times (Fig. 5B). Longer compression of cartilage results in more water exudation from the matrix, increasing the real contact area and enhancing the formation of more adhesive asperity microcontacts. As the tissue is gradually depleted of water, its physical and chemical properties (e.g., permeability and protein and ionic concentrations) should also be altered (Quinn et al., 1998; Nugent et al., 2006; Leddy and Guilak, 2008). These structural changes in the tissue could also affect the adhesive and friction properties of cartilage. Additionally, compressive contact stresses may cause molecules at the surface, such as SZP, hyaluronan, aggrecan, and other GAGs, to undergo conformational changes and, in addition, promote chemical changes in the tissue (Chang et al., 2008). These molecular alterations may, in turn, increase friction and adhesion in articular cartilage (Han et al., 2007; Chang et al., 2008).

The results of this study suggest that the fluid in cartilage is exuded from the matrix under relatively high loads (contact pressures) and long equilibration times, causing boundary lubrication to become the dominant mode of lubrication. Under these contact conditions, conformal boundary films play dominant roles in protecting the tissue from damage and wear. The findings of this study underscore the significant difference in lubrication of high- and low-load-bearing cartilage and the role of trapped lubricants (either fluid pools or boundary films) in preventing high adhesion and friction under mixed and boundary modes of lubrication after sustained compressive loading.

Conflict of interest statement

The authors confirm that there are no conflicts of interest.

Acknowledgments

The authors thank Neil Willetts, Senior Statistician, Department of Mathematics, University of California, Davis, for assistance in statistical analysis.

References


