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COMPARISON OF THE EFFECTS OF BOUNDARY LUBRICANTS ON THE TRIBOLOGICAL REHYDRATION OF ARTICULAR CARTILAGE

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INTRODUCTION

Articular cartilage is unique in that it retains exceptionally low frictional properties through the millions of articulation cycles we expose it to yearly. Healthy cartilage is composed of ~80% water, with the remaining ~20% consisting mostly of a deformable matrix of collagen and negatively charged proteoglycans[1], and it is thought that cartilage's unique tribomechanical properties stem from this biphasic composition. A major goal of the field has been to understand how this soft biological material can maintain phenomenally low friction values ($\mu<0.02$) under usage conditions that can be considered extreme[2–8]. Recently, our team rediscovered an *ex vivo* cartilage testing configuration, the convergent stationary contact area (cSCA), that helps to shed light on these mechanisms due to its unique ability to promote and sustain physiologically-consistent levels of cartilage strain, deformation recovery, hydration, and lubricity over unprecedentedly-long time scales (hours). The cSCA configuration uses large (∅19mm) articular cartilage explants that retain the joint's natural radius of curvature thought necessary for promoting hydrodynamically-driven interfacial behaviors when explants are slid again a counterface. These hydrodynamic forces, through a mechanism termed 'tribological rehydration'[8–10], can 'pump' fluid exuded from cartilage during compression back into the tissue during sliding, enabling the recovery of interstitial fluid pressures and fluid-load support needed to maintain interstitial lubrication and physiologically low friction coefficients.

Previous cSCA studies have used 1X phosphate buffered saline (PBS) as the hydrating/lubricating solution, representing a 'worst-case' lubrication condition. Despite this, we have consistently observed remarkable tribological rehydration-driven strain recovery and frictional outcomes. However, the effect of other bathing solutions containing lubricants such as synovial fluid (SF), hyaluronic acid (HA), bovine serum albumin (BSA), or culture media, on tribological rehydration have not been fully explored. The *goal of this study was to investigate the effect of boundary lubricants on tribological rehydration and tribomechanical outcomes in the cSCA configuration.* Four 'lubricants' were chosen for their presence *in vivo* (SF), and as common additives to articular cartilage testing (HA) and explant culture solutions (BSA, media). We hypothesized that the more viscous lubricants, HA and SF, would increase both the effectiveness of tribological rehydration and lubrication in articular cartilage by enhancing fluid uptake (strain recovery) and reducing friction over sustained bouts of sliding. We also wished to ask the question of *whether low viscosity PBS and standard chondrogenic culture media are suitable bathing solutions for ex vivo tribology studies or if boundary lubricants should be added to produce physiological 'safe' frictional responses*.

METHODS

Tissue Specimens and Tribological Testing: ∅19mm osteochondral cores were removed from the femoral condyles of mature bovine stifle joints (procured from Bowman's Butcher Shop, Churchville, MD)[10]. Cores were trimmed to ~12mm in height and the *in vivo* sliding direction noted[11]. Following extraction, explants were stored in PBS + protease inhibitors (referred to herein as PBS) at 4°C[12]. Explants were tested in a custom-built reciprocating materials device (a.k.a. tribometer)[8] in which explants were compressed and slid against a reciprocating slide.

Boundary Lubricants: Four different boundary lubricants were tested in this study: SF, PBS+HA, PBS+BSA, and chondrogenic culture media. SF was extracted from healthy equine stifle joints within 2 hrs of euthanasia and stored at -20°C. PBS+HA was prepared by dissolving high-molecular weight (1350 kDa, 5mg/mL) hyaluronan in PBS. PBS+BSA was prepared at 3% wt/vol. Chondrogenic culture media was prepared using DMEM supplemented with 1% ITS+Premix (Corning), 20 μg/mL ascorbic acid, 1% pen-strep, and 1% amphotericin-B.

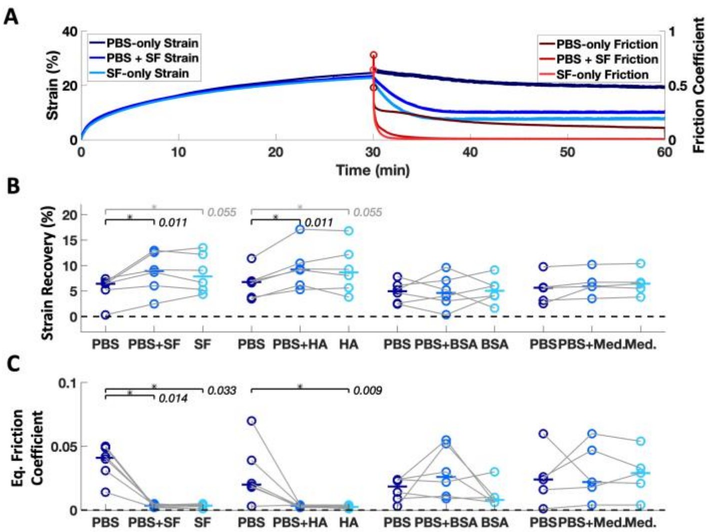

Figure 1. A) Overlaid traces from a random SF explant show increased strain recovery and lower friction after addition of lubricant. Collated data shows B) sliding-induced strain recovery (i.e. tribological rehydration) is greater, and C) sliding equilibrium friction coefficient is lower in in the samples treated with the more viscous lubrication solutions (SF, HA).

Testing Protocols: Prior to testing, each sample was inspected for surface damage using India Ink and a stereomicroscope; visible damage was an exclusionary criterion. Samples then underwent a diagnostic test consisting of 10min compression at 7N (~0.25MPa), followed by 2min of reciprocal sliding at 80mm/s (~walking speed) to identify if samples exhibited adequate tribological rehydration for inclusion in the study, i.e. friction coefficients $\mu<0.2$, and sliding-induced recovery/reversal of deformation (<10% of samples were excluded in this manner). Samples were then assigned to one of four lubricant study arms.

A tribological rehydration characterization scheme, with a repeated-measures design, was used for each lubricant testing group. This protocol started with 30min of static compression at 7N, followed by 30min of reciprocal sliding at 80mm/s under 7N compression[11]. Characterization was first performed in the presence of a *PBS-only* bath, followed by a 1-hr free-swell period in PBS. Next, characterization was repeated with the PBS bath replaced by one of the four lubricant baths immediately prior to sliding. After these *PBS+lubricant* tests, each explant was free-swelled in lubricant bath for 1-hr before a final characterization in the presence of *lubricant only*. Repeated-measures testing was utilized to improve statistical power and to determine if brief lubricant application or extensive lubricant soaking differentially influenced tribomechanical outcomes in the cSCA. After testing, explants were again assessed for damage using stereomicroscopy, and then bisected to measure cartilage thickness (h).

Data Analysis: Deformation (δ), normal force (F_N), and friction coefficients (μ) recorded and analyzed using MATLAB[11]. Tissue strain ($\varepsilon = \delta/h$) and friction magnitudes were analyzed at the beginning and end of active sliding, and strain recovery (i.e. tribological rehydration) during sliding was calculated. Friedman's Test, a nonparametric test with replication, was used to identify statistically significant changes between the repeated *PBS-only*, *PBS+lubricant*, and *lubricant-only* tests. Kruskal-Wallis' test was used to identify differences among the *PBS-only* test groups and the *lubricant-only* test groups.

RESULTS

The most pronounced changes occurred in samples where SF and HA were present (representative SF deformation and friction traces shown in **Fig. 1A)**; BSA and culture media did not meaningfully affect the tribomechanical behavior of explants. In the repeated tests, strain recovery increased when SF (p=0.011) and HA (p=0.011) were added as lubricants (**Fig. 1B)**. Friction coefficients at the end-of-sliding (i.e. sliding equilibrium) were, relatively speaking, quite low for all groups (on average <0.04), but high-viscosity lubricants (SF and HA) drove a significant reduction in sliding equilibrium friction (μ = 0.03 to 0.003, p=0.033 & 0.009, respectively; **Fig. 1C**). There were no significant changes in strain recovery or end-of-sliding friction for the samples lubricated with BSA or chondrogenic media.

When comparing across lubricant testing groups, no significant differences in strain recovery magnitude (i.e. tribological rehydration) or rate of strain recovery were observed either prior to the addition of lubricant (*PBS-only* tests) or following the addition of the lubricants (*PBS+lubricant* or *lubricant-only*). However, the presence of both SF and HA led to, on average, significantly reduced end-of-sliding and time averaged friction compared to PBS, BSA, and culture media (BSA and culture media outcomes were no different from PBS outcomes). Start-of-sliding strain, end-of-sliding strain, time-average strain, start-of-sliding friction, and deformational time-constants were also investigated (data not shown), and showed similar results to strain recovery and end-of-sliding friction, with the majority of changes occurring when SF and HA were present as lubricants.

DISCUSSION

These results illustrate that viscous lubricants, e.g. SF and HA, create an environment that enhances tribological rehydration and lubrication during high-speed sliding in the cSCA. This was reflected in increased overall strain recovery and reduced sliding equilibrium friction when compared to the BSA, chondrogenic media, and PBS-lubricated groups. These findings suggest that SF and HA may aid cartilage function through two distinct, but complementary mechanisms. First, as traditionally-understood boundary-mode lubricants[1], and second, as viscous bathing solutions that enhance fluid pressurization (and thus tribological rehydration) within the convergent contacts (e.g. the cSCA) by purely hydrodynamic means. Enhanced tribological rehydration supports both the recovery of tissue deformation (i.e. strain recovery) and the promotion of very-low friction coefficients via the maintenance and replenishment of interstitial lubrication. Lastly, we have seen that even PBS and chondrogenic media can mediate, under high-speed sliding in the cSCA, equilibrium friction outcomes (μ<0.03) that are below those previously shown to induce chondrocyte 'dysfunction' (μ>0.1)[13]. Together, these findings suggest that highly-viscous solutions like HA and SF *enhance* the lubrication of articular cartilage *ex vivo,* however these *lubricants are not necessary for maintaining physiologically-consistent and safe tribomechanical behavior in the cSCA*. When possible, we recommend adding lubricants to bathing solutions, however due to increased cost of these solutions, lubrication with PBS and media is sufficient.

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