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EFFECT OF COUNTERFACE SURFACE ROUGHNESS ON TRIBOLOGICAL REHYDRATION OF ARTICULAR CARTILAGE

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INTRODUCTION — Articular cartilage comprises the load-bearing region of synovial joints and, in the absence of injury or degradation, is able to support smooth articulation over decades of repetitive use. Cartilage's multiphasic structure, consisting of ~80% fluid and ~20% solid collagen and proteoglycan matrix, allows for its exceptionally low coefficient of friction (μ <0.02) and high wear resistance.[1-8] The fluid phase in particular is key to the tissue's phenomenal lubrication properties becaue sit can support large fractions of contact stress while also shielding the solid matrix from excessive shear. In previous studies, our team has characterized usage of an ex vivo testing configuration known as the convergent stationary wedge (cSCA) that allows assessment of cartilage biomechanics under sustained, physiologically consistent values of cartilage strain, strain recovery, hydration, and lubricity over multiple hours of testing.[6] In the cSCA configuration, glass counterfaces are slid against large (⌀19mm) articular cartilage explants, which retain the cartilage's natural radius of curvature and allow hydro-dynamically driven fluid flow from the bathing solution into the tisse during sliding. Through a mechanism we have termed 'tribological rehydration,' hydro-dynamically driven fluid flow during sliding competes with, and often exceeds, the rate of load-inuduced fluid exudation associated with stationary compression. As a result, high interstitial fluid pressure is retained in the tissue during sliding and provides sufficient load support for sustaining cartilage hydration and low coefficients of friction over the duration of active sliding.[8] In our previous cSCA-based studies, only one type of glass counterface, standard lass microscope slides was used. In order to further characterize the biomechanical response of cartilage under the cSCA configuration, *the goal of this study was to investigate the effect of glass counterfaces of different surface roughnesses on tribological rehydration and resultant cartilage tribomechanical outcomes.* Specifically, counterfaces of three asperity heights were investigated, including super-polished quartz slides, plain microscope slides (those used in our previous investigations), and frosted microscope slides.

METHODS — Tissue Specimens and Tribological Testing: ⌀19mm osteochondral cores were removed from the femoral condyle centerline 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 using a custom-built reciprocating materials device ('tribometer')[8] in which explants were compressed and slid in succession against different glass surfaces with one of three different asperity heights: super-polished quartz microscope slides (Fisher Scientific), plain microscope slides (company), and frosted microscope slides (Fisher). Testing Protocols: Prior to testing, each isolated osteochondral 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 10 min compression at 7N, followed by 2 min of reciprocal sliding at 80 mm/s (~walking speed) to identify if individual samples exhibited adequate tribological rehydration for inclusion in the study, i.e. friction coefficients μ<0.2, and sliding-induced recovery/reversal of deformation (<10% of samples were excluded by this criteria). A tribological rehydration characterization scheme, with a repeated-measures design, was used for each counterface testing group. This protocol started with the application of 30 min of static compression at 7N (~0.25 MPa), followed by 30 min of reciprocal sliding at 80 mm/s (~walking speed) under 7N compression[11]. Characterization was first performed sliding against a plain microscope slide, followed by two hours of free swelling in PBS. Characterization was then repeated with polished, plain, and frosted

slides, in that order. Between each sliding test, each explant was free-swelled in PBS for two hours. 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 by the tribometer were analyzed using MATLAB.[11] Measures of tissue strain (ε = δ/h) were calculated, and strain and friction magnitudes were analyzed at the beginning and end of active sliding, and strain recovery (i.e. tribological rehydration) during sliding was calculated. Characteristic deformation rates were obtained from linear regression fits of the time-dependent deformation data. Negative deformation rates are representative of recovery of compressive deformation (i.e., tribological rehydration), while positive deformation rates are representative of pure compression-induced deformation (during static loading) or continued compressive deformation, and thus, compromised tribological rehydration (during sliding). Friedman's Test, a nonparametric test with replication, was used to identify statistically significant changes between the repeated surface roughness tests

RESULTS — The deformation and friction responses were similar when matched samples were slid against regular and super-polished slides, with similar strain recovery (i.e., tribological rehydration) being observed under both of these conditions (p=0.97). The most pronounced changes occurred when explants were tested against the rough glass counterface, under which strain recovery (i.e., tribological rehydration) did not occur. For regular and rough glass surfaces, explants recovered an average of 14.5 and 17.6 μm, respectively. However, when slid against the rough counterface, strain recovery was significantly impaired relative to regular and polished surfaces, showing no recovery of deformation at the end of 30 minutes of sliding against the rough glass (p<0.0001). Friction coefficients at the end of sliding (i.e., sliding equilibrium, were similar for all three surfaces (μ = 0.04 - 0.07). However, there was a significant increase in start of sliding friction coefficient for sliding against rough glass (μ = 0.5288, p<0.0001) compared to regular and rough surfaces (μ= 0.288 and 0.289, respectively; p=0.9996). Increased surface roughness had a significant effect on both start-of-static and start-of-sliding deformation time constant relative to regular and polished glass surfaces (p=0.01 and p=0.0001, respectively), consistent with the lack of strain recovery observed under this condition.

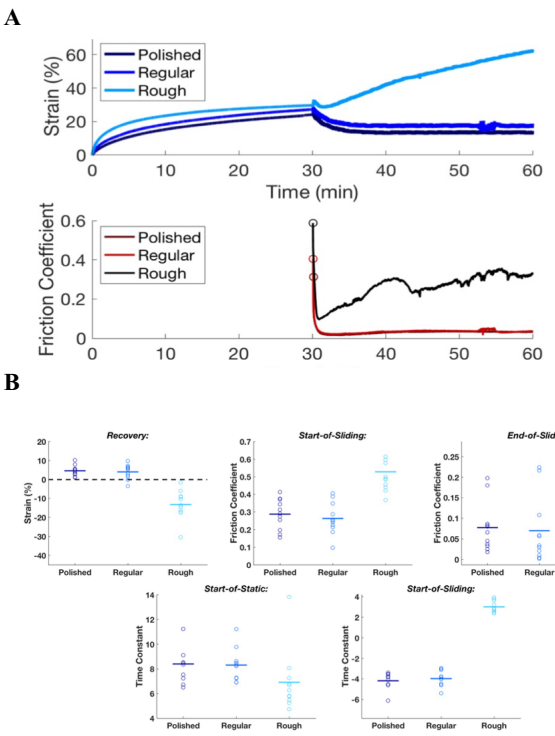

Figure 1. A) Overlaid traces from a representative explant show similar strain and friction behavior for regular and polished glass, and continued compressive deformation during sliding for rough glass. B) Sliding-induced strain recovery occurs with both polished and regular, but not rough glass (top row); the static compression time constants were similar for polished and regular glass while explants reached characteristic deformation faster when compressed against rough glass; end-of-sliding deformation time constants show that strain recovery occurred for polished and regular glass while sliding against rough glass resulted in continued deformation (bottom row).

DISCUSSION — The results of this study illustrate that decreasing surface roughness of the glass counterface from that of a standard microscope glass to ultra-polished quartz glass has no appreciable effect on the tribomechanical response of articular cartilage under the cSCA testing configuration. This was reflected in similar values of strain recovery, and start- and end-of-sliding friction coefficients for both regular glass and ultra-polished glass. These findings suggest that regular microscope slides are of sufficient surface properties to assess the tribological behavior of articular cartilage under the cSCA and ultra-polished glass is not necessary for these experiments. Conversely, the use of frosted glass, which is of a greater asperity height, and thus, surface roughness, prevents cartilage from sustaining tribological rehydration under reciprocal sliding in the cSCA configuration. Under this condition of increased microscale porosity of the counterface surface, reciprocal sliding led to wearing of the cartilage surface, and as the uppermost layer of cartilage matrix is being removed, it appeared that tribological rehydration was not appreciably being driven. This is reflected in an overall increase in deformation during sliding for the rough surface, whereas a recovery of deformation is seen for polished and regular glass, meaning that tribological rehydration is not occurring when cartilage is slid against rough glass. In addition, increased surface roughness (i.e., increased nanoscale porosity) leads to a greater number of fluid flow pathways, thereby increasing compression-induced exudation and preventing tribological rehydration during reciprocal sliding. These findings suggest that ultra-polished glass is not necessary for creating physiologically analogous behavior under the cSCA configuration and that plain microscope slides provide a sufficient surface smoothness to sustain tribological rehydration in these experiments, while increasing counterface surface roughness defeats the tissue's ability to recover compressive deformation under sliding.

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