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Cartilage shear dynamics during tibio-femoral articulation: effect of acute joint injury and tribosupplementation on synovial fluid lubrication

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Summary

Objective: To determine the effects of acute injury and tribosupplementation by hyaluronan (HA) on synovial fluid (SF) modulation of cartilage shear during tibio-femoral articulation.

Methods: Human osteochondral blocks from the lateral femoral condyle (LFC) and tibial plateau (LTP) were apposed, compressed 13%, and subjected to sliding under video microscopy. Tests were conducted with equine SF from normal joints (NL-SF), SF from acutely injured joints (AI-SF), and AI-SF to which HA was added (AI-SF + HA). Local and overall shear strain (E_{xz}) and the lateral displacement (Δx) at which E_{xz} reached 50% of peak values ($\Delta x_{1/2}$) were determined.

Results: During articulation, LFC and LTP cartilage E_{xz} increased with Δx and peaked when surfaces slid, with peak E_{xz} being maintained during sliding. With AI-SF as lubricant, surface and overall $\Delta x_{1/2}$ were ~40% and ~20% higher, respectively, than values with NL-SF and AI-SF + HA as lubricant. Also, peak E_{xz} was markedly higher with AI-SF as lubricant than with NL-SF as lubricant, both near the surface (~80%) and overall (50–200%). Following HA supplementation to AI-SF, E_{xz} was reduced from values with AI-SF alone by 30–50% near the surface and 20–30% overall. Magnitudes of surface and overall E_{xz} were markedly (~50 to 80%) higher in LTP cartilage than LFC cartilage for all lubricants.

Conclusion: Acute injury impairs SF function, elevating cartilage E_{xz} markedly during tibio-femoral articulation; such elevated E_{xz} may contribute to post-injury associated cartilage degeneration. Since HA partially restores the function of AI-SF, as indicated by E_{xz}, tribosupplements may be beneficial in modulating normal cartilage homeostasis.

Key words: Synovial fluid, Lubrication, Hyaluronan, Acute injury, Cartilage mechanics, Shear deformation.

Introduction

Within the knee joint, articular cartilage lining the femoral condyle articulates against articular cartilage of the tibial plateau that is uncovered by meniscus [Fig. 1(A)] to facilitate diarthrodial joint movement[1]. Following various physical activities such as knee bending, impact loading, and running, cartilage compresses ~3 to 20%[2–4], with compression typically being higher in cartilage from the tibial plateau than the femoral condyle[3,4]. Under such magnitudes of physiologic compression, tibio-femoral cartilage compression and shear are depth-varying, being highest near the surface and decreasing monotonically with increasing depth, and greater in tibial cartilage than femoral cartilage[5]. However, the shear kinematics as well as the effects of synovial fluid lubrication remains to be elucidated for physiologically apposed cartilage surfaces. Such characterization would further the understanding of cartilage contact mechanics during joint loading and motion by elucidating the boundary conditions at the interface and the intra-tissue shear deformation within physiologically articulating femoral and tibial cartilage surfaces.

In healthy joints, synovial fluid (SF) is present between articulating cartilage surfaces, functioning as an effective boundary lubricant. During cartilage articulation, interstitial fluid pressurization[6,7] and boundary mode lubrication[8] may both mediate friction between articulating surfaces. In boundary lubrication, load is supported by surface-to-surface contact and friction between articular surfaces is thus dictated by bound surface lubricant molecules, which may become increasingly important with prolonged loading as interstitial fluid pressure diminishes[9]. Two major constituents of SF identified to lubricate the articular surface are hyaluronan (HA)[10] and proteoglycan 4 (PRG4)[11,12]. In a configuration to reveal boundary lubrication effects, the surface interactions, as indicated by friction[8] and shear deformation[13,14] of articulating cartilage, are reduced by SF relative to phosphate buffered saline (PBS). The SF lubricant molecules, HA and PRG4, contribute, independently and in combination, to reduce articulating cartilage friction under boundary lubrication conditions[15]. Thus, altered lubricant molecule concentrations may diminish the boundary lubrication function of SF and cause elevated tissue shear, predisposing cartilage to accelerated wear and degradation.

Following acute injury, the friction-reducing function of SF is compromised[16–18], possibly due, in part, to reduced HA

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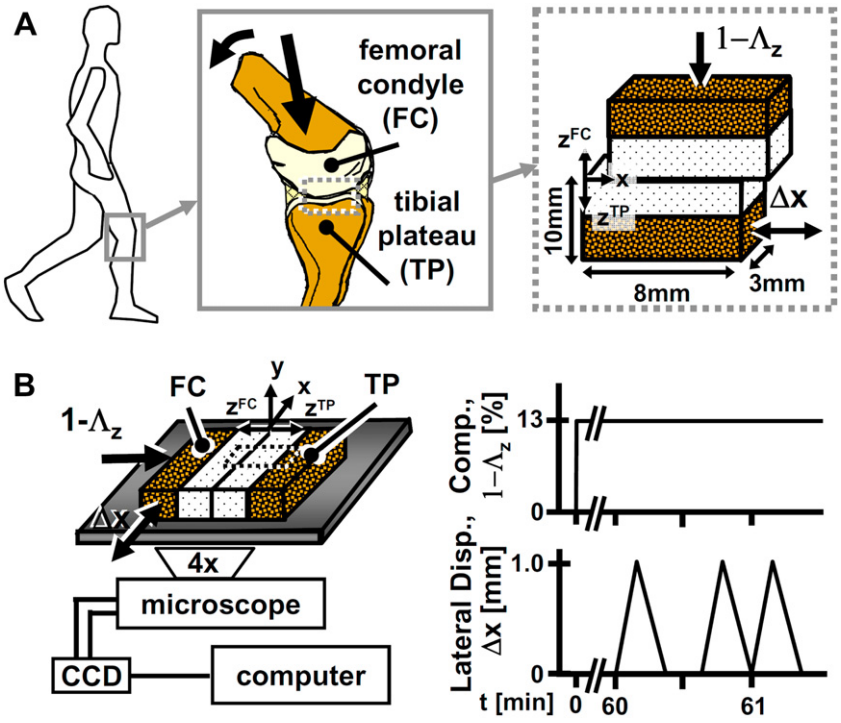

Fig. 1. Schematic of (A) knee joint movements at multiple scales and (B) of experimental setup and loading protocol for microshear testing. Definition of loading variables: compressive axial strain ($1-\Lambda_z$) and lateral displacement (Δx).

concentration. The HA concentration in SF of acutely injured equine joints was decreased from normal (~0.3 mg/ml to 0.2 mg/ml), while PRG4 and surface-active phospholipid (SAPL) concentrations were increased[19]. When SF was tested between articulating cartilage surfaces to reveal boundary mode lubrication, friction was markedly higher with SF from acutely injured joints (AI-SF) than that from contra-lateral normal joints (NL-SF). When AI-SF was augmented with HA (AI-SF + HA), friction coefficient, μ (a dimensionless measure which describes the ratio of frictional force and the normal force between two surfaces), was reduced towards normal values, suggesting lowered HA concentrations reduced SF function. Such alterations in the friction-reducing function of SF following acute injury may alter the normal mechanobiology of articulating cartilage since tissue shear deformation markedly regulates lubricant and matrix metabolism[20–22].

Femoral and tibial human articular cartilage exhibit shear deformation and interaction during tibio-femoral cartilage articulation[5], as revealed by video microscopy[23] to track the displacement of fluorescently labeled cells[24]. With this experimental approach, osteochondral samples from the tibia and femoral condyle were compressed with the cartilage surfaces in apposition and subjected to lateral shearing motion to mimic the biomechanics of tibio-femoral joint articulation [Fig. 1B]. Resultant compressive and shear strains of cartilage were determined locally and overall, with an effective resolution of 40 μm. Using such a configuration, biomechanical microscale analysis can be used to assess the effects of synovial fluid lubrication on local and overall shear deformation of femoral and tibial cartilage during tibio-femoral cartilage articulation.

The hypothesis of this study was that during tibio-femoral articulation, cartilage lubrication by AI-SF elevates tissue shear deformation, while lubrication by AI-SF with augmented HA reduces shear deformation towards normal magnitudes. To test this hypothesis, the objectives of this study were to determine, during tibio-femoral cartilage articulation (1) the effects of acute injury on SF lubricant function and (2) the ability of HA addition to AI-SF to enhance lubricant function, with lubricant function assessed as peak cartilage shear deformation during sliding motion.

Materials and methods

SAMPLE ISOLATION AND PREPARATION

Six osteochondral cores, each with a 10 mm diameter, were isolated, one from each anterior lateral femoral condyle (LFC) of six fresh cadaveric human male ($n=3$) and female ($n=3$) donors (mean ± standard error of the mean (S.E.M) age of 46 ± 1.5 yrs). In addition, six osteochondral blocks (each with a chondral surface area of ~1 cm^2) were harvested from the region of the donor-matched lateral tibial plateau (LTP) not covered by the meniscus. LFC cores with grossly normal surfaces (modified Outerbridge grade of 1[25]) were selected, while all donor-matched LTP blocks displayed mild surface fibrillation and were modified Outerbridge grades of 2–3[25]. The harvested specimens were immersed in phosphate buffered saline (PBS) containing proteinase inhibitors (PIs)[26] and stored at −70°C until use.

The osteochondral specimens were thawed and further processed on the day of testing. The LFC core and LTP block were each trimmed using a low-speed saw with a 0.3 mm thick diamond edge blade (Isomet™, Buehler, Lake Bluff, IL) to yield approximately one rectangular block for biomechanical testing. Each rectangular block had a cartilage surface area of ~3×8 mm^2 and a total thickness of ~1 cm. Blocks were created such that their 8 mm lengths were parallel to the direction of articulation in the joint from which they were isolated from [Fig. 1(A)]. Samples consisted of one LFC and one donor-matched LTP block and were each rinsed with PBS + PI overnight prior to mechanical testing[15].

From macroscopic images[5], thickness measurements of the present samples were made at three separate locations and averaged to yield a full cartilage thickness measurement. For the LTP samples, cartilage thickness (2.88 ± 0.53 mm, mean ± S.E.M, $n=6$ blocks) was somewhat thicker than the cartilage thickness of the LFC samples (2.20 ± 0.15 mm). In addition, the LFC and LTP samples used in this study were characterized by histopathology[5]. LFC cartilage was normal, while LTP cartilage exhibited typical features of very mild degeneration. Both LFC and LTP cartilage exhibited

normal cellularity and an absence of cell cloning. LTP cartilage exhibited mild surface irregularity, occasional transverse clefts, and mildly reduced glycosaminoglycan staining.

LUBRICANTS

Soon after an acute injury (within 3 weeks), synovial fluid (SF) was aspirated from joints of six mature (2–4 yr old) horses (with IACUC approval) during arthroscopic surgery by one of the authors (CWM). SF was aspirated from the injured joint (AI-SF) as well as the contra-lateral uninjured joint (NL-SF) which showed no clinical signs of injury. Equine SF was classified as from an acute injury if the horse was presented for surgery within 3 weeks of clinical diagnosis. Collected samples were cleared off cells and debris by centrifugation (3000*g*, 30 min) and resultant supernatants were frozen at −70°C until mechanical testing.

The synovial fluid samples were characterized for boundary lubrication properties of lubricant molecules, confirming NL-SF and AI-SF were characteristic of SF from NL-SF and AI-SF, respectively. For lubricant samples used in this study, the coefficient of kinetic friction and the biochemical composition were determined previously with a cartilage-on-cartilage friction test and biochemical analyses, respectively[27]. From these tests, the coefficient of friction was 0.040 ± 0.003 for AI-SF and 0.022 ± 0.001 for NL-SF. Levels of HA were lower in AI-SF (0.19 ± 0.05 mg/ml) than NL-SF (0.31 ± 0.11 mg/ml) samples, while proteoglycan 4 concentrations were higher in AI-SF (90.9 ± 90.4 μg/ml) than NL-SF lubricants (17.6 ± 5.3 μg/ml), differences that are distinctive of synovial response to injury. Lubricant samples used in this study were (1) SF from the contralateral non-injured joint (NL-SF), (2) SF from the injured joint (AI-SF), and (3) AI-SF to which HA was added (AI-SF + HA). For AI-SF + HA lubricant samples, high molecular weight (800 kDa) HA (SUPARTZ®, Seikagaku Corporation, Tokyo, Japan) was added to AI-SF to create a final concentration of supplemented HA to be 1 mg/ml. All lubricant samples in the present study had PI added prior to mechanical testing.

EXPERIMENTAL DESIGN

To assess the effect of acute injury on SF function in terms of cartilage shear deformation, lubricants were tested on cartilage samples in microshear tests described below. In particular, each of the lubricant samples (AI-SF, AI-SF + HA, NL-SF) from one equine animal were paired and tested sequentially between cartilage tissue from one human donor to reduce donor–donor variability of lubricant and tissue. Prior to mechanical testing, cartilage was completely immersed in ~0.5 ml of test lubricant containing PI and 20 μg/ml propidium iodide to fluorescently highlight cell nuclei at 4°C for 12–16 h. Subsequently, AI-SF, AI-SF + HA, and then NL-SF were tested sequentially in microscale shear tests. In between lubricant bathing and mechanical testing, cartilage samples were rinsed, allowed to reswell, and reincubated in PBS + PI for ~4 h at 4°C. As a result, each cartilage sample was mircoscale shear tested a total of 3 times (one time per lubricant sample) at room temperature.

MICROSCALE SHEAR TESTING

Samples were tested for effects of lubricant on cartilage shear as described previously[5]. Briefly, each LFC and LTP pair was apposed in a custom bi-axial loading chamber mounted onto an epi-fluorescence microscope for digital video imaging [Fig. 1(B)][28]. The chamber secured the LFC block at the bone and allowed in-plane movement of the apposing mobile LTP block with orthogonally positioned plungers interfaced with either a micrometer (for axial displacement; Model 262RL; Starrett Co., Athol, MA) or motion-controller (for lateral displacement; Model MFN25PP; Newport, Irvine, CA). Subsequently, an axial displacement was applied (~40 μm/s) to the bone portion of the LFC sample to induce 13% compression ($1 - \Lambda_z$, where Λ_z is the stretch ratio[29]) of the overall (i.e., LFC and LTP) cartilage thickness determined from gross images. Samples were then allowed to stress relax for 1 h which was confirmed to be sufficient to reach an approximate equilibrium stress for the current sample geometries[13].

Following axial compression, cartilage deformation was assessed separately in the LFC and LTP tissue during shear loading as previously described[5]. Three sets of applied lateral displacements (Δx), each consisting of +1 mm and then −1 mm (returning to initial position) was applied to the bone portion of the LFC block [Fig. 1(B)] at a rate of 0.1 mm/s to induce relative lateral motion. The first set of sliding motion was for preconditioning[8], while tissue motions during the second and third set were recorded for analysis of LTP and LFC cartilage, respectively. Before and during the application of lateral displacements, sequential fluorescence (Nikon G-2A filter) images were taken separately for LFC and LTP cartilage at increments of 33 μm to capture the shear deformation and sliding during tibio-femoral cartilage articulation. Resultant images with a field of view of $\sim 3 \times 2$ mm^2 encompassed the entire cartilage thickness of the LFC (or LTP) and a partial view of the apposing surface. The same regions of tissue were imaged and analyzed for each of the three lubricant conditions.

DATA COLLECTION AND CALCULATIONS

Digital fluorescence images from microscale tests were analyzed as described previously[13,24] to determine the depth-varying and overall shear deformation in cartilage. Briefly, an evenly distributed set of cell nuclei (~250 cells/mm^2) was selected to serve as fiducial markers and tracked by maximizing cross-correlation of regions surrounding each marker to the preceding, and then initial frames. For each recorded image, local affine mappings of nuclei were used to calculate the displacement of uniformly spaced (10 pixel) mesh points in the region of interest (~1 mm × full thickness) during deformation. Subsequently, displacement gradients were determined by finite difference approximation, from which, Lagrangian shear strains (E_{xz}) relative to the compressed cartilage thickness at compressive equilibrium were determined for both LFC and LTP cartilage during lateral shearing[30].

Local and overall E_{xz} as well as measurements to assess the rates at which E_{xz} reached their peaks were determined separately for LFC and LTP samples. For each image frame, E_{xz} of the LFC and LTP were each consolidated by averaging E_{xz} at the same normalized depth (0, surface and 1, tidemark) and then interpolating E_{xz} linearly at every 0.05 times the normalized tissue thickness near the articular surface (i.e., 0–0.3) and 0.1 for remaining regions of the tissue depth (i.e., 0.3–1). Surface E_{xz} was defined as that occurring at the top 5% of the cartilage thickness, while overall E_{xz} was determined as half the lateral tissue displacement near the articular surface normalized to the compressed cartilage thickness. Surface and overall E_{xz} determined at Δx increments of 0.1 mm (Δx ranging between 0 and 0.8 mm) were used to determine the Δx when surface and overall E_{xz} reached 50% of their peak values ($\Delta x_{1/2}$).

STATISTICAL ANALYSIS

Data are reported as mean ± S.E.M, unless noted otherwise. Repeated measures ANOVA was used in Systat 10.2.05 (Systat Software, Richmond, CA) to determine the effects of joint location (LFC, LTP) and lubricant (AI-SF, AI-SF + HA, NL-SF) on local and overall E_{xz}. To account for sample variances being approximately proportional to the amplitude of the data, $\Delta x_{1/2}$ data were log transformed prior to analysis in Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA). Planned comparisons (AI-SF vs NL-SF, AI-SF + HA vs NL-SF, and AI-SF vs AI-SF + HA) were then made between lubricant groups for a given joint location in Systat 10.2.05.

Results

SHEAR DEFORMATION KINEMATICS

Similar to previous qualitative[13] and quantitative[14] descriptions of cartilage-on-cartilage articulation, tibio-femoral cartilage articulation resulted in a sequence of four main events for all experimental conditions during shear loading. Qualitatively at compressive equilibrium (1) LFC and LTP surfaces adhered and began to move laterally in unison to initiate E_{xz} in both apposing tissues near the onset of Δx. (2) With increasing Δx, LFC and LTP E_{xz} increased while surfaces remained adhered. (3) Eventually with sufficient Δx, LFC and LTP E_{xz} reached a peak just as their respective surfaces detached and slid relative to each other. (4) With additional Δx, cartilage E_{xz} in both LFC and LTP tissues maintained their steady-state peak.

During shear loading, cartilage E_{xz}, near the articular surface and overall, increased markedly with Δx and was significantly higher for LTP than LFC cartilage ($P < 0.05$). For both LFC and LTP cartilage and for all lubricant conditions, E_{xz} near the surface and overall increased markedly ($P < 0.001$) with increasing Δx, eventually reaching a maximum that was maintained even as Δx continued to increase (Fig. 2). These quantitative trends were consistent with the qualitative observations described above. When AI-SF was used as the lubricant, the resultant E_{xz} near the articular surface of LFC samples reached a peak value during articulation that was higher than that when samples were tested with AI-SF + HA and NL-SF as lubricants [Fig. 2(A)].

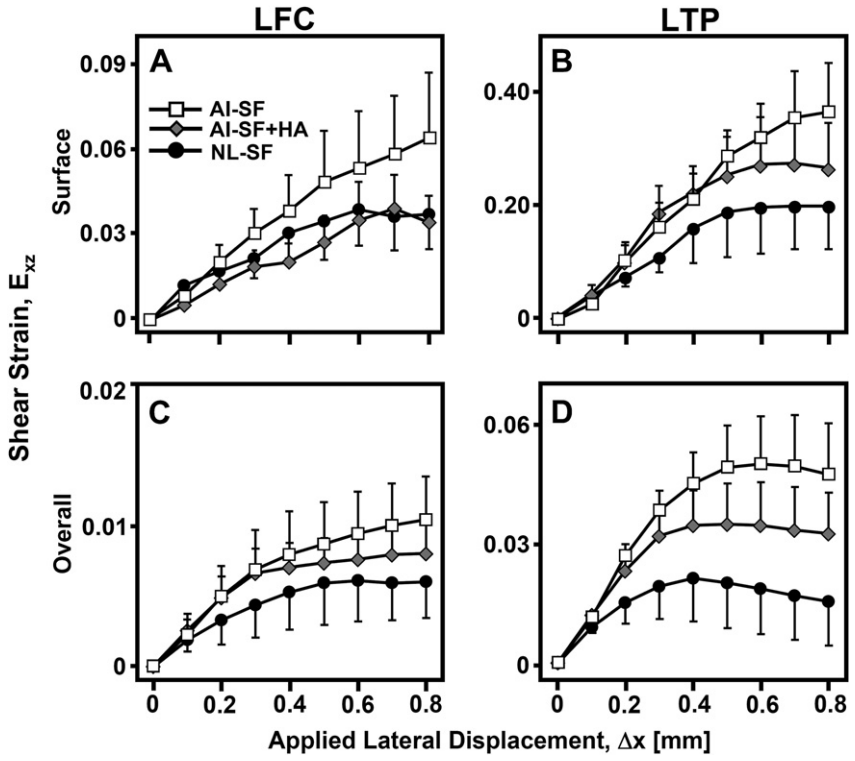

Fig. 2. (A and B) Surface and (C and D) overall shear strain (E_{xz}) vs applied lateral displacement (Δx) for (A and C) LFC and (B and D) LTP cartilage tested with AI-SF, AI-SF + HA, and NL-SF.

Analogously, overall E_{xz} of LFC cartilage also reached a higher peak magnitude for AI-SF as lubricant than AI-SF + HA and NL-SF as lubricants [Fig. 2(C)]. Typically, E_{xz} near the surface and overall of LFC samples reached peak values that were highest, second highest, and lowest during articulation with AI-SF, AI-SF + HA, and NL-SF, respectively, as lubricant. Similar trends in LTP E_{xz} during tibio-femoral articulation were found; however, the E_{xz} magnitudes of LTP cartilage were markedly higher near the articular surface ($P < 0.01$) and overall ($P < 0.05$) than those of the LFC samples [Fig. 2(B and D)]. For all cases, E_{xz} reached a steady-state peak at a Δx of 0.8 mm. Thus, effects of lubrication on tissue E_{xz} were further compared at this point as described below (Peak shear deformation).

The rate at which surface and overall E_{xz} reached their peak values during articulation, as indicated by $\Delta x_{1/2}$, was significantly dependent upon lubricant. Near the articular surface, $\Delta x_{1/2}$ was markedly affected by lubricant ($P < 0.05$), tending to be higher with AI-SF (70−80%, $P = 0.13$) and AI-SF + HA (20−60%, $P = 0.07$) as lubricant than with NL-SF [Fig. 3(A)]. Overall, $\Delta x_{1/2}$ was also markedly affected by lubricant ($P < 0.05$), being 65−80% higher ($P < 0.05$) with AI-SF as lubricant than with NL-SF as lubricant [Fig. 3(B)]. However, with AI-SF + HA as lubricant, overall $\Delta x_{1/2}$ was similar to that with NL-SF as lubricant ($P = 0.7$). Differences in $\Delta x_{1/2}$ near the surface ($P = 0.5$) and overall ($P = 0.10$) were not statistically significant between LFC and LTP cartilage for all cases (Fig. 3).

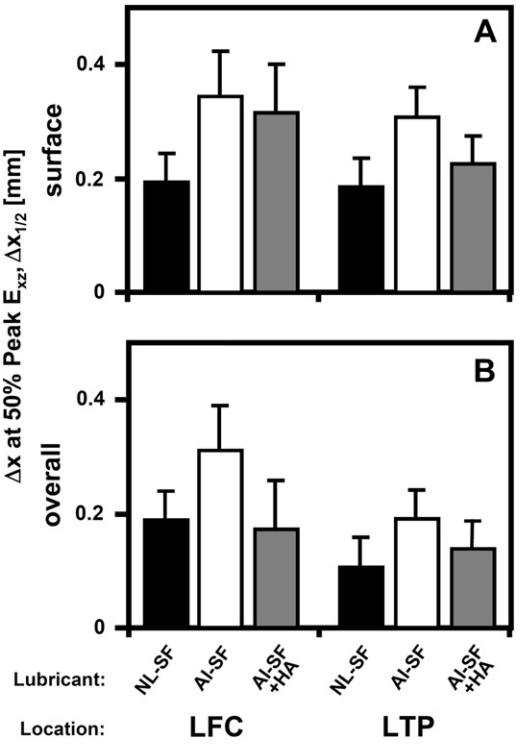

Fig. 3. Effect of acute injury lubrication on Δx at 50% peak ($\Delta x_{1/2}$) (A) surface and (B) overall shear strain for LFC and LTP samples.

PEAK SHEAR DEFORMATION

When tibio-femoral surfaces were sliding and E_{xz} was at a peak, lateral tissue displacements and deformation were

depth-varying, being highest near the articular surface and lowest (almost negligible) near the tidemark for LFC and LTP cartilage [Fig. 4(A–C)]. For LTP cartilage, the maximum lateral displacement when tibio-femoral surfaces were lubricated with NL-SF (76 ± 48 μm) increased when lubricated with AI-SF (to 193 ± 63 μm, $P < 0.05$), but not significantly when lubricated with AI-SF + HA (143 ± 48 μm, $P = 0.06$). For LFC cartilage, trends were similar but not statistically significant with the maximum lateral displacement when tibio-femoral surfaces were lubricated with NL-SF (31 ± 11 μm) increased when lubricated with AI-SF (to 48 ± 13 μm, $P = 0.4$) and AI-SF + HA (to 41 ± 6 μm, $P = 0.7$). Differences in shear deformation between LFC and LTP cartilage were also apparent, with shear deformation of LFC [Fig. 4(A–C); top images] cartilage being much lower than LTP shear deformation [Fig. 4(A–C); bottom images] during tibio-femoral articulation.

Peak E_{xz} varied with tissue depth, lubricant, and joint location [Fig. 4(D)]. With tissue depth, E_{xz} markedly ($P < 0.001$) decreased monotonically with increasing depth from the articular surface for all experimental conditions. There was an interactive effect between lubricant and tissue depth ($P < 0.001$) on LFC and LTP E_{xz}, as indicated by the differences in E_{xz} between lubricant conditions being greatest near the surface and lowest (almost indistinguishable) in the deeper regions. A similar interactive effect between cartilage location and depth on E_{xz} was also evident ($P < 0.001$) for all lubricant cases, with LTP E_{xz} being markedly higher in magnitude than LFC E_{xz} near the surface and being similarly low to LFC E_{xz} near the tidemark.

Depending on the joint location from which cartilage samples were isolated, peak E_{xz} near the surface and overall were affected by lubrication. Near the surface and overall, interactive effects between joint location and lubrication on E_{xz} were marked ($P < 0.05$). In the LTP, peak E_{xz} increased markedly and significantly with acute injury lubricant and reduced towards normal with HA supplementation. LTP cartilage E_{xz} near the surface and overall were 0.2 and 0.02, respectively, when tibio-femoral surfaces were lubricated with NL-SF, and increased markedly ($P < 0.05$) to 0.37 and 0.05, respectively, when surfaces were lubricated with AI-SF [Fig. 5(A and B)]. When AI-SF was supplemented with HA (AI-SF + HA), surface and overall E_{xz} of LTP cartilage were reduced ($P < 0.05$) to 0.26 and 0.03, respectively, during tibio-femoral articulation, and the resultant E_{xz} were statistically indistinguishable ($P = 0.3$–0.5) from that with NL-SF as lubricant. In contrast, in the LFC, while surface and overall E_{xz} tended to be lowest for NL-SF, highest for AI-SF, and second highest for AI-SF + HA surface lubricants, differences in peak E_{xz} were relatively small (<0.01) and not statistically significant near the surface ($P = 0.2$) and overall ($P = 0.25$).

Discussion

The present results indicate that synovial fluid function, as reflected by intra-tissue cartilage shear deformation during tibio-femoral cartilage articulation, is impaired by acute injury, while tribosupplementation with HA partially restores SF function. As in a previous study[14], tibio-femoral cartilage articulation involved four sequential events: adherence, adherence and shear deformation, surface detachment as shear deformation peaks, and sliding with maintenance of peak shear [Figs. 2 and 6(A)]. When samples were lubricated with AI-SF, surface and overall $\Delta x_{1/2}$ increased to ~1.8-fold, indicating surfaces detached [Fig. 6(B), III)] at greater Δx magnitudes than when NL-SF was used as a lubricant [Figs. 3 and 6(B)]. Concomitantly peak cartilage E_{xz}, near the surface and overall, increased ~1.6 to 2.9-fold when samples were lubricated with AI-SF compared

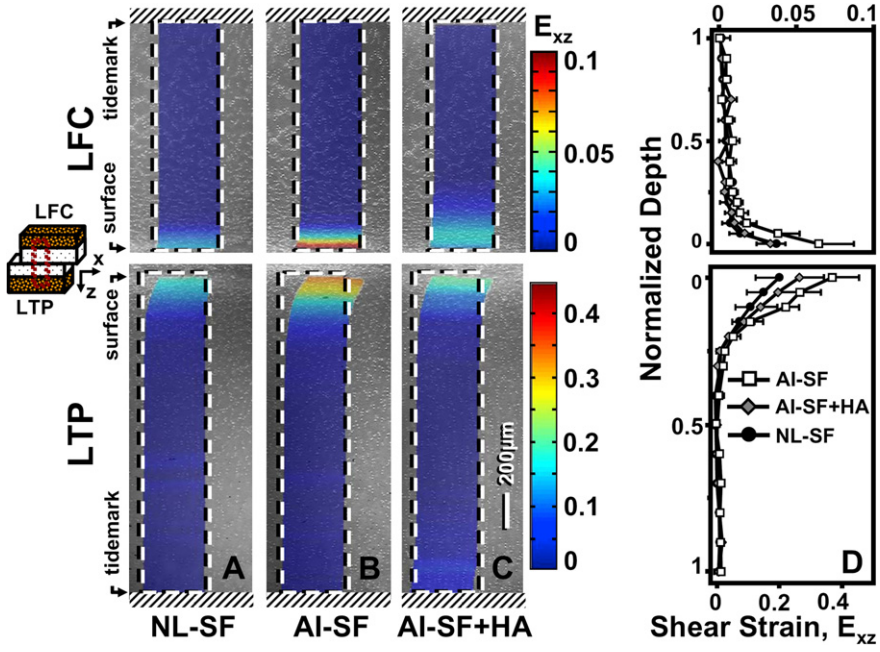

Fig. 4. (A–C) Representative micrographs with superimposed colormaps of shear strain taken of apposing LFC (top images) to LTP (bottom images) samples after achieving maximum shear strain with NL-SF (A), AI-SF (B), and AI-SF + HA (C) as lubricants. Cell nuclei tracking method was used to determine maps of shear strain (color maps, A–C). Dashed lines (- -) encompass the analyzed regions on the undeformed images, while strain map boundaries encompass the corresponding deformed states. (D) Local shear strain (E_{xz}) averaged depth-wise vs normalized tissue depth for LFC (top graph) and LTP (bottom graph) samples. Values are mean ± S.E.M.

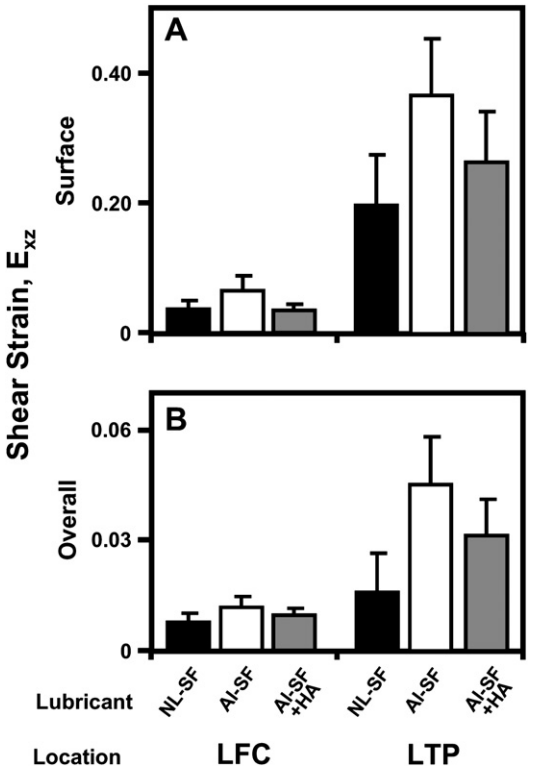

Fig. 5. Effect of acute injury lubrication (NL-SF, AI-SF, or AI-SF + HA) on peak (A) surface and (B) overall shear strain for both LFC and LTP samples.

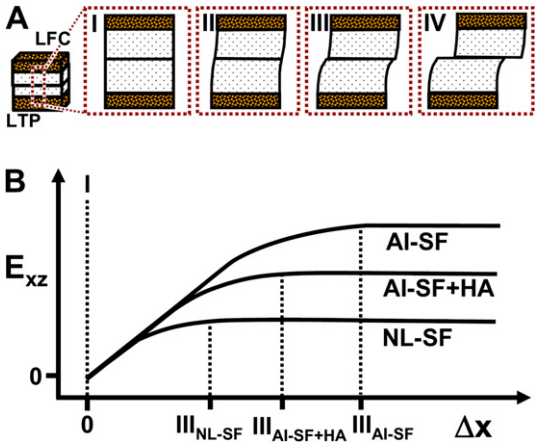

Fig. 6. (A) Schematic of the four sequential events [(I) adherence, (II) adherence and deformation, (III) detachment, and (IV) sliding] that occurs during tibio-femoral cartilage articulation and (B) representative shear strain (E_{xz}) vs applied lateral displacement (Δx) diagrams with markers indicating where detachment (III) occurs for each lubricant condition.

to NL-SF (Fig. 4). When AI-SF was supplemented with HA (AI-SF + HA), surface and overall $\Delta x_{1/2}$ were consistently reduced despite the magnitude of reduction being variable. Such variation may be due in part to the sample variability and absolute peak E_{xz} magnitudes. Concomitantly, E_{xz} near the surface and overall were reduced with AI-SF + HA as lubricant compared to AI-SF. Magnitudes of E_{xz} were consistently greater in LTP cartilage than LFC cartilage for all experimental cases, being ~7- and ~3-fold greater near the surface and overall, respectively.

The present study addresses the biomechanical environment (i.e., compression and sliding) of articulating tibio-femoral cartilage during normal joint loading. Osteochondral samples were taken from the LFC and donor-matched LTP, whose surfaces anatomically articulate against one another within the joint[31]. Such regions are load-bearing, and tibio-femoral cartilage undergoes a wide range of compression amplitude (3–20%)[2–4] and sliding velocity (0–0.1 m/s) during normal activities (estimated from Refs. [32] and [33]). The loading parameters of present study mimic relatively high compression (13%) and low sliding velocity (0.1 mm/s) corresponding to the contra-lateral toe-off and heel rise phases of gait[32], when surface interaction is likely to be high. In addition, anatomically articulating cartilage surfaces (femoral vs tibial cartilage) were analyzed.

In the present study, measures were taken to reduce the influence of undesired factors on cartilage E_{xz} during testing. To ensure lubrication effects were due to the lubricants tested, residual lubricant molecules were cleared from the articular surface by rinsing each sample with PBS + PI for ~12–16 h prior to mechanical testing[15]. Since samples were tested sequentially from worst lubricating SF (AI-SF) to best lubricating SF (NL-SF) based on friction tests, residual effects of prior lubricants were reduced since lubrication would likely be most dependent upon the better performing lubricant. Furthermore, E_{xz} significantly reduced in this sequence of repeated tests, indicating degenerative effects that might occur with repeated testing were likely minimal. While the samples used had been previously frozen, a single freeze–thaw cycle does not result in marked changes in compressive properties of normal articular cartilage[34] or its structure[35], and is thus unlikely to have marked effects on shear properties. Lastly, the influence of the glass interface [Fig. 1(B)] on cartilage E_{xz} was likely minimal since lubricants were present on this surface and the through plane (y-direction) forces on the cartilage was minimal.

Furthermore, the role of equine SF lubricant component of the boundary condition of human cartilage articulation was analyzed in the present study. Articular cartilage surfaces were lubricated with NL-SF and AI-SF to mimic normal and injured lubrication conditions during loading. Samples were also tested with AI-SF supplemented with HA (AI-SF + HA) to analyze a tribosupplementation type of therapy[36]. The concentration of HA ranges between 0.3 and 1.3 mg/ml in normal equine SF[37–40] and reaches as high as 4 mg/ml in normal human SF[41]. Thus, HA was added to AI-SF to bring the total supplemented HA concentration to be ~1 mg/ml and so that final levels of HA would be within normal ranges. Since PRG4 concentrations were elevated in the present lubricant samples, PRG4 supplementation was not studied. Whether PRG4 or other tribosupplements, such as LUB-1[42], the engineered variant of PRG4, have similar effects, remains to be established and should be considered for future studies. In addition, equine SF was tested for lubrication of human articular cartilage since SF lubrication of articular cartilage appears to be largely species independent. Normal bovine SF reduces boundary mode friction between bovine cartilage surfaces[8] and human cartilage E_{xz}[13] compared to saline. Since the shear-reducing function of equine SF was preserved in this study compared to that of saline[5], the lubricating function of equine SF appears to be species independent. This is consistent with findings that equine SF lubricates bovine cartilage, as indicated by boundary mode friction, just as well as bovine SF[19].

The elevation in E_{xz} with AI-SF as lubricant is consistent with the increased friction found between surfaces lubricated with SF from acutely injured joints. Friction coefficient increases with AI-SF as lubricant compared to NL-SF for both cartilage-on-cartilage[19] and latex-on-glass[17] boundary mode friction tests. Assuming cartilage material properties (i.e., compressive and shear modulus) are maintained with the uses of AI-SF and NL-SF, higher friction would be predicted to result in an elevation of tissue E_{xz}. Furthermore, increased cartilage E_{xz} with AI-SF as lubricant during articulation in the present study corresponded to the increased coefficient of friction measured previously[27] in friction tests for AI-SF. Such elevated tissue E_{xz} may contribute to the cartilage wear and degeneration associated with impaired SF function[17,43].

The molecular mechanisms and consequences of tribosupplementation of AI-SF by HA remain to be fully established. Tribosupplementation by HA alone may not fully restore SF function following acute injury since the resultant E_{xz} was generally higher ($\sim$0 to 98%) when samples were tested with AI-SF + HA than NL-SF as lubricant. Other identified (i.e., PRG4[11,12] and SAPL[44]) and unidentified lubricant molecules may be necessary for supplementation to fully restore SF function following acute injury. Furthermore, changes in lubricant molecule structure may occur following acute injury, as found in arthritis[45], and alter the ability of the molecules to function. The benefit of tribosupplementation (AI-SF + HA), associated with reduction of E_{xz} by $\sim$20 to 50% compared to lubricant conditions without HA supplementation (AI-SF) (Fig. 4), appear to have beneficial consequences in animal models of arthritis[42].

The greater shear deformation in cartilage from the tibia than the femoral condyle is consistent with previous studies on *in vitro* tibio-femoral cartilage shear deformation. Analogous to the current study, osteochondral samples from the tibial plateau and femoral condyle were previously tested mechanically with articular surfaces in apposition, but with PBS as lubricant[5]. Resultant cartilage E_{xz} near the surface and overall were higher in tibial cartilage than femoral cartilage, consistent with variation in E_{xz} between LFC and LTP samples for the lubricants tested in the present study. Such consistent differences in shear deformation between LFC and LTP cartilage reflect cartilage from femoral condyles being stiffer in shear than the cartilage from the tibial plateau[5].

The present study provides insight into the effects of acute injury on SF function and the ability of tribosupplementation to restore SF lubricant function, as indicated by cartilage mechanics, during tibio-femoral joint articulation. Reduced SF function, as indicated by increased friction, is positively associated with cartilage wear in mice joints[43], suggesting altered SF function contributes to cartilage degeneration. Excessive E_{xz} that results from impaired SF function may damage cells and matrix, analogous to excessive compression[46–49], and contribute to post-injury cartilage degeneration and wear. Tribosupplementation of biomechanically deficient SF may also be beneficial by restoring cartilage mechanobiology.

Conflict of interest

The authors have no conflicts of interest to report.

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