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Biodynamic Performance of Hyaluronic Acid versus Synovial fluid of the Knee for Osteoarthritic Therapy

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Abstract

Hyaluronic acid (HA), a natural biomaterial present in healthy joints but depleted in osteoarthritis (OA), has been employed clinically to provide symptomatic relief of joint pain. Joint movement combined with a reduced joint lubrication in osteoarthritic knees can result in increased wear and tear, chondrocyte apoptosis, and inflammation, leading to cascading cartilage deterioration. Therefore, development of an appropriate cartilage model and evaluation for its friction properties with potential lubricants in different conditions is necessary, which can closely resemble a mechanically induced OA cartilage. Additionally, the comparison of different models with and without endogenous lubricating surface zone proteins, such as PRG4 promotes a well-rounded understanding of cartilage lubrication. In this study, we present our findings on the lubricating effects of HA on different articular cartilage model surfaces in comparison to synovial fluid, a physiological lubricating biomaterial. The mechanical testings data demonstrated that HA reduced average static and kinetic friction coefficient values of the cartilage samples by 75% and 70%, respectively. Furthermore, HA mimicked the friction characteristics of freshly harvested natural synovial fluid throughout all tested and modeled OA conditions with no statistically significant difference. These characteristics led us to exclusively identify HA as an effective boundary layer lubricant in the technology that we develop to treat OA [Singh *et al*. 2104].

Keywords

Cartilage; Osteoarthritis; Hyaluronic Acid; Lubricin; Synovial Fluid

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Disclosure

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Conflict of Interest Statement:

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

1.0 Introduction

Articular cartilage is a deformable and wear-resistant connective tissue that allows joint articulation with low friction despite continuous stresses on the order of ~1-4 MPa [2]. In addition, daily activities such as bending the knee and ambulation compress articular cartilage by approximately 5% to 20% [3-6]. Under compressive loads, synovial fluid within and around the healthy cartilage is forced between the articulating surfaces to reduce surface interaction and friction [6,7]. Synovial fluid contains HA, proteoglycan-4 (PRG4), and surface-active protein lipids [7-16], which provide lubricating properties and prevent joint wear. Reduced levels of these synovial components can deteriorate articular cartilage causing osteoarthritis.

Osteoarthritis (OA), or degenerative joint disease, is the most common joint disorder, affecting approximately 630 million people worldwide [17]. A possible cause of OA is the loss of natural lubrication due to a gradual loss of surface-active proteins from the cartilage tissue [1,18]. The lack of lubrication between surfaces directly influences the amount of wear and damage. Increased cartilage friction due to cartilage damage under various conditions is an instigating theory in the bovine models used in this study [19-21]. The mechano-biological pathways that are triggered in response to injury and age, are especially important to be considered when studying cartilage lubrication for osteoarthritis and have raised significant interest in OA scientific community. A few studies have suggested that the concentrations of boundary layer lubricants also decrease with the onset and progression of OA, either through injury or advancing age [22,23].

High molecular weight HA has been shown to improve joint lubrication because of its viscoelastic properties and other biological functions, such as its interaction with ECM and maintenance of tissue homeostasis [24]. As a result, a common clinical treatment for OA is direct injection of cross-linked HA into the diseased joint to improve synovial lubrication. The rationale behind this approach is that use of HA as a lubricant creates a viscous boundary layer between the articulating surfaces, reducing the progression of wear and inflammation. However, boundary layer lubrication is not always the main mode of lubrication. When materials have not yet depressurized, interstitial pressurization is mostly involved in lubricating the surfaces; however, after depressurization, boundary layer lubrication is more involved.

In the current work, we developed *in vitro* OA cartilage models to approximate the properties and characteristics of OA cartilage, such as the abrupt topographical changes in the surface and texture of the cartilage layers and the presence of reduced amounts of lubricating agents. The topographical roughness found on the surface of OA cartilage was modeled by fibrillating cartilage samples with an emery paper to the matching degradation level of OA. A second OA model was created to simulate the inflammatory response in arthritic joints, either as a secondary effect of age and injury or mechanical degradation, or as the primary source of cartilage damage (as in autoimmune-related OA). This is a relatively complex model, which was generated by first fibrillating the surface to induce mechanical degradation, and second by incubating the mechanically degraded cartilage in chondroitinase ABC to induce enzymatic degradation of the cartilage's extracellular matrix.

To study cartilage lubrication, the experimental groups were subjected to testing in HA conditions and compared with natural synovial fluid. It has been previously shown that in OA cartilage, surface zone protein lubricin, or proteoglycan 4 (PRG4), a critical component for cartilage surface lubrication, is present in reduced amounts compared to the healthy cartilage [1]. To validate the lubricating efficacy of HA, we included one test group that was treated to remove lubricin, and its frictional mechanics was compared to that of normal cartilage and other tested conditions. To further model the degradation of OA, we included another experimental test group that was microtomed to remove the superficial zone by eliminating the top 20% of the cartilage from the samples, and by using only the transitional stratum. These cut cartilage samples mimicked a total loss of superficial layer allowing the friction results to be directly correlated to the lubricant environment rather than the depth of the testing surface, which is often altered in OA conditions.

The lubricating properties and topography of both mechanically degraded and enzymatic combined with mechanically degraded cartilage were studied by tribological and histological methods compared to undamaged control samples (Fig. 1A-F). We were able to evaluate the effect of HA and other lubricants on two major modes of cartilage lubrication, interstitial fluid pressurization [13,26,27] and boundary layer lubrication (Fig. S1) by allowing samples to depressurize for a sufficiently long time under an equilibrium compressive force (N_{eq}) [10,11,26]. The results of this study provide insight into an HA binding technology as a OA therapy that we developed earlier [1]. Furthermore, this study also provides a biomechanical explanation for future osteoarthritis therapies involving HA and can explain how this biomaterial can serve as a critical boundary lubricant.

2.0 Materials and Methods

2.1 Preparation of Cartilage Specimens

Bovine articular cartilage was harvested from juvenile bovine femoral knee joints 24 hours postmortem (Research 87 Inc., Boylston, MA). Immediately after harvesting, the cartilage was washed in PBS at 4 °C to ensure that residual synovial fluid and endogenous lubricants were washed away; natural proteins in the superficial layer can cause a natural binding of these endogenous materials [28-30]. The cartilage was then cut into 12-mm-diameter disks and annuli with D_o = 8 mm and D_i = 3 mm [2] and trimmed and flattened using a Leica microtome (Leica Biosystems, Nussloch, Germany). To ensure that the surfaces were level for tribological testing, thickness for all samples were measured at three locations and averaged using a micrometer and later confirmed using the Rheometer (ARES G2 rheometer, TA Instruments, New Castle, DE).

The prepared cartilage samples were divided into five groups. The first group (n=4) represented healthy cartilage and was used as a control (no degradation). The second group (n=12) was mechanically degraded using emery paper of three different grit sizes (120, 320 and 600) (Fig. 2B) using a force of 174 kPa controlled with a custom-made spring action holder. Cartilage was placed in the holder with a set protrusion from the device that applied the tested 174 kPa of force onto the emery paper. The length of each abrasion was fixed to 2200 mm. The third group (n=4) was degraded mechanically (using the same method as group 2) and enzymatically (incubated in chondroitinase ABC type II solution for 50 h at

(37 °C, 5% CO_2). Cartilage samples in the fourth group (n=4) were treated to remove lubricin (please see methods section), The fifth group (n=4) underwent superficial layer removal by microtoming the top surfaces (1 mm - 2 mm).

All samples after preparation were washed in PBS at 4 °C, shaked on a laboratory shaker and incubated in their designated lubricants overnight and tested on the next morning. Human OA cartilage was collected (NDRI, Philadelphia, PA) 24 hours after surgical removal from a female Caucasian patient 72 years of age. The sample was cut into with 12 mm disks (n=4) and 8 mm annuli (n=4) using biopsy punches. The OA human cartilage was washed overnight in PBS at 4 °C and tested the next morning.

2.2 Lubricants

HA (MW=975 kDa; Lifecore Biomedical LLC, Chaska, MN) in PBS, 5 mg/mL, was mixed for 45 min to 60 min at ~100 rpm prior to use. 15 mL of 5 mg/mL was used in each mechanical test for groups incubated with HA lubricant. Synovial fluid (Lampire Biological Laboratories, Inc., Pipersville, PA) was harvested 24 hours post-mortem and filtered through a 100 micron cell strainer under vacuum. Lubricated samples were incubated overnight on a shaker before mechanical testing.

2.3 Measurement of Static and Kinetic Friction

The normal and tangential force values were measured using the ARES rheometer, from these values the friction coefficients were derived based on the geometry of the tested sample. After loading, samples were first equilibrated with an applied a load conditioning step (3600 seconds), which allowed the material and normal forces to stabilize before data collection [3]. After the stabilization step, a pre-shear ramp was used to initialize boundary flow. Recorded testing immediately followed with pre-sliding times (1.2, 12, 120, 1200, 2400, and 3600 seconds) to allow the interstitial fluid pressurization to recede and measure possible variability in static and kinetic friction after a period of immobility. For each measurement, the cartilage samples were under 18% compression and rotated during each ramp at a constant rate (0.3 mm/s), twice in each direction.

2.4 Mathematical Analysis

Friction was calculated from the basic Newtonian equations [10,11]. The most important considerations were the tangential and axial forces. However, our geometry consists of an annulus in contact with a disk; therefore, the radius of the annulus was included in the equation as described previously by Schmidt *et al* [10,11]. In this study, given that the inner radius is 1.5 mm and the outer radius is 4 mm; we calculate the effective radius to be 2.939 mm. The effective radius was applied to determine effective velocity, which is related to the strain rate. The rheometer uses strain rate(s) to guide the kinematics of the experiment and maintain a constant speed. The strain rates are proportional to the thickness of the sample or loading height. The same formula as mentioned by Schmidt *et al* [10,11] was used to calculate the appropriate strain rate(s) given the effective velocity (V_{eff}) on each sample tested, and the subtle differences in loading height (H_L). Since radial geometries did not change between samples (D_o=8 mm, D_i=3 mm), effective velocity was essentially constant for all measurements (0.3 mm/s).

2.5 Statistical Analysis

Statistical significance was calculated for Group A, D, and E by a Bonferroni's multiple comparison one-way ANOVA. Significance was set to 99.9 % confidence for each analysis. Groups B and C were statistically analyzed using a paired t-test at a 99.9 % confidence. RT-PCR was statistically analyzed using a multiple t-test at a 99.9 % confidence.

2.6 Lubricin Extraction

Lubricin was extracted through the use of 1.5M NaCl in PBS followed by 4M guanidine HCl with a pH adjusted to 6.2. Cartilage designated for extraction was generously shaken in NaCl solution for 20 minutes followed by 20 minutes in guanidine solution [4,5]. The cartilage was then removed from the solution and stored at 4°C until testing.

2.7 Histological Staining and Immohistochemistry

The model groups were coated with India ink (Fig. 1A-F) (Becton, Dickinson and Company, Franklin Lakes, NJ) and qualitatively assessed by the severity of fibrillation caused by the mechanical abrasion compared to normal cartilage samples also stained with India ink. The samples were then cleaned of residual staining reagent and imaged using a Zeiss Discovery V8 dissection microscope (Carl Zeiss, Jena, Germany).

Safranin O stain was used to assess the topography of the degraded cartilage and proteoglycan content of the tissue. Slides were rehydrated to continue the staining process: specimens were saturated in fast green (Sigma Aldrich, St. Louis, MO) for 3.5 min, acetic acid (1%) for 10 seconds, Safranin O 0.01% (Sigma Aldrich, St. Louis, MO) for 10 minutes, and sequentially dehydrated with increasing concentrations of ethanol (1-2 minute each). After dehydration, the samples were fixed on slides with Permount (Sigma Aldrich, St. Louis, MO). The human OA cartilage samples were prepared in the same manner as the bovine cartilage samples. The prepared slides were imaged using a Carl Zeiss Imager A2 (Carl Zeiss, Jena, Germany).

For lubricin staining, prepared slides were left to warm at 60 °C for two hours to promote strong tissue adhesion to the slides before being deparaffinized in Citrisolv and rehydrated. The rehydrated slides were topped with several drops of hydrogen peroxide for 10 min to reduce endogenous peroxidase activity. The sections were then washed with PBS and, to unmask antigenic sites, transferred into capped polypropylene slide holders that contained citrate buffer solution (pH 6.0) that had been heated to 80 °C in a microwave oven (700 W) for 15 min. The non-specific binding sites were then blocked through the application of 5% horse serum (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA) for 30 min.

Primary antibody, rabbit polyclonal anti-lubricin antibody (Santa Cruz Biotechnology Inc., Dallas, Texas), was applied onto the sections. After application, the sections were incubated in a moist chamber at 4 °C overnight. Post-incubation, the sections were treated with the secondary antibody, biotinylated anti-mouse/anti-rabbit IgG (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, CA, USA) and left for 30 min at room temperature, followed by the avidin-biotin-peroxidase complex for another 30 min at room temperature.

The immunoreaction was visualized using the DAB substrate kit, peroxidase (Vector Laboratories, Burlingame, CA, USA) and then counterstained with Mayer's Haemotoxylin (Sigma Aldrich, St Louis, MO) and secured in Polymount (PolySciences, Warrington, PA).

2.8 Western Blot

Four different samples were run on the Western Blot: Normal, OA, Normal Lubricin extracted (LE) and OA LE. The samples were collected using the lubricin extraction method. For the Normal and OA samples, 3 mm biopsy punches of cartilage were added to solutions of NaCl and guanidine from the lubricin extraction method. The cartilage was soaked according to the protocol and the resulting solutions of both NaCl and guanidine were combined to ensure collection of all the extracted lubricin. To determine the validity of the lubricin extraction process, Normal LE and OA LE were tested. The Normal LE and OA LE samples were created by running the lubricin extraction procedure on the cartilage twice, discarding the solution from the initial run, and collecting the solutions from the second run.

The solutions were loaded onto SDS-PAGE gel along side the protein marker (HiMark Pre-Stained Protein Standard Novex by Life Technologies Fredrick, MD) and ran at 100 mV for 20 minutes followed by 130 mV in running buffer. The gel was then electro-transferred to a PVDF membrane blot and run at 100 mV for 1 hr. After the transfer, the blot was washed with 25 ml of TBS/T for 5 minutes and then incubated in molecular biology grade milk at room temperature for 5 min. The blot was then washed three separate times for 5 minutes each with TBS/T. After the washes, the blot was incubated with the anti-lubricin antibody with the primary antibody buffer (Pierce – Thermo Scientific Waltham, MA) overnight at 4 °C. The wash sequence was then repeated before the blot as incubated with the rabbit-anti mouse horseradish peroxidase, to detect biotinylated protein markers in molecular biology grade blotting milk for 1 h at the room temperature under gentle agitation. It was then washed twice with TBS/T and the blot was then incubated with 2 ml SuperSignal West Pico Chemiluminescent (Thermo Scientific Waltham, MA) substrate for 5 min at room temperature. The blot was then drained and manually exposed for 20 sec using Kodak (Kodak Rochester, NY).

2.9 Polymerase Chain Reaction (PCR)

Samples were flash frozen and broken down by mortar and pestle and homogenized with TRIzol. The resulting RNA cluster extracted by TRIzol method was dissolved in 8 μl of DEPC treated water and quantified with a Qubit Fluorimeter (Life Technologies Fredrick, MD). The cDNA synthesis was done using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) by following the kit manufacturer's protocol, and RT-PCR was run using SYBR green. GAPDH, Aggrecan, and PRG4 primers were tested before use (Integrated DNA Technologies Coralville, IA.).

2.10 Diffusivity/Penetration

Tissue was harvested from a young bovine calf 24 h post mortem. Samples were prepared under the same conditions for mechanical testing. Only 8 mm punches were used to save fluorescent solution. Each group was placed in a 48 well plate that contained fluorescent rhodamine tagged HA and fluorescent BODIPY (Life technologies Fredrick, MD) tagged

synovial fluid. In each well 100 μl of solution was added. Samples were then flipped and placed experimental side down into the fluid and incubated for approximately 24 h. Samples were then taken briefly washed in PBS and sectioned via cryo-sectioning. After samples were mounted they were dehydrated and cover slipped with GVA aqueous mounting media (Genemed San Francisco, CA). Slides were imaged with a Zeiss LSM 510 confocal imager and Zeiss A2 imager.

3.0 Results

3.1 Histology

The histological studies showed the topographical differences in cartilage samples as well as proteoglycan levels after each type of degradation. The human OA stain shows a significant reduction in proteoglycan levels that is only mimicked by the cartilage with enzymatic degradation and mechanical fibrillation (Fig. 1E). Although the proteoglycan levels in A, B, C, and D are less reduced, the topographical changes are evident in both Safranin-O staining and macroscopic images; the sample with 120 grit showed the strongest representation for mechanical degradation on the surface of the tissue. The human OA sample did not show much fibrillation, due to the specific slice that was stained.

Immunohistochemistry was performed on cartilage Groups A, D, and E as well as a negative control to determine the expression of lubricin in each case. The 40x imaging showed significant lubricin expression in Group A, proposing the presence of the surface protein. In both Group D and E, there were no evident stains to suggest lubricin expression, indicating a successful removal of the protein. The negative control also displayed no lubricin expression, a control in validating the results for the three tested groups.

3.2 Friction Force Mechanical Testing

Samples treated with HA resulted in lower friction values across all test groups in both static and kinetic categories compared than those in a PBS environment. Both synovial fluid and HA were significant lubricants on cartilage in Groups A, D, and E. Under static conditions, there was no statistical difference in the decrease of friction values between synovial fluid and HA. Synovial fluid had average friction values of 7.48×10^{-2} 8.79×10^{-2}, and 6.25×10^{-2} for Groups A, D, and E respectively, and HA presented corresponding average friction values of 7.62×10^{-2} 10.3×10^{-2}, and 7.71×10^{-2}. The lubricity ($\mu_{HA/SF}$- μ_{PBS}) for static frictions in Group A was from synovial fluid's 0.150 to HA's 0.149; in Group D, synovial fluid lubricity (μ_{SF}- μ_{PBS}) to HA (μ_{HA}- μ_{PBS}) lubricity was 0.0575 to 0.0525, and in Group E, the lubricities ($\mu_{HA/SF}$- μ_{PBS}) were 0.110 to 0.096 for synovial fluid and HA, respectively. However under kinetic conditions, though both lubricants succeed in decreasing the friction, synovial fluid is more effective during low pre-sliding times. Then, as time surpasses 1200 seconds and approaches boundary layer levels, the synovial fluid and HA arrive at similar values with no statistical difference. On average, synovial fluid resulted in average kinetic frictions of 2.61×10^{-2} for Group A, 2.75×10^{-2} for Group D, and 2.54×10^{-2} for Group E, while HA exhibited frictional results of 3.54×10^{-2}, 6.24×10^{-2}, and 4.20×10^{-2} for those same groups. The kinetic lubricities in Group A for synovial fluid (μ_{SF}- μ_{PBS}) and HA (μ_{HA}-

μ_{PBS}) were 0.070 to 0.061; in Group D these were 0.058 to 0.050; in Group E these were 0.066 to 0.049.

3.3 PCR and Western Blot

We performed PCR to determine if lubricin is expressed in both human normal and OA cartilage Lubricin expression in normal cartilage was much more higher than in OA cartilage. Fig. 3A shows ΔΔCt analysis with normalization to GAPDH expression in both samples. Aggrecan was used as a positive control for both tissue samples.

A Western Blot was performed to determine the presence of the surface protein, lubricin, with and without the application of lubricin extraction procedure in both normal and OA cartilage. In comparison to the normal cartilage, a less dense Western Blot band characterized the OA cartilage by use of densitometry quantification. By percent density, OA had only 35.3% after normalization. In both LE samples, once the lubricin was extracted, the amount of protein became negligible: 0.9% for lubricin-extracted normal and 4.4% for lubricin extracted OA.

3.4 Diffusivity/Penetration

Penetration was determined through the use of fluorescent imaging. Synovial fluid was tagged with BODIPY and the images showed that the natural lubricant penetrated the cartilage, creating a gradient throughout the sample. This resulting gradient was found across all the images for synovial fluid. Only a very minimal fluorescence or penetration can be observed in any of the test groups, for the samples tested in HA-rhodamine.

4.0 Discussion

To understand the mechanical performance of HA versus the synovial fluid of the knee, we tested bovine cartilage samples in various conditions that modeled OA. The mechanical data validated that HA is a critical component of synovial fluid for the cartilage lubrication and acts as both boundary layer and pressurized lubricants between cartilaginous tissues under heavy loads. HA reduced the frictional values of the samples during mechanical testing throughout all modified and control conditions (Groups A through E, please see Table 1). Different groups resulted in very different outcomes and provided us an insight into the performance of HA in the damaged conditions that may resemble OA. Group B roughened with a 120 grit emery paper produced a highly fibrillated surface usually found in OA (Fig. 1A), showed high frictional coefficient values under control conditions; however, they were well lubricated with HA, resulting in a 50% decrease in the friction coefficient values from control conditions (Fig. S1C-D). Other mechanically degraded sample groups (roughened with 320g and 600g) were histologically analyzed but were excluded from the mechanically testing due to the lack of a significant fibrillation or topological differences (Fig. 1B and C). After the enzymatic incubation, samples in Group C resulted in very low friction coefficients most likely due to the soft and elastic nature of the treated tissue (Fig. S1E-F) and did not closely resemble OA physiological condition; therefore, they were also excluded from the study.

Mechanical testings were performed on harvested samples that were flat and standardized to an overall height of 3–5 mm. Low and higher pre-sliding time points represented different lubrication mechanisms; lower time points offered relatively more pressurized interstitial lubrication than the boundary lubrication, while higher time points represented boundary lubrication mechanism. During the depressurization step in mechanical testing, the samples were completely depressurized at approximately 600 seconds, which acted as a threshold time for the samples to undergo boundary lubrication instead of the pressurized interstitial lubrication. We conservatively considered the time points from 1.2 s up to 1200 s to be less dominated by the boundary lubrication mechanism and extended pre sliding time points as much as to 2400 and 3600 seconds to make sure that samples are undergoing boundary lubrication mechanism.

Groups A, D and E offered interesting characteristics that were valuable for physiological resemblance of OA. These conditions were the most studied and were inspired by the importance of superficial zone protein, PRG4 or lubricin in cartilage lubrication. PRG4 has been identified as being a vital part of the lubrication mechanisms that occur within the knee [15]. The results from PCR, Western Blot, and Immunohistochemistry data (Fig. 1G-J, Fig. 3A-C) showed that PRG4 content in OA cartilage is significantly diminished and the adopted procedure could successfully extract lubricin from the cartilage samples. Densitometry was used to quantify the Western Blot, which resulted in a 75 % density difference between OA and normal bands, while lubricin extracted bands had differences of close to 100 %. The results support the hypothesis that OA has reduced levels of lubricin, which further complicates the diseased cartilage. Removal of lubricin (Group D) profoundly affected the lubrication efficiency of the articular cartilage, which resulted in higher friction coefficients in comparison to normal cartilage (Fig. 2B,E). However, HA continued to lubricate cartilage samples even in the samples with lubricin removed, similar to synovial fluid in pre 1200 second time points and more superiorly in post 1200 second time points, which is more prevalent of the lubrication mechanism of boundary lubrication. We found that HA performed relatively better in final time points compared to synovial fluid itself although marginally (Fig. 2G-J), specifically for group E, suggesting that HA lubricates cartilage surfaces even in boundary layer lubrication condition. Samples treated with HA showed higher lubricity values (μ_{HA}-μ_{PBS}) than the samples treated with SF (μ_{SF}-μ_{PBS}). Group E served to completely eliminate the superficial zone of the cartilage (Fig. 1K) resembling severe OA, where subchondral bone is partially exposed due to severe erosion or loss of cartilage tissues [7,32].

In this study, HA exhibited relatively strong lubrication characteristics and maintained a statistically insignificant difference in lubrication efficiency than the natural lubricant found in healthy joints. Current OA therapy employs articular injections of the cross-linked HA to improve the lubrication of the damaged cartilage surfaces. The data from this study suggest that HA is a critical component of the synovial fluid for cartilage lubrication and even perform well in boundary layer lubrication conditions. However, the questions that how far in cartilage tissue HA can penetrate post articular injection of HA and how long it can stay to provide boundary lubrication in a diseased environment. As seen in the fluorescent images (Fig. 3D – I), HA did not penetrate the cartilage tissue and mostly remained on the surface. While this may be beneficial for boundary lubrication, over time HA might not be

retained especially when physiological residence time of HA is short. Recently, we developed an HA binding technology that can prolong HA retention and joint lubrication [1]. Therefore, this study was the basis to understand HA as a valuable therapy for OA and to incorporate this biomaterial into our further studies and technology found in our other publications.

5.0 Conclusion

In this study, we studied the effect of HA on cartilage lubrication mechanics in a variety of conditions and further supplemented that OA has reduced levels of a known protein lubricant, lubricin/PRG4. Furthermore, we demonstrated that HA lubricated cartilage samples that lacked lubricin, even in the boundary lubrication conditions. Throughout all tested and model OA conditions, HA mimicked the lubrication performance of synovial fluid with no statistically significant difference. This study provides a basis for understanding the lubrication mechanism in articular cartilage by HA for our recently published and patented HA binding technology. We believe that these results can further help OA scientific community for finding a hopeful therapy to treat degenerative joint disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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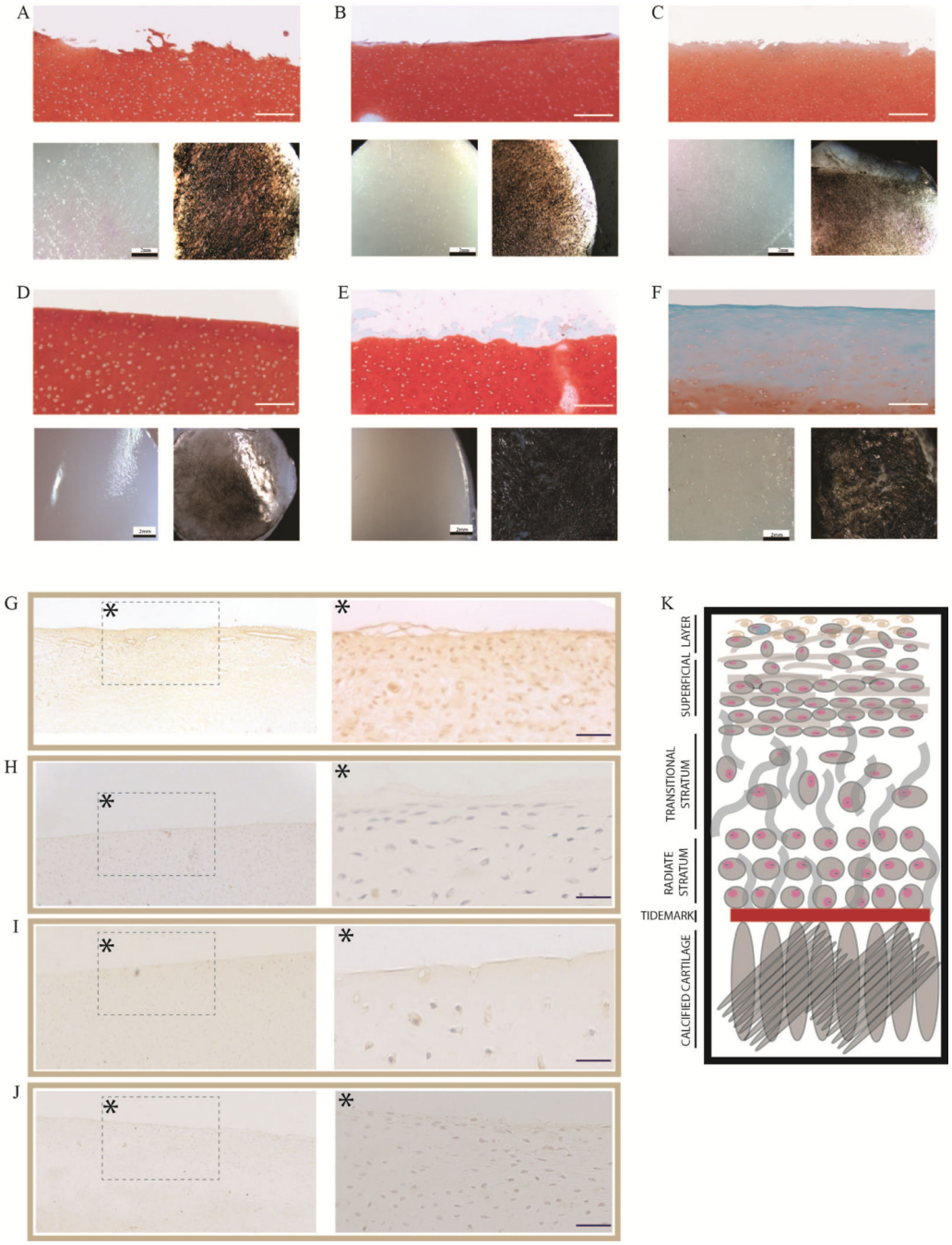

Figure 1.

Cartilage composition and topography of various test groups. **A-F**. (top) Safranin O staining of cartilage surface, (bottom left) macroscopic image, and (bottom right) India ink stained macroscopic image to show topographical differences between sample groups. **G-J**. Immunohistochemistry for lubricin at 10x (right) and 40x (left), showing lubricin expression in different conditions (**G**, Normal bovine cartilage, **H**, 120 grit mechanically degraded surface, **I**, cartilage sample with removed superficial zone layer and **J**, cartilage with lubricin extracted). **K**. Digital rendering of cartilage components.

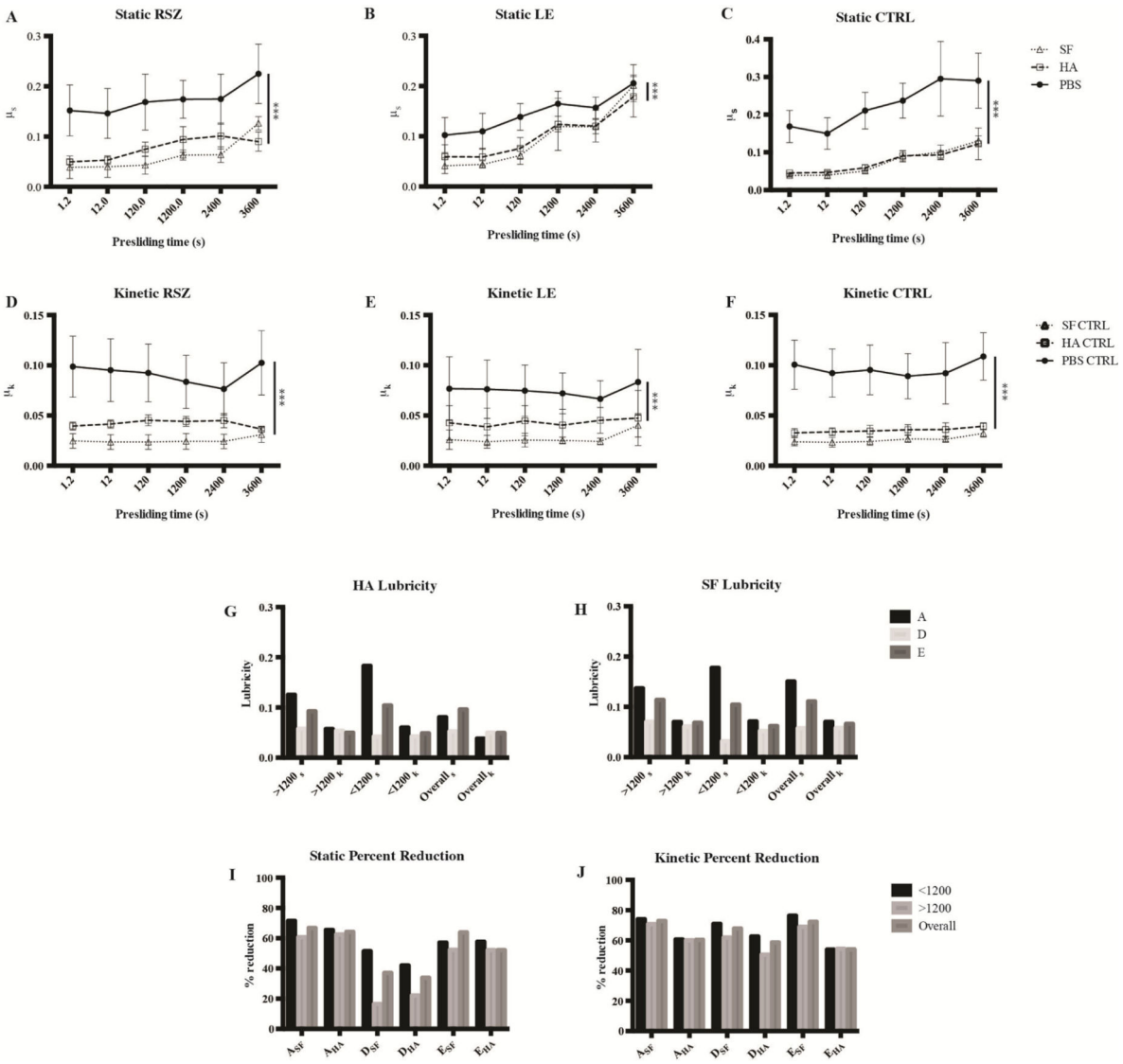

Figure 2.

Friction measurement for different cartilage groups in various environments. Cartilage incubated and tested in HA exhibits comparable lubrication to that of cartilage under equal treatment in synovial fluid. **A-F**. Representative graphs for static and kinetic friction against pre-siding time for cartilage in groups E, D, and A. The error bars indicate standard deviation; unbroken lines represent a PBS control environment, broken lines represent a HA environment, and dotted lines represent a synovial fluid environment. **G**. The lubricity of different cartilage groups under HA (μ_{HA}- μ_{PBS}) at different time periods. **H**. The lubricity (μ_{SF}- μ_{PBS}) of different cartilage groups under synovial fluid at different time periods. **I-J**. Percent reduction of μ_s and μ, respectively for the cartilage groups during different time periods.

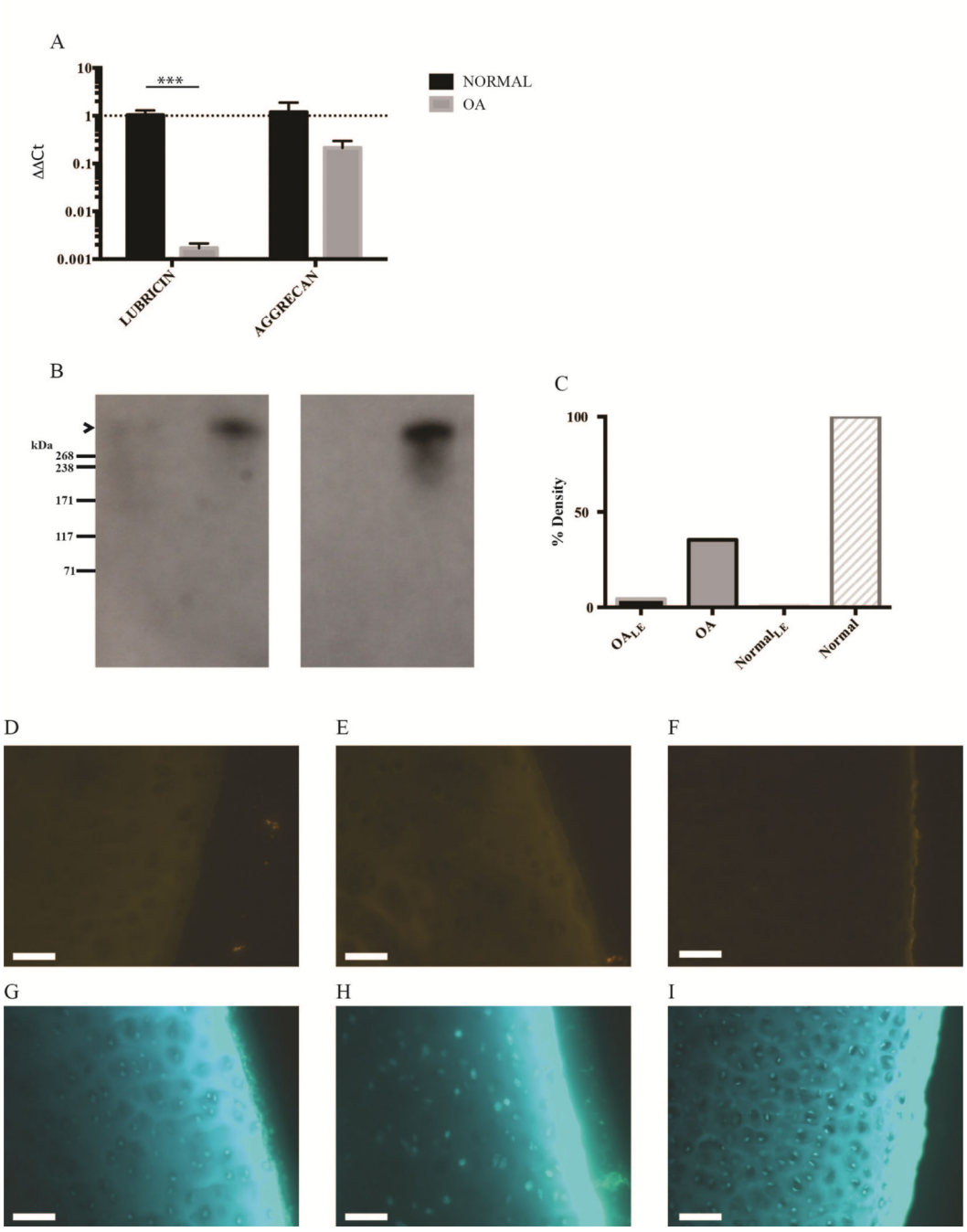

Figure 3.

PRG4 expression in normal and OA cartilage, and penetration of lubricant into the cartilage tissue. **A**. PCR for human normal cartilage compared to cartilage with osteoarthritis. **B**. Western blot from left to right osteoarthritis with lubricin extracted, osteoarthritis, normal lubricin extracted, normal. **C**. Percent density from Western blot in B. **D-I**. Fluorescence images for HA in **D**, control, **E**, sample with superficial zone layer removed, **F**, sample with

lubricin removed, and synovial fluid in **G**, control, **H**, sample with superficial zone layer removed, **I**, sample with lubricin removed.

Table 1

Cartilage experimental groups in various testing environments. The groups are referred by letters in the article.

Tested Groups	Description	Sample Size	Lubricants
A	Healthy Normal (control)		
B	Mechanical	N = 4	PBS
C	Enzymatic and Mechanical	Per cartilage	HA (5mg/mL)
D	Lubricin Extracted	group	Bovine SF (filtered)
E	Removed Superficial Zone		

Table 2

Average static and kinetic friction coefficients for each experimental group. Averages were calculated from all pre-sliding time points

Group	Average Static Friction	Average Kinetic Friction
A_{CTRL}	22.5×10^{-2}	9.63×10^{-2}
A_{HA}	7.62×10^{-2}	3.54×10^{-2}
A_{SF}	7.48×10^{-2}	2.61×10^{-2}
D_{CTRL}	14.6×10^{-2}	7.50×10^{-2}
D_{HA}	10.3×10^{-2}	6.24×10^{-2}
D_{SF}	8.79×10^{-2}	2.75×10^{-2}
E_{CTRL}	17.3×10^{-2}	9.14×10^{-2}
E_{HA}	7.71×10^{-2}	4.20×10^{-2}
E_{SF}	6.25×10^{-2}	2.54×10^{-2}