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Biodynamic performance of hyaluronic acid versus synovial fluid of the knee in osteoarthritis

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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 that can be evaluated for its friction properties with potential lubricants in different conditions is necessary, which can closely resemble a mechanically induced OA cartilage. Additionally, a 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., 2014).

1. 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 5–20% [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 interests 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 [22–25].

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 [25]. 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 the 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

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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. Materials and methods

2.1. Preparation of cartilage specimens

Bovine articular cartilage was harvested from juvenile bovine femoral knee joints 24 h postmortem (Research 87 Inc., Boylston, MA). Immediately after harvesting, the cartilage was washed in PBS at 4 °C to ensure that the 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 (1–2 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 the next morning. Human OA cartilage was collected (NDRI, Philadelphia, PA) 24 h 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 h 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 s), 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 s) 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