Lubrication Of Degraded Cartilage By Hyaluronic Acid

Edward D. Bonnevie¹, Cynthia Secchieri², Devis Galesso², Lawrence Bonassar, PhD¹.
¹Cornell University, Ithaca, NY, USA, ²Fidia Farmaceutici SpA, Padua, Italy.

Disclosures:
E.D. Bonnevie: 6; Fidia Farmaceutici SpA. C. Secchieri: 3A; Fidia Farmaceutici SpA. D. Galesso: 3A; Fidia Farmaceutici SpA. L. Bonassar: 5; Fidia Farmaceutici SpA.

Introduction: After the onset of osteoarthritis (OA), it is difficult clinically to both restore normal joint function and slow the propagation of damage across a joint surface. It is likely that a tribological cascade occurs after the onset of tissue damage that predisposes the joint surface for further damage. Clinically, hyaluronic acid (HA) injections have been used to treat OA symptoms, but its mechanisms to lubricate degraded tissue have not been fully explored. One marker for tissue deterioration that is characteristic of OA is loss of proteoglycan content - an extracellular matrix constituent that is important to the mechanical properties of cartilage. Many researchers have shown in vitro culture of cartilage explants with the catabolic cytokine interleukin-1β (IL-1β) causes a time-dependent loss of proteoglycan [1]. Culture with IL-1β is also known to increase boundary mode friction coefficients of articular cartilage [2]. In a cartilage degradation model, we have cultured cartilage explants in the presence of IL-1β to determine the effects of degradation on the lubrication mechanisms of cartilage. To determine the effects of degradation on the lubrication mechanisms, we have developed Striebeck curves comparing the friction coefficients from both healthy and IL-1β cultured cartilage. These curves describe lubrication modes by presenting friction coefficients as a function of the Hersey dimension (sliding speed * lubricant viscosity / normal pressure) [3]. We hypothesize that degradation of cartilage tissue can alter both boundary mode lubrication as well as the transitions to other lubrication modes (i.e. mixed and hydrodynamic lubrication).

Methods: Full thickness articular cartilage samples were extracted from the patellofemoral groove of neonatal bovines. IL-1β degraded samples were cultured for time periods of 4 and 8 days in DMEM supplemented with 10% FBS, 5% antibiotics/antimycotics, and 10 ng/ml IL-1β. To determine the effects of IL-1β on proteoglycan content cultured samples were analyzed histologically with Safranin-O staining and biochemically with a modified DMMB assay. A custom-built tribometer was used to measure friction coefficients of articular cartilage sliding against polished glass while bathed in a lubricant solution. Briefly, cartilage samples were compressed to 20% strain and the normal load was allowed to reach steady-state equilibrium resulting in normal stress on the order of 100 kPa. Samples were articulated at sliding speeds from 0.1 to 10 mm/s. To develop the Striebeck curves, altering sliding speeds alone is not sufficient, so lubricant viscosity was also altered by using three different lubricants. The lubricants used were PBS, 500-700 kDa HA at 10 mg/ml, and a more viscous HA derivative (HYADD) at 8 mg/ml with dynamic viscosities of 1, 156, and 72038 mPas respectively.

Results: Explants cultured with IL-1β contained significantly decreased proteoglycan content. A decrease in proteoglycan content was evident from histological staining after 4 days of culture, and this effect was magnified after 8 days of culture (figure 1, left). The respective decreases in proteoglycan content by wet weight were 14% and 30% for four and eight days (figure 1, right). The HA lubricant formulations significantly reduced friction coefficients compared to PBS. For the 500-700 kDa HA, these reductions were most evident at increased sliding speeds, and the HYADD lubricant reduced friction over the entire range of speeds. By normalizing sliding speed with lubricant viscosity and normal pressure, a Striebeck curve was generated from the three lubricant solutions (Figure 2, left). Three lubrication modes are present in the data which are boundary, mixed and hydrodynamic modes. Degradation of the tissue altered the Striebeck curve (Figure 2, right). Degradation increased the boundary mode friction coefficient by 36% after 4 days of culture and by 60% after 8 days. While HA reduced friction for all groups, the shift to hydrodynamic lubrication occurred at higher Hersey dimensions for the degraded tissue. Friction coefficient minima were present in the uncultured tissue at a Hersey dimension of 530 nm and at 2100 nm for the four day culture. But, the 8 day cultured tissue was unable to transition to hydrodynamic lubrication by Hersey dimension of 10^4 nm.

Discussion: The data presented here shed new light on the consequences of cartilage degradation by IL-1β. We have seen similar magnitude and kinetics of proteoglycan loss as previous researchers [1,2], and we observed similar increases in boundary friction after IL-1β degradation compared to previous studies [2]. Unlike previous studies, the work here explored different modes of lubrication by using HA as a lubricant. Degradation by IL-1β affected all lubrication modes and tended to shift the transition to different lubrication modes to higher Hersey dimensions. Hindering the transitions between lubrication modes may predispose cartilage to further damage and degradation because more time will be spent in boundary mode lubrication which is characteristic of the highest friction coefficients and wear. Qualitatively, the action of HA was similar regardless of level of degradation. HA tended to reduce friction by facilitating the transitions between lubrication modes. However, quantitatively this effect is somewhat reduced in degraded cartilage. These effects of degradation may also be convolved with the loss of lubricin which is induced by IL-1β exposure.

Significance: This study shows how degradation of articular cartilage as described by loss of internal proteoglycans affects
lubrication by both elevating boundary mode friction as well as hindering the transitions between lubrication modes. HA lubricated both normal and degraded tissue with the most notable change being a shift in the speed necessary to achieve hydrodynamic lubrication after degradation.

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**Figure 1:** Left: Safranin-O staining of control and IL-1β cultured explants shows proteoglycan loss. Triangles denote tissue surface. Right: Fraction of GAG retained compared to control tissue by wet weight for four and eight days of culture. Significance set at p < 0.05 (n = 3-4).

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**Figure 2:** Left: Striebeck curve of uncultured cartilage showing lubrication modes. Right: Friction coefficient versus Hershey dimension for different culture durations. Arrows mark friction minima (n = 1-5).