Polyacrylamide Hydrogel Viscosupplements Lubricate Cartilage After Mechanical Injury and Biochemical Degradation

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Introduction: Hyaluronic Acid (HA) injections have been the cornerstone of arthritis treatments for many decades. This therapeutic modality, known as viscosupplementation, aims to restore the viscosity of synovial fluid and improve lubricity of articular cartilage in patients with mild to moderate osteoarthritis (OA). However, viscosupplementation with HA and HA derivatives is expensive and has been shown to have little to no clinical benefit over placebo injections. HA can also be cleaved by the enzyme hyaluronidase, leading to low residence time after application [1-3]. Unlike existing viscosupplements, synthetic lubricants have been shown to (i) lubricate cartilage just as effectively as HA, (ii) can be chemically modified to resist enzymatic degradation, and (iii) cost a fraction to manufacture. Polyacrylamide (PAAm) hydrogels (NoltrexVet® - RC Bioform LLC) are synthetic, bioinert, and biocompatible materials that have been used as viscosupplements in equine subjects. The PAAm gel is a high molecular weight (10 MDa), highly viscous polymer hydrogel designed to mimic the viscoelastic and lubricating properties of synovial fluid. While the gel has shown a sustained lowering of lameness in equine subjects, its mechanism of action and lubricating ability is unclear. The goals of this study were to characterize the frictional properties of the PAAm hydrogels and determine if they can treat cartilage samples degraded in an IL-1β *in vitro* degradation model and in samples that have been injured via a previously developed impact injury model [4]. We hypothesize that the PAAm hydrogels improve the lubricating ability of IL-1β degraded and impact injured cartilage samples.

Methods: Cylindrical cartilage explants (6 mm diameter x 2 mm thick) were collected from the femoral condyles of 6 neonatal bovids 24 hours after sacrifice. One set of explants was cultured for 7 days in DMEM media with or without 10 ng/mL of IL-1β to induce degradation. A second set of explants was subjected to injury using a previously described, spring-loaded impactor system [4]. Briefly, a cylindrical indenter was used to impact articular cartilage explants in unconfined compression. Impact was delivered at a peak stress of 16 MPa to cause impact trauma and cell death, without causing full thickness cracking. As described previously, all explants were mated against a polished glass surface and bathed in lubricating baths consisting of PBS or PAAm hydrogels described above. Both sets of samples (n=3 IL study, n=3 Impact injury study) were compressed to 30% strain and allowed to depressurize for 1 hour. The glass surface was then reciprocated at sliding speeds ranging from 0.1-10 mm/s and the coefficient of friction μ was recorded as the ratio of shear force to normal force, as measured by a biaxial load cell. Immediately after sliding, explants from all groups were fixed in 10% phosphate buffered formalin for 5-7 days. To determine the effects of IL-1β degradation and impact injury on proteoglycan content, all samples lubricated in PBS and PAAm were analyzed histologically with Safranin-O staining and imaged under a light microscope. Statistical analysis was done using a three-way ANOVA with repeated measures and significance was evaluated at $p < 0.01$. Tukey's HSD test was used for comparing friction at the different speeds between lubricants (arrows in bottom of Figure 2).

Results: PAAm hydrogels lubricated both degraded and injured cartilage, and reduced friction effectively across the range of sliding speeds as seen in Figure 1A and 1B. PBS-lubricated samples showed significantly higher coefficient of friction compared to the PAAm-lubricated samples at 0.1 and 10 mm/s sliding speed (Fig. 1A, *** denotes $p < 0.001$). The IL-1 degraded cartilage that was lubricated in PBS baths showed a significantly higher coefficient of friction than control samples in PBS. The impacted cartilage lubricated in PBS showed a higher coefficient of friction compared to the control samples, but this difference was not statistically significant (Figure 1B). PAAm lowered the coefficient of friction in the impacted group ($p < 0.05$). Histology images of the samples from both injury groups indicate proteoglycan loss and increase in roughness at the surface of both IL-1 degraded and impacted cartilage groups (Figure 2A and 2B). Interestingly, the samples that were tested with the PAAm hydrogel showed localization of the lubricant on the surface of the tissue.

Discussion: This study demonstrated that the coefficient of friction of both damaged and native cartilage was lowered by almost 60% in presence of the polyacrylamide hydrogels. In previous studies, we observed similar increases in the coefficient of friction of samples that underwent IL-1β degradation and impact injury [4,5]. Like conventional HA viscosupplements, the PAAm hydrogel significantly lowered the coefficient of friction of both IL degraded and impacted cartilage samples (***, $p < 0.001$) across a range of sliding speeds. But the key difference between the frictional characterization of existing HA formulations and the PAAm gels is most evident in the histology after sliding. Histology of the explants exposed to IL-1β or impact injury models reveals that there is an increase in the roughness of the surface of the cartilage. But more interestingly, both IL-1 degraded and impacted samples lubricated by the PAAm gel showed retention and localization of the lubricant on the surface, even after histologic processing (Figure 2A, 2B). It is possible that the disruption of the surface layer from cartilage degradation or injury facilitates a greater localization of the hydrogel at the surface during depressurization and after sliding. Future studies will evaluate the mechanism of gel localization and will attempt to quantitatively assess the amount of gel that is trapped.

Significance: This work is the first to characterize the lubricating ability of a novel polyacrylamide hydrogel viscosupplement.

References: [1] Altman+ Am J Sports Med, 2015; [2] Maheu+ Clin Exp Rheum, 2011; [3] Vitanzo+ Am J Orthop 2006; [4] Bonnevie+ J Biom, 2016; [5] Bonnevie+ JOR, 2018;

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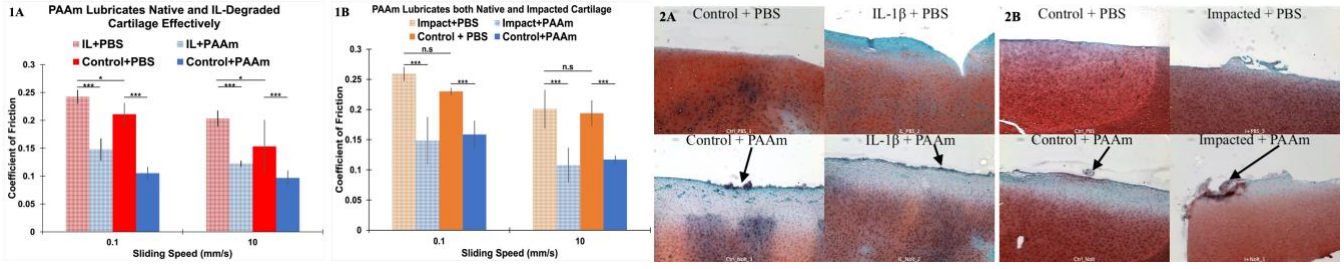

Figure 1: (A) Friction at 0.1 mm/s and 10 mm/s for IL-1β degraded and impacted samples tested in PAAm gels is significantly lower compared to samples in PBS (n=3, *** denotes $p < 0.001$, * denotes $p < 0.05$). The difference between the impacted and control samples tested in PBS (2B) was not significant ($p > 0.05$). **Figure 2:** Histology of explants from both injury groups. (A) Samples cultured in IL-1β show increased proteoglycan loss (in blue) and higher surface roughness compared to control cultured samples, (B) Evidence of surface fissuring in the impacted samples (right half of Fig 2B), samples tested in PAAm show a thin film of the lubricant being localized at the surface.