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Polyacrylamide Hydrogel Lubricates Cartilage After Biochemical Degradation And Mechanical Injury

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AUTHOR CONTRIBUTIONS STATEMENT

K.V. contributed to research design, data acquisition, analysis, and interpretation, as well as the authoring and editing of this manuscript. S.M. contributed to the data analysis, and interpretation, as well as the editing of this manuscript. L.J.B. contributed to research design, analysis, and interpretation, as well as the authoring and editing of this manuscript.

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Abstract

Intra-articular injections of hyaluronic acid have been a mainstay of osteoarthritis treatment for decades. However, controversy surrounds the mechanism of action and efficacy of this therapy. As such, there has been recent interest in developing synthetic lubricants that lubricate cartilage. Recently, a synthetic 4 wt% polyacrylamide hydrogel was shown to effectively decrease lameness in horses. However, its mechanism of action and ability to lubricate cartilage is unknown. The goal of this study was to characterize

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the lubricating ability of this hydrogel and determine its efficacy for healthy and degraded cartilage. The study utilized previously established IL-1β-induced biochemical degradation and mechanical impact injury models to degrade cartilage. The lubricating ability of the hydrogel was then characterized using a custom-built tribometer using a glass counterface and friction was evaluated using the Stribeck framework for articular cartilage. The polyacrylamide (pAAm) hydrogel was shown to significantly lower the friction coefficient of cartilage explants from both degradation models (30-40% reduction in friction relative to controls). A striking finding from this study was the aggregation of the polyacrylamide hydrogel at the articulating surface. The surface aggregation was observed in the histological sections of explants from all treatment groups after tribological evaluation. Using the Stribeck framework, the hydrogel was mapped to higher Sommerfeld numbers and was characterized as a viscous lubricant predominantly in the minimum friction mode. In summary, this study revealed that pAAm hydrogel lubricates native and degraded cartilage explants effectively and may have an affinity for the articulating surface of cartilage.

Introduction

Intra-articular hyaluronic acid (HA) viscosupplementation has been a mainstay of osteoarthritis (OA) treatment for many decades[1,2]. Viscosupplementation involves the restoration of the viscosity and the lubricating ability of synovial fluid from injured or inflamed joints. However, the clinical efficacy and potential mechanism of action of viscosupplementation are still controversial. Meta-analyses of clinical studies have shown that viscosupplementation with HA offers no significant benefit over placebo injections, and does not lead to better clinical outcomes in patients with mild to moderate OA[1,2]. This has led prominent orthopedic associations like the American Academy of

Orthopedic Surgeons (AAOS), American Orthopedic Society for Sports Medicine (AOSSM) to offer conflicting guidance and recommendations to clinicians about the use of viscosupplements[3,4]. Another potential drawback of viscosupplementation is the susceptibility of HA to be degraded by enzymes such as hyaluronidase in osteoarthritic joints. Equine studies involving intra-articular HA therapy have shown that the clearance time of HA in the joint after injection is only 11-12 hours in subjects with OA and 20 hours in a healthy joint[5,6]. Notably, the clinical benefits of HA greatly outlast reported residence times, suggesting localization at the tissue surface or other biological mechanisms may be contributing to the action of injectable HA.

Consequently, the uncertainty about mechanism has led to an increased interest in the development of synthetic viscosupplements that can lubricate cartilage as effectively as HA and resist enzymatic degradation. Recently, a biocompatible, chondroprotective HA analog was shown to lubricate cartilage as effectively as native synovial fluid with an increased residence time *in vivo*[7]. There is also interest in inducing HA localization at the surface of cartilage mediated by HA-binding peptides. An injectable peptide-polymer composed of HA and collagen binding peptides was shown to lubricate both native and damaged cartilage effectively, enhancing the localization of endogenous and exogenous HA at the surface of the cartilage[8,9].

Polyacrylamide (pAAm) hydrogels are highly crosslinked, three-dimensional networks of acrylamide that can be tuned to have a wide range of physical and chemical properties[10]. Owing to the three-dimensional porous architecture and excellent biocompatibility, pAAm hydrogels have found applications in a wide range applications such as novel materials for contact lenses, cartilage replacement candidates in tissue engineering, substrates to probe cell mechanobiology, and injectable materials for tissue

augmentation[11–17]. Further, pAAm hydrogels have been shown to exhibit low coefficients of friction as intact gels for contact lenses and as acellular cartilage implants[11,13]. Additionally, a 4 wt% injectable pAAm hydrogel was shown to decrease symptoms of OA in horses[18]. However, the ability of this injectable formulation to lubricate native cartilage is not known.

Recently, the Stribeck-like framework has been applied to study the lubrication of cartilage tissues in various biolubricants[19,20]. In this framework, lubrication is mapped into three distinct modes as a function of the lubricant viscosity, applied normal load, sliding velocity and the sample geometry. This technique enables the identification of distinct mechanisms of lubrication such as boundary and viscous lubrication, which reflect changes in tissue or the lubricating fluids. Notably, this approach yields parameters that correlate with clinical outcomes of lubrication therapies[21]. While such effects have been documented for HA lubricants, the performance of polyacrylamide in this system is unclear.

The goal of this study was to characterize the ability of the 4 wt% pAAm hydrogel to lubricate healthy cartilage. Additionally, the study will evaluate the ability of the polyacrylamide hydrogel to lubricate cartilage samples degraded in an IL-1β *in vitro* degradation model and in samples that have been injured via a previously developed impact model[22,23]. We hypothesize that the pAAm hydrogel will lower friction for healthy articular cartilage as well as biochemically degraded and mechanically injured cartilage.

Materials and Methods

To assess the ability of pAAm to lubricate healthy and degraded cartilage we used established IL-1β-induced biochemical degradation and mechanical impact injury models, which have been shown to reliably hinder cartilage lubrication[22–25].

IL-1β Biochemical Degradation Model

Cylindrical cartilage explants (6 mm diameter x 2 mm thickness) were harvested from the femoral condyles of 10 neonatal bovine stifles (1-3 days old, Gold Medal Packing, Rome, NY) within 24 hours of processing. Explants were cultured in DMEM (Mediatech, Manasas, VA) and supplemented with 1% fetal bovine serum (Mediatech), 100 U/mL penicillin and 100 μg/mL streptomycin (Mediatech), and with or without 10 ng/mL of rhIL-1β (SinoBiological, Beijing, China) at 37°C and 5% CO_2 with media changes every 3 days. One set of explants (n=14) was cultured for 7 days in media with IL-1β to induce biochemical degradation. To serve as controls in the biochemical degradation group, additional explants (n=14) were cultured in the absence of IL-1β for 7 days. Eight samples from the biochemical degradation groups were prepared for tribological evaluation and tested immediately after culture. The remaining samples from both culture groups (n=20 total) were frozen at -20 C and friction tested within one week. The frozen samples were thawed in PBS with protease inhibitors (Sigma-Aldrich) at 37° C prior to being friction tested. Previous studies with this tribometer have shown no changes to the coefficient of friction between fresh and frozen tissue[19,20].

Impact injury model

To assess the effect of mechanical injury on the lubricating ability of pAAm, we used an established model of impact injury[23,26]. We imposed a peak stress rate of 25 GPa/s which is known to cause sub-critical cracking of the cartilage surface, increases the surface roughness, and leads to significantly higher friction when tested in a wide array

of lubricants. Twelve explants were subjected to mechanical injury and twelve were left unimpacted as a control. A cylindrical indenter (8 mm diameter) was used to impact the explants in unconfined compression. All explants from the impacted and unimpacted groups were tribologically evaluated immediately after mechanical injury.

Tribological Evaluation of the Polyacrylamide Hydrogel

Frictional characterization of the pAAm hydrogel was performed using a previously described, custom built tribometer[19]. Briefly, explants from both degradation models were mated against a polished glass surface and bathed in lubricating baths consisting of PBS or a 4 wt% solution of crosslinked pAAm hydrogel (NoltrexVet - RC Bioform). Samples from both degradation models (*n=14* IL-1β degraded, *n=12* impact injury) were compressed to 30% strain and allowed to depressurize for 1 hour. Once the fluid pressure was equilibrated with the ambient pressure to negate effects of interstitial fluid pressurization, the glass surface was reciprocated at sliding speeds ranging from 0.1-10 mm/s using a DC motor. These compression levels and sliding speeds were chosen based on the strong correlation of the reported friction data to clinical outcomes[21]. The coefficient of friction μ was recorded as the ratio of shear to normal force measured by a biaxial load cell. Coefficients were calculated at the end of sliding when friction reached an equilibrium value and then averaged in the forward and reverse sliding directions to give one value for the coefficient of friction at each speed.

The Stribeck framework of tribological analysis was then applied to characterize the primary lubrication mode of the pAAm hydrogel[13,19,27]. In this framework, the coefficient of friction was presented as a function of the Sommerfield number S, which is a dimensionless number that is a ratio between the product of the sliding speed (v), the

lubricant viscosity (η), and the contact width (a = 6 mm) and the applied normal load (F_N):

$$S = v\eta a/F_N \quad (1)$$

The boundary mode friction coefficient, μ_b, the minimum friction coefficient, μ_{min} and the Sommerfeld number at the midpoint (transition number S_t) were all determined through fitting the friction data of the biochemically degraded and mechanically injured groups in PBS and the pAAm by using the following equations of friction coefficient μ, as a function of the Sommerfeld number S:

$$\mu(S) = \mu_{min} + (\mu_b - \mu_{min})e^{-(S/S_t)^d} \quad (2)$$

Where d is a fitting parameter that controls the slope of the transition. These parameters (μ_b, μ_{min}, S_t and d) obtained from the curve fit of the friction data are characteristic for a lubricating fluid and can be used to assess changes to both cartilage tissue as well as the fluid[19,28].

Histological Evaluation

Immediately after frictional characterization, explants from both degradation models were fixed in 10% phosphate buffered formalin for 5-7 days. Samples were embedded in paraffin, sectioned to 5 μm, mounted on glass slides and then stained with Safranin-O/Fast Green to observe changes to proteoglycan content. Samples from all treatment groups lubricated in PBS and pAAm were analyzed via brightfield microscopy and imaged under a light microscope.

Confocal Imaging

To determine the extent of association of pAAm to the surface of the cartilage, fluorescein isothiocyanate (FITC, Sigma-Aldrich) was used to label the free amines in the

pAAm backbone as per the manufacturer's instructions (Thermo Fisher Scientific, amine reactive probe labelling protocol). A brief overview of the conjugation protocol has been outlined in the supplementary document. Cartilage explants from both degradation models were incubated in the pAAm hydrogel conjugated with FITC (90% purity, Sigma-Aldrich) for 10 minutes and then washed thoroughly in PBS for one hour. After incubation, samples were cut into hemicylinders, mounted on a glass slide and a cross-sectional view of the explant was imaged on a Zeiss LSM 880 confocal/multiphoton inverted microscope using a C-Apochromat 10x/0.45 W water immersion objective. Images consisted of 2 channels: a red channel to image collagen fibers (cellular autofluorescence was captured between 495 nm and 585 nm) present in the cartilage explants using second harmonic generation (SHG), and a green channel at an excitation wavelength of 488 nm, which represented the fluorescently labeled pAAm hydrogel. Confocal z-stacks of each sample were collected from the cut plane and consisted of 50 slices over 600 um. The z-stacks were projected onto a 2D plane and an image of the average intensity of the stacks was obtained for each sample. All images were compiled using Zeiss ZEN 2.3 (blue edition) and images were processed in ImageJ v2.0.

Statistical Analysis

Statistical analysis for the friction comparisons between the IL-1β degraded and control cultured, and the impacted and unimpacted samples was performed by first fitting the data to a linear mixed effects regression model with the linear sliding velocity, condition of the tissue (degraded vs control) and the lubricant (PBS vs pAAm) as the fixed effects. The random effect in the model accounted for the experimental trial number on the tribometer (i.e., number of times the experiment was conducted on the system). A three-way ANOVA with repeated measures was then applied on the fitted linear models

and significance was evaluated at $p < 0.05$. Tukey's HSD test was used for comparing friction at the different speeds between lubricants within a treatment group (i.e. impacted samples tested in pAAm vs PBS). All statistical analyses were performed in R Studio (R Version 3.4.4, R Studio, Boston MA).

Results

Frictional Characterization of Polyacrylamide Hydrogel

The pAAm hydrogel lubricated native, biochemically degraded, and mechanically injured cartilage. The gel effectively lowered the coefficient of friction across the range of sliding speeds from 0.1 to 10 mm/s (Figure 1). IL-1β cultured explants lubricated in pAAm exhibited a 40% lower the coefficient of friction (μ=0.11-0.15, Figure 1A) compared to similarly lubricated in PBS (mu = 0.2-0.24). Similarly, pAAm lubricated explants exposed to impact injury showed a 35% reduction in the coefficient of friction compared to injured explants lubricated in PBS. Polyacrylamide treated samples were significantly more lubricating than PBS irrespective of the cartilage treatment group (Figure 1, *** denotes $p<0.001$).

Control cartilage explants from the IL-1β degradation group lubricated in PBS had a 15% lower coefficient of friction (μ=0.18-0.19, Figure 1A) than the IL-1β treated explants in PBS (μ=0.2-0.24, Figure 1B). Similarly, unimpacted cartilage explants lubricated in PBS had a 13% lower coefficient of friction (μ=0.19-0.22, Figure 1C) than mechanically impacted explants in PBS (μ=0.21-0.26, Figure 1D), paralleling previously published results for this injury model[22,29]. Interestingly, pAAm lubricated cartilage explants from both degradation models had a sustained lowering in the coefficient of friction across the range of sliding speeds (shown in blue). The pAAm hydrogel

lubricated IL-1β degraded explants (μ=0.11-0.15, Fig. 2B) 40% better than PBS, and lubricated impacted explants (μ=0.14-0.15, Figure 1D) 35% better than PBS.

The IL-1β degraded cartilage that was lubricated in PBS showed a higher coefficient of friction than the respective control samples in PBS, however the difference was significant only between 0.1 and 5 mm/s (***, $p < 0.0001$, Table S1, Supplementary file). Similarly, PBS lubricated explants from the impacted group showed a significantly higher coefficient of friction compared to the control across the entire range of sliding speeds (***, $p < 0.001$, Figure 1C and Table S1, Supplementary file). The pAAm hydrogel significantly lowered the coefficient of friction in the impacted and unimpacted group (***, $p < 0.001$, Figure 1C-D) relative to the control PBS. There was also a significant difference in the coefficient of friction between the IL-1β degraded and control samples lubricated in pAAm between at the lower range of sliding speeds (**, $p<0.01$, between 0.1 and 1 mm/s, Table S1, Supplementary file). But there was no significant difference in the friction between the impacted and unimpacted cartilage samples tested in pAAm (Table S1, Supplementary file).

Using the Stribeck framework to evaluate the lubricating ability, the control samples lubricated in pAAm and PBS from both cartilage degradation models were fit to equation (2) with variation in RMS Error under 5%. PBS lubricated control samples were located in boundary mode whereas the pAAm lubricated samples were in the minimum friction mode at higher Sommerfeld numbers ($S > 10^{-4}$, Figure 2). The reported Sommerfeld numbers for the controls is in agreement with previously reported data for these models[22,23]. Experimentally determined coefficients of friction for both IL-1β and impact injury explants were fit to equation (2) in a similar manner. The models showed an elevation in the boundary mode friction coefficients (μ_b) compared to their respective

controls (Figure 2). The impacted samples had an elevated boundary mode coefficient of friction but the transition number was not significantly higher than the control unimpacted samples. The IL-1β degraded samples showed a 81% increase in the transition number compared to the control culture samples, indicating that the transition from boundary mode to the minimum friction mode happens at higher sliding speeds or viscosities. Coefficient of friction as a function of the Sommerfeld number for both degradation models (and their controls) fit equation (2) well; the coefficient of variation of the root mean square error CV(RMSE) was determined to be 4.5% for the IL-1β cultured samples, 5.1% for the control culture, 6.7% for the impacted samples and 8.0% for the unimpacted cartilage samples. Curve fits from both Stribeck curve models and their confidence intervals can be found in the supplementary document to this manuscript (Table S2 and Table S3, Supplementary file).

Histology Sections

Safranin-O stained histological sections of the control cultured samples from the IL-1β group lubricated in PBS (Figure 3-A) were consistent with previously reported data, exhibiting tissue roughness and GAG content typically observed after sliding on the tribometer[22]. Similarly, explants cultured with IL-1β and lubricated in PBS (Figure 3-B) showed increased proteoglycan loss (seen as white bands, Figure 3-B) at the surface of the tissue and are similar to previous findings after tribological evaluation.

The histological sections of the unimpacted explants from the impact injury group that were lubricated in PBS (Figure 4-A) were consistent with the reported histological data for healthy control tissue. Contrastingly, impacted samples tested in PBS (Figure 4-B) showed substantially higher surface damage, increased surface roughness and

fibrillation at the surface of the tissue, consistent with previous histology reported for this model[23].

Interestingly, histological sections of all pAAm hydrogel lubricated explants from both degradation models showed surface retention and localization of the gel as seen in Figure 3-C and Figure 4-D and Figure 4-C and Figure 4-D.

Polyacrylamide Hydrogel Localization at Surface

To further investigate the presence of material at the cartilage surface in the pAAm lubricated samples, polyacrylamide hydrogel fluorescently labeled with FITC were used to image a cross-sectional view of the explants.

Control cultured explants from the IL-1β degradation model showed a thin layer of the pAAm-FITC conjugate at the surface of the cartilage (Figure 5-A). Interestingly, explants cultured in the presence of IL-1β (Figure 5-B) showed a much higher penetration of the conjugate into the surface zone and tissue, which paralleled the penetration of pAAm into the tissue observed in the histology sections of these explants (Fig. 3-B). Unimpacted cartilage explants were similar to the control culture explants (Figure 5-C). Contrastingly, explants exposed to impact injury showed a disruption in the surface zone (Figure 5-D) and aggregation of the gel on the surface of the explant.

Discussion

The objective of this study was to evaluate and characterize the ability of the 4% pAAm hydrogel to lubricate healthy cartilage, and cartilage that has been exposed to mechanical injury or biochemical degradation. The hydrogel was shown to be an effective lubricant and significantly lowered the coefficient of friction of healthy, IL-1β degraded and mechanically impacted cartilage explants. On the Stribeck curve, the PBS lubricated control and degraded explants were consistent with previously published data

for these models[19,21–23]. Increases to the boundary and minimum friction coefficients and transition number (S_t) were observed after biochemical degradation or injury. The pAAm hydrogel consistently lowered friction across all treatment groups. The lubricating ability of the pAAm hydrogel was associated with the aggregationof gel at the articulating surface. This surface aggregation was robust even in samples exposed to either mechanical injury or biochemical degradation. Fluorescently labeled pAAm penetrated into the cartilage ECM, particularly in the IL-1β treated samples. Collectively, these results show that the pAAm hydrogel is a suitable candidate for cartilage lubrication and has a unique ability to aggregate at the articulating surface of the cartilage.

The friction coefficients of the hydrogel were reported to be between μ=0.1-0.14, with no significant dependence on the sliding speed. On the Stribeck curve, the pAAm lubricated explants were mapped out in the minimum friction mode at higher Sommerfeld numbers. Analogous to previous studies involving HA lubricants with damaged cartilage, the effect of biochemical degradation and impact injury on the lubricating ability of the pAAm hydrogel is most prominent at the lower range of sliding speeds (0.1-0.5 mm/s)[22,23,25]. These degraded samples exhibited an increase in the minimum friction coefficient (μ_{min} = 0.114 for IL, μ_{min} = 0.133 for impacted) compared to their respective experimental controls (μ_{min} = 0.105), where the pAAm lubricated explants appears to be in the mixed mode of lubrication. One reason for this increase in friction could be attributed to the drainage of the pAAm hydrogel from the surface of the tissue during sliding, preventing efficient shear load transfer between the cartilage and glass, ultimately causing an increase in the friction coefficient. These friction data suggest that the pAAm hydrogel serves as a viscous lubricant in the minimum friction regime at most speeds, with exceptions at low sliding speeds where the gel is in the mixed lubrication mode. A

notable concern is the shape of the Stribeck curve from both degradation models. While the generated curves and Stribeck parameters fit the experimental data well with CV(RMSE) < 10% (Table S2 and S3, Supplementary file), the gap between Sommerfeld numbers 10^{-8} to 10^{-4} allows the possibility of multiple curve shapes and wide ranges in the fitted parameters. Notably, only the fitting parameter *d*, which represents the slope of the Stribeck curve strongly affects the transition region and overall shape. The slope was determined to be within the same order of magnitude as previously published data for these degradation models[22,23] (Table S2, S3, Supplementary file). Additionally, the 95% confidence intervals of these fitted parameters are tightly bound and within the same order of magnitude (Table S3, Supplementary file). However, altering the chemical composition of the existing pAAm hydrogel to generate new Stribeck curves in this intermediate region would not be representative of the intra-articular dose used clinically. The changed formulation might raise other questions about the therapeutic efficacy of this material *in vivo*.

A relevant question is whether synthetic lubricants like pAAm lower friction as effectively as other existing commercial viscosupplements. The 4 wt% pAAm hydrogel (μ_b = 0.1-0.14) is more lubricious than low-molecular weight formulations (μ_b = 0.17-0.22) but does not lubricate cartilage as effectively as the high-molecular weight partially hydrophobic forms of HA (μ_b = 0.05-0.1)[21]. The boundary and minimum friction coefficients of the pAAm hydrogel are similar to the high-molecular weight crosslinked HA gels (μ_b=0.12-0.15). But since the zero-shear viscosity of pAAm (~1000 Pa.s, Table S4, Supplementary file) is an order of magnitude larger than the most viscous commercial HA formulations (η_0 =100 Pa.s), it is mapped to higher Sommerfeld numbers on the

Stribeck curve, with the exception of the lower sliding speeds where it is in the mixed mode.

The boundary and minimum coefficients of friction of PBS lubricated explants from both IL-1β (μ_b = 0.21, μ_{min} = 0.12, Figure 2) and impact injury (μ_b = 0.23, μ_{min} = 0.13, Figure 2) models are consistent with previously reported data[22,23]. The increase in boundary and minimum coefficients of friction of explants cultured for 7 days in 10 ng/mL IL-1β parallel the increase in friction observed after 8 days of culture. Additionally, the histological changes to the explants from both degradation models are also consistent with previously reported data. While not directly measured in this study, the observed tissue characteristics and surface roughness changes in the histology of biochemically degraded and impacted samples are equivalent to the histology reported for both models[22]. These outcomes in friction and histology serve to bolster the reliability and robustness of these degradation models to evaluate the lubricating ability of synthetic viscosupplements.

A striking finding from this study is the aggregation of the pAAm hydrogel on the surface of the cartilage after friction testing. While HA has been shown to entangle with lubricin at the surface of cartilage[28], it is unclear whether HA localization at the surface of cartilage has been observed at this length scale. One possible factor that might hinder HA localization is the electrostatic repulsion between anionic HA and the negatively charged proteoglycans and overall negative fixed charge density of the cartilage matrix, preventing entanglement or aggregation at the surface of the tissue[30,31]. In contrast, the pAAm hydrogel in this study were shown to localize at the surface of the cartilage explants after tribological analysis. This localization is evident from the histology of the sectioned cartilage explants after friction testing (Figure 3, Figure 4). The hydrogel

survived exposure to formalin, ethanol and xylene, and localization was observed in all explants irrespective of the injury and treatment group. Second harmonic generation (SHG) images of healthy, mechanically impacted and IL-1β degraded cartilage explants incubated in fluorescein isothiocyanate (FITC) labeled pAAm also exhibited a greater presence of the gel at the surface (Figure 5, pAAm-FITC conjugate in green), contrasted against the reflectance of the collagen in the tissue (in red). However future experiments are required to understand the mechanism of gel localization as well as the microscale mechanics and interaction of the pAAm hydrogels with the surface zone of the cartilage.

The localization behavior of pAAm has been previously reported *in vivo* for other tissues. A 4 wt% pAAm hydrogel solution was injected into 6 healthy equine fetlock joints and was shown to be on the surface of the synovium 7 days after injection, and remained visible in the histology of one synovial membrane 56 days after being injected[32]. Transmission electron micrographs (TEM) of the synovial membrane 7 days and 56 days after injection revealed the presence of the gel in the interstitial spaces as well as being phagocytosed by synoviocytes[32,33]. In another study, histological analysis of 4 rabbit knee joints and 13 osteoarthritic equine joints treated with a 2.5 wt% pAAm hydrogel solution showed the presence of the pAAm on the surface of the synovium as well as in the synovial folds of the tissue for up to 14 days after injection[33]. Such localization effects at the surface of the cartilage and synovial membrane may be clinically beneficial due to the increased residence time of the gel in the joint.

While this study characterized the lubricating ability of a novel polyacrylamide hydrogel viscosupplement, there are some limitations to be addressed. Lubrication measurements in this study utilized cartilage explants obtained from neonatal bovine femoral condyles. While neonatal cartilage may not be structurally representative of adult

human, bovine or equine cartilage explants, it has been previously used in many studies involving IL-1β and impact injury[22,23,26,29,34]. Additionally, friction measurements involving neonatal bovine cartilage are comparable to adult human-on-human and equine-on-equine cartilage systems and have recently been shown to be predictive of *in vivo* outcomes[21]. Furthermore, this study used a stationary contact area cartilage on glass tribometer configuration, where polished glass was used as the counter surface. The benefits of using the polished glass counter face against cartilage provides similar boundary coefficients of friction for PBS, lubricin and synovial fluid when measured on a cartilage on cartilage configuration[35].

In conclusion, we have shown the 4 wt% polyacrylamide hydrogel can lubricate native, biochemically degraded and mechanically injured cartilage explants effectively. The hydrogel was found to aggregate to the surface of the tissue after frictional characterization and this was evident in the histological sections of tissue from both degradation models. Collectively, these results indicate that the pAAm hydrogel is a suitable cartilage lubricant. The localization of the hydrogel at the articulating surface may be of clinical benefit with a possibility of increased residence time in the joint.

Acknowledgments

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Conflicts of Interest

Dr. Bonassar is a cofounder of and holds equity in 3DBio Corp and is a consultant for Fidia Farmaceutici, SpA. Dr. McClure has served as a scientific consultant for Nucleus ProVets, LLC.

Figure Legends

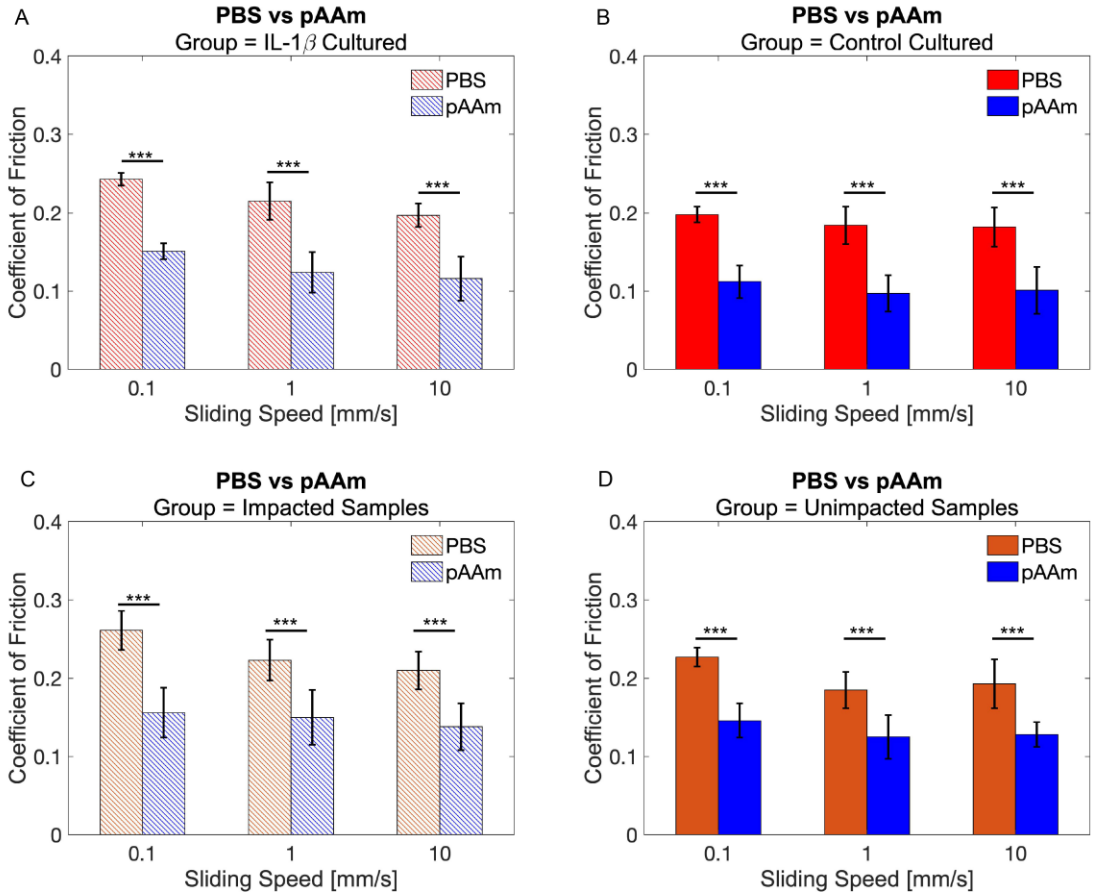

Figure 1: Friction data of polyacrylamide lubricated explants from both degradation models.

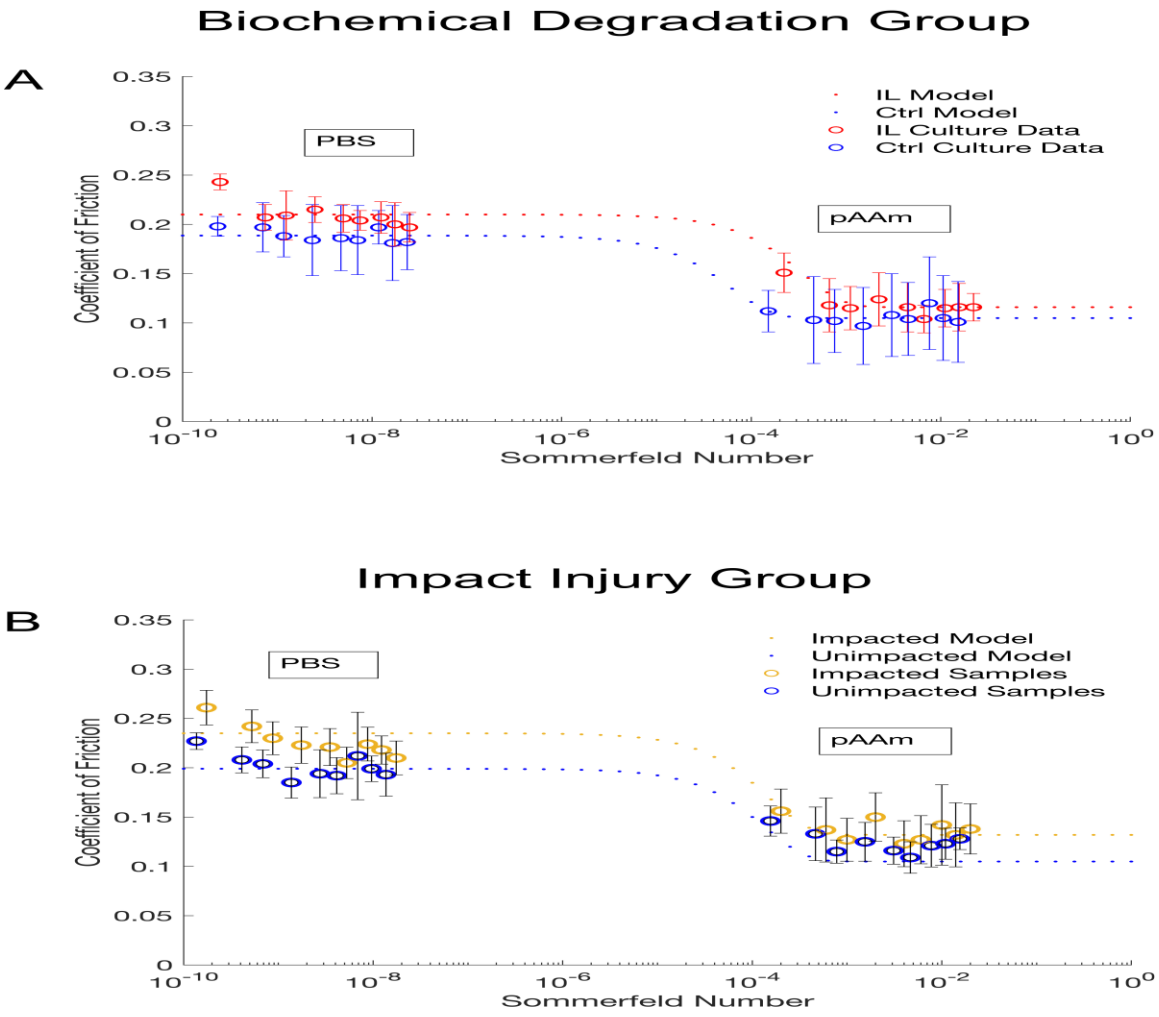

Figure 2: Stribeck curves for explants lubricated in PBS and the pAAm hydrogel from both degradation groups.

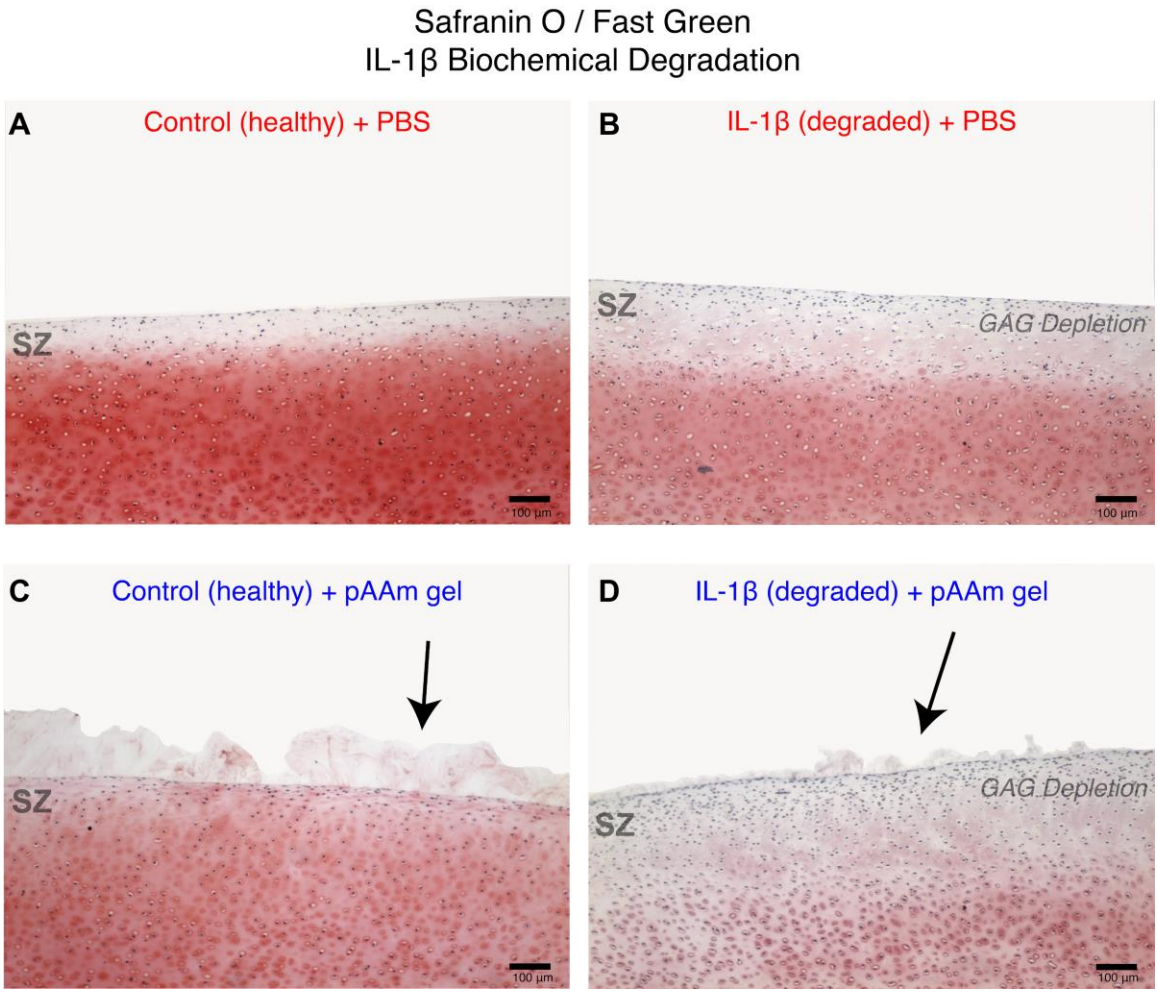

Figure 3: Safranin-O/Fast Green stained histological sections of cartilage explants from the IL-1β degradation model. Scale bar = 100 µm.

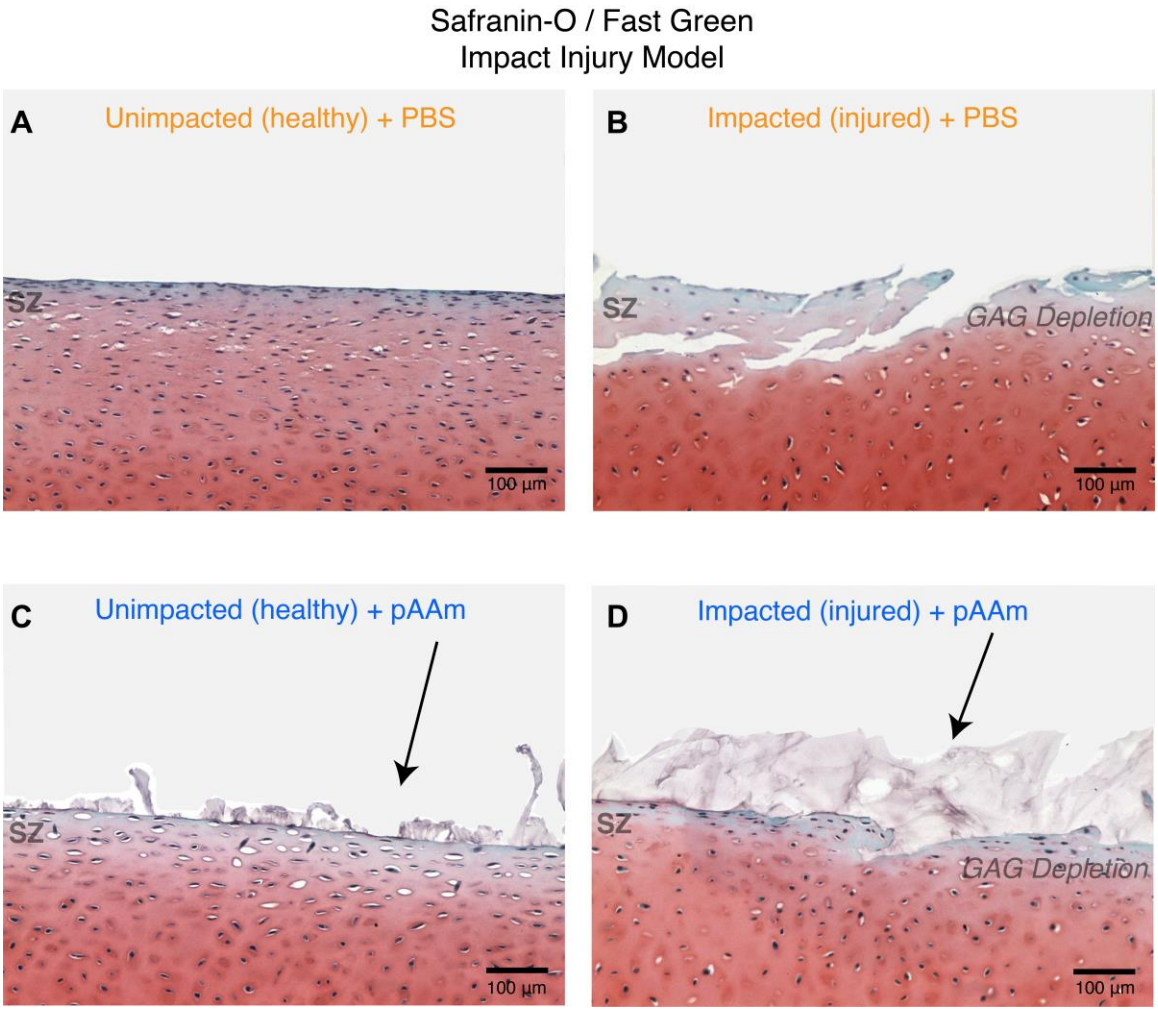

Figure 4: Safranin-O/Fast Green stained histological sections of cartilage explants from the impact injury model. Scale bar = 100 µm.

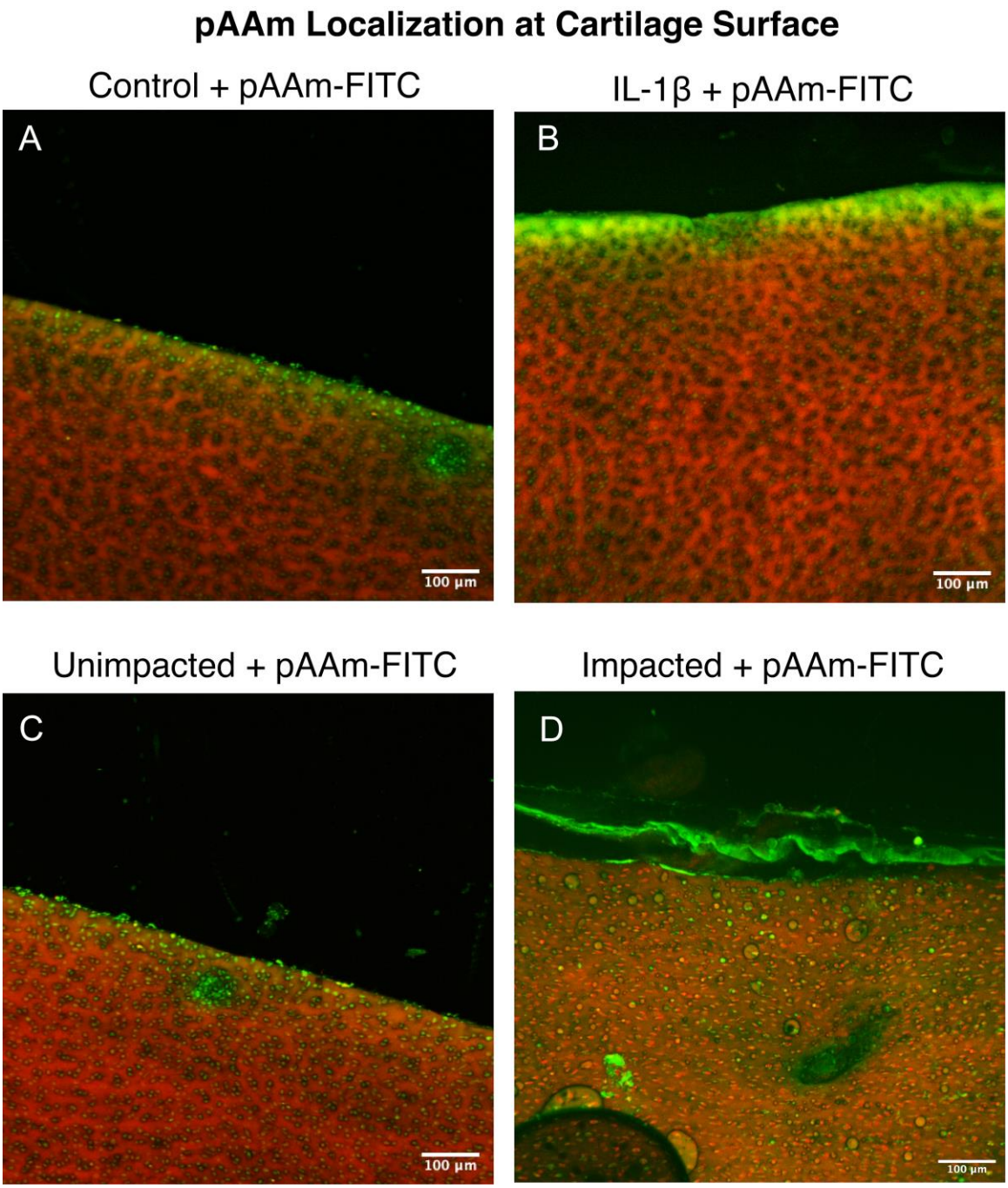

Figure 5: Second harmonic generation confocal images of explants incubated in fluorescently labeled polyacrylamide. Scale bar = 100 µm.

References

1. Balazs EA, Denlinger JL. 1993. Viscosupplementation: A new concept in the treatment of osteoarthritis. Journal of Rheumatology 20(SUPPL. 39):3–9.

2. Bellamy N, Campbell J, Robinson V, et al. 2006. Viscosupplementation for the treatment of osteoarthritis of the knee. Cochrane Database Syst Rev (2):CD005321.

3. Trojian TH, Concoff AL, Joy SM, et al. 2016. AMSSM Scientific Statement Concerning Viscosupplementation Injections for Knee Osteoarthritis: Importance for Individual Patient Outcomes. Clin J Sport Med 26(1):1–11.

4. Strauss EJ, Hart JA, Miller MD, et al. 2009. Hyaluronic Acid Viscosupplementation and Osteoarthritis: Current Uses and Future Directions. Am J Sports Med 37(8):1636–1644.

5. Jevsevar DS. 2013. Treatment of osteoarthritis of the knee: evidence-based guideline, 2nd edition. J Am Acad Orthop Surg 21(9):571–576.

6. Gupta RC, Lall R, Srivastava A, Sinha A. 2019. Hyaluronic Acid: Molecular Mechanisms and Therapeutic Trajectory. Frontiers in Veterinary Science 6:192.

7. Wathier M, Lakin BA, Cooper BG, et al. 2018. A synthetic polymeric biolubricant imparts chondroprotection in a rat meniscal tear model. Biomaterials 182(August):13–20.

8. Singh A, Corvelli M, Unterman SA, et al. 2014. Enhanced lubrication on tissue and biomaterial surfaces through peptide-mediated binding of hyaluronic acid. Nature Materials 13(10):988–995.

9. Faust HJ, Sommerfeld SD, Rathod S, et al. 2018. A hyaluronic acid binding peptide-polymer system for treating osteoarthritis. Biomaterials 183(August):93–101.

10. Baker BA, Murff RL, Milam VT. 2010. Tailoring the mechanical properties of polyacrylamide-based hydrogels. Polymer 51(10):2207–2214.

11. Bonyadi SZ, Atten M, Dunn AC. 2019. Self-regenerating compliance and lubrication of polyacrylamide hydrogels. Soft Matter 15(43):8728–8740.

12. Kandow CE, Georges PC, Janmey PA, Beningo KA. 2007. Polyacrylamide Hydrogels for Cell Mechanics: Steps Toward Optimization and Alternative Uses. Methods in Cell Biology 83(07):29–46.

13. Dunn AC, Sawyer WG, Angelini TE. 2014. Gemini Interfaces in Aqueous Lubrication with Hydrogels. Tribol Lett 54(1):59–66.

14. [Date unknown]. A low friction, biphasic and boundary lubricating hydrogel for cartilage replacement | Elsevier Enhanced Reader. [cited 2021 Oct 20] Available from: https://reader.elsevier.com/reader/sd/pii/S1742706117306773?token=FC28FB66E90E128D34F2552436B9A9AFDB7B5EF3151EB93AB29F834136F8E7FFA2FC81C55A6A16A8CD2133E94045DB45&originRegion=us-east-1&originCreation=20211020191504.

15. Yashima S, Takase N, Kurokawa T, Gong JP. 2014. Friction of hydrogels with controlled surface roughness on solid flat substrates. Soft Matter 10(18):3192–3199.

16. Reale ER, Dunn AC. 2017. Poroelasticity-driven lubrication in hydrogel interfaces. Soft Matter 13(2):428–435.

17. Shoaib T, Espinosa-Marzal RM. 2020. Advances in Understanding Hydrogel Lubrication. Colloids and Interfaces 4(4):54.

18. McClure SR, Wang C. 2017. A Preliminary Field Trial Evaluating the Efficacy of 4% Polyacrylamide Hydrogel in Horses With Osteoarthritis. Journal of Equine Veterinary Science 54:98–102.

19. Gleghorn JP, Bonassar LJ. 2008. Lubrication mode analysis of articular cartilage using Stribeck surfaces. Journal of Biomechanics 41(9):1910–1918.

20. Gleghorn JP, Jones ARC, Flannery CR, Bonassar LJ. 2009. Boundary mode lubrication of articular cartilage by recombinant human lubricin. Journal of Orthopaedic Research 27(6):771–777.

21. Bonnevie ED, Galesso D, Secchieri C, Bonassar LJ. 2019. Frictional characterization of injectable hyaluronic acids is more predictive of clinical outcomes than traditional rheological or viscoelastic characterization. PLOS ONE 14(5):e0216702.

22. Bonnevie ED, Galesso D, Secchieri C, Bonassar LJ. 2018. Degradation alters the lubrication of articular cartilage by high viscosity, hyaluronic acid-based lubricants. Journal of Orthopaedic Research 36(5):1456–1464.

23. Bonnevie ED, Delco ML, Galesso D, et al. 2017. Sub-critical impact inhibits the lubricating mechanisms of articular cartilage. Journal of Biomechanics 53:64–70.

24. Larson KM, Zhang L, Elsaid KA, et al. 2017. Reduction of friction by recombinant human proteoglycan 4 in IL-1α stimulated bovine cartilage explants. J Orthop Res 35(3):580–589.

25. DuRaine G, Neu CP, Chan SMT, et al. 2009. Regulation of the friction coefficient of articular cartilage by TGF-β1 and IL-1β. Journal of Orthopaedic Research 27(2):249–256.

26. Ayala S, Delco ML, Fortier LA, et al. 2021. Cartilage articulation exacerbates chondrocyte damage and death after impact injury. Journal of Orthopaedic Research 39(10):2130–2140.

27. Hersey MD. 1914. The laws of lubrication of horizontal journal bearings. Journal of the Washington Academy of Sciences 4(19):542–552.

28. Bonnevie ED, Galesso D, Secchieri C, et al. 2015. Elastoviscous Transitions of Articular Cartilage Reveal a Mechanism of Synergy between Lubricin and Hyaluronic Acid. PLOS ONE 10(11):e0143415.

29. Gleghorn JP, Jones ARC, Flannery CR, Bonassar LJ. 2009. Alteration of articular cartilage frictional properties by transforming growth factor β, interleukin-1β, and oncostatin M. Arthritis & Rheumatism 60(2):440–449.

30. Lesperance LM, Gray ML, Burstein D. 1992. Determination of fixed charge density in cartilage using nuclear magnetic resonance. J Orthop Res 10(1):1–13.

31. Palmer AW, Guldberg RE, Levenston ME. 2006. Analysis of cartilage matrix fixed charge density and three-dimensional morphology via contrast-enhanced microcomputed tomography. PNAS 103(51):19255–19260.

32. McClure SR, Yaeger M, Wang C. 2017. Clinical and Histologic Evaluation of Polyacrylamide Gel in Normal Equine Metacarpal /Metatarsal-Phalangeal Joints. Journal of Equine Veterinary Science 54:70–77.

33. Christensen L, Camitz L, Illigen KE, et al. 2016. Synovial incorporation of polyacrylamide hydrogel after injection into normal and osteoarthritic animal joints. Osteoarthritis and Cartilage 24(11):1999–2002.

34. Kar S, Smith DW, Gardiner BS, et al. 2016. Modeling IL-1 induced degradation of articular cartilage. Arch Biochem Biophys 594:37–53.

35. Schmidt TA, Sah RL. 2007. Effect of synovial fluid on boundary lubrication of articular cartilage. Osteoarthritis and Cartilage 15(1):35–47.