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RESEARCH ARTICLE

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Loss of effective lubricating viscosity is the primary mechanical marker of joint inflammation in equine synovitis

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Abstract

Inflammation of the synovium, known as synovitis, plays an important role in the pathogenesis of osteoarthritis (OA). Synovitis involves the release of a wide variety of pro-inflammatory mediators in synovial fluid (SF) that damage the articular cartilage extracellular matrix and induce death and apoptosis in chondrocytes. The composition of synovial fluid is dramatically altered by inflammation in OA, with changes to both hyaluronic acid and lubricin, the primary lubricating molecules in SF. However, the relationship between key biochemical markers of joint inflammation and mechanical function of SF is not well understood. Here, we demonstrate the application of a novel analytical framework to measure the effective viscosity for SF lubrication of cartilage, which is distinct from conventional rheological viscosity. Notably, in a well-established equine model of synovitis, this effective lubricating viscosity decreased by up to 10,000-fold for synovitis SF compared to a ~4 fold change in conventional viscosity measurements. Further, the effective lubricating viscosity was strongly inversely correlated ($r = -0.6$ to -0.8) to multiple established biochemical markers of SF inflammation, including white blood cell count, prostaglandin E_2 (PGE_2), and chemokine ligand (CCLs) concentrations, while conventional measurements of viscosity were poorly correlated to these markers. These findings demonstrate the importance of experimental and analytical approaches to characterize functional lubricating properties of synovial fluid and their relationships to soluble biomarkers to better understand the progression of OA.

KEYWORDS
biomarkers, biomechanics, cartilage, inflammation, osteoarthritis

1 | INTRODUCTION

The pathogenesis of osteoarthritis (OA) is commonly monitored using clinical outcome measures like patient reported outcomes (PROs), imaging techniques like radiography and magnetic resonance imaging (MRI), and synovial fluid (SF) biomarker analyses.[1–4] However, a major hurdle in understanding the pathology of OA is the lack of a set of fundamental markers or indicators that effectively couple biological changes that occur within the joint with functional mechanical outcomes.

Synovial inflammation is a hallmark of OA, with established links to indices of pain and function.[5] SF aspirated from patients with OA commonly exhibits upregulated levels of inflammatory markers such as plasma protein, PGE_2, and cytokines like TNF-α, IL-1β, and IL-6.[6–8] These markers have been used as clinical indicators and potential therapeutic targets (e.g., interleukin-1 receptor antagonist, IL-1Ra) in

the diagnosis and treatment of OA. However therapeutics that entirely reverse the progression of disease are still unavailable. While SF analysis can reveal specific changes to biomarker content, the link between these biochemical changes and functional mechanical outcomes like joint lubrication is complex and unclear.

Synovial fluid lubricates the joint through a wide range of well characterized mechanisms, mediated by a combination of surface chemistry and viscous fluid mechanics.[9–11] The efficacy of SF lubrication is assessed using established metrics such as the coefficient of friction, and rheological properties such as viscosity. Notably, the lubricating ability of SF has been found to vary widely after injury and disease.[12–15] Loss of lubrication has been shown to damage cartilage and may play a substantial role in the progression of OA. However, the reported outcomes of these studies did not directly investigate changes to mechanical and inflammatory markers on the acute timescale (i.e., 1–4 days after the onset of injury or inflammation). Additionally, the lubrication outcomes of these studies varied greatly owing to differences in tribological evaluation methods, choice of animal model, biochemical analyses of SF composition, and time points chosen after injury or disease.[13,16,17] Thus, there is a need for a unifying approach to understand the link between transient changes in SF inflammation on the acute timescale, and the associated changes to SF mechanical function.

A challenge in identifying changes in cartilage lubrication is that coefficient of friction is a state variable and depends on several operating parameters.[18] The Stribeck framework of tribological analysis is a useful tool to distinctly characterize the modes of lubrication exhibited by a lubricant. Originally developed for hard materials, this framework has been used reliably in many studies to characterize the modes of lubrication of cartilage, termed the boundary, mixed, and minimum friction modes.[18–20] In this framework, the coefficient of friction is mapped as a function of a dimensionless Sommerfeld number (S) which is calculated from the viscosity of the lubricant (η), the characteristic length of the contacting geometry (a), the sliding speeds (v), and the normal force applied on the cartilage (F_N), as shown in equation (1).

$$S = \frac{v\eta a}{F_N}. \quad (1)$$

For decades, intra-articular HA therapies have been termed viscosupplements with the idea that they apparently restore the viscosity to injured SF.[21,22] Despite many clinical studies and meta-analyses, the relationship between measured viscosity of HA and clinical outcomes is unclear[23–25] due to the low half-life of exogenous HA in the joint. Notably, for these studies, the viscosities of these HAs were determined via conventional rheometry, in which lubricants are in contact with stiff impermeable materials such as glass or stainless steel, that have vastly different surface chemistries than cartilage. Recent work has shown that HA in particular has a significantly altered viscosity in the presence of cartilage, and its viscosity is a function of the molecular weight and contact gap width.[26] To address this disparity in viscosity, a novel analytical framework was developed to determine the effective lubricating viscosity (η_{eff}) of lubricants based on their position on the Stribeck curve.[27,28] This effective viscosity has also been shown to be more predictive of improved lubrication and clinical outcomes than conventionally measured viscosity.[28] While the effective lubricating viscosity framework has been used to characterize viscosupplements like HA, the transient changes in the effective viscosity of inflamed synovial fluid is not known. We hypothesize that the effective viscosity of pathological SF will change over the course of synovitis, and will be more in line with the transient changes in the classical biochemical and inflammatory markers in the SF compared to conventional measurements of viscosity.

Here, we demonstrate the application of the effective viscosity framework to characterize the transient changes in the lubricating ability of pathological SF from a large animal model known to be relevant to human disease. In an established model of equine IL-1β-induced synovitis known to cause acute changes to SF pathology, we show that the effective viscosity is orders of magnitude more sensitive to SF pathology than conventional indices of lubrication like viscosity or friction. Additionally, the effective lubricating viscosity is highly correlated to multiple established clinical markers of inflammation such as PGE_2, white blood cell count (WBC) count, TP content, and chemokine ligands CCL2 and CCL11, while conventional viscosity is not correlated. The combined analysis of temporal variation in inflammatory biomarkers, SF composition, and mechanical markers may explain how changes to SF biochemistry and inflammation can mediate changes in lubrication. These results will help inform parameters for targeted delivery of therapeutics, and suggest that there is a critical time window where viscosupplementation treatment may be most effective.

2 | MATERIALS AND METHODS

2.1 | Study design and sample collection

To characterize the temporal changes to lubrication and biomarker presence after synovitis, synovial fluid samples from the synovitis and contralateral control limbs of $N = 6$ horses were aspirated at 0, 48, 72, and 672 h after a single 10 ng/mL IL-1β injection to assess SF biochemistry, inflammation, and composition as described previously.[29] The 0-h timepoint served as the presynovitis baseline. The 48 and 72 h SF samples were selected for tribological evaluation since prior biochemical analyses revealed that they had the highest lubricin content, lower fractions of high molecular weight HA, and had the lowest measured viscosities.[29] All SF samples from these time points were frozen at −80°C and thawed in a water bath at 37°C before tribological evaluation.

2.2 | Tribological evaluation of synovial fluid samples

Frictional characterization of the SF was performed using a previously described, custom cartilage-on-glass tribometer.[30] Briefly,

cylindrical cartilage explants (from $N = 17$ joints, 6 mm diameter and 2 mm thickness) were compressed to 30% axial strain and allowed to depressurize for 1 h. Once the fluid pressure was equilibrated with the ambient pressure, the glass surface was reciprocated at linear sliding speeds ranging from 0.1 to 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 from this system to clinical outcomes.[28] Coefficients of friction 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 a mean value at each speed for each SF sample.

2.3 | Stribeck analysis and effective viscosity

The Stribeck framework of tribological analysis was applied to characterize the temporal variation in lubrication modes of the SF from the synovitis and contralateral control limbs. The equine SF 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 all SF samples at all timepoints by using the cftool package on MATLAB. These parameters (μ_b, μ_{min}, S_t and d) were obtained via a nonlinear least squares curve fit of the friction data in MATLAB (MathWorks) with the goal of minimizing RMS error. These fitted parameters are characteristic for equine SF and were used to assess the effective viscosity (η_{eff}).

The effective viscosity of the SF from both groups at each time point was determined based on a technique described previously.[27,28] Briefly, a predicted Sommerfeld number (S_{pred}) was calculated using Equation (1), in which the viscosity of the SF was allowed to vary based on a model Stribeck curve for equine SF. Predicted friction coefficients for all SF groups were then calculated relative to this model curve using Equation (2). A custom MATLAB RMS error minimization function was applied on the difference between the predicted and experimental friction data for each time point, allowing the predicted Sommerfeld number and viscosity from Equation (1) to vary, enabling the calculation of an effective viscosity for each sample from each group.

$$\mu(S) = \mu_{\min} + (\mu_b - \mu_{\min}) \cdot e^{-\left(\frac{S}{S_t}\right)^d}. \quad (2)$$

2.4 | Statistical analyses

Statistical analyses were conducted using a linear mixed effects regression model to account for the hierarchical nature of the data. The fixed effects in the model included the treatment group (synovitis vs. control), the categorical timepoint (0, 48, 72 and 672 h) and each linear sliding speed. Random effects included the leg from which the SF was obtained, nested within the specific animal to account for the nonindependence of the observations. *Post hoc* pairwise comparisons (Student's *t*-test) were conducted to compare the estimated marginal means of the friction coefficient for the synovitis and control SF at each sliding speed. Pearson correlation coefficients were used to determine the relationships between the measured mechanical outcomes and inflammatory and biochemical markers in these SF samples. The associated *p-values* of each correlation were then calculated from the corrplot package in R (RStudio Inc. v 4.3.2) to determine the statistically significant correlations.

3 | RESULTS

3.1 | Synovitis transiently impairs SF lubrication

To assess temporal changes to lubrication after the induction of synovitis, SF samples from the synovitis and contralateral control limbs at 0, 48, 72, and 672 h from a previous study were selected for tribological evaluation.[29]

Synovitis SF exhibited transiently inferior lubrication at 48 and 72 h with a nearly 75% increase in coefficient of friction relative to contralateral control SF (**, $p < 0.01$, Figure 1). At 48 and 72 h, the coefficient of friction of the synovitis group was significantly higher compared to the 0 h synovitis SF group (denoted as B, $p < 0.05$, Figure 1). The measured coefficients of friction at 48 and 72 h ranged from $\mu_{meas} = 0.06–0.075$ with coefficient of friction decreasing as a function of the sliding speed. Interestingly at the 672 h timepoint, the coefficients of friction returned to pre-synovitis levels with no significant differences between the control and synovitis groups (n.s., Figure 1). The measured coefficients of friction at 672 h ranged from $\mu_{meas} = 0.035–0.045$ across all sliding speeds.

Control SF at each time point exhibited coefficients of friction ranging from $\mu_{meas} = 0.05–0.06$ at low speeds (0.1–0.3 mm/s, Figure 1), and decreasing by approximately 30% to coefficients of friction ranging from $\mu_{meas} = 0.035–0.045$ at higher sliding speeds (3–10 mm/s, Figure 1). While the coefficients of friction at the 48 and 672 h timepoints were not different than the presynovitis 0-h timepoint (denoted by A, n.s., Figure 1), the control SF samples at 72 h exhibited significantly higher coefficients of friction ranging from $\mu_{meas} = 0.05–0.06$ across all sliding speeds tested (denoted as B, $p < 0.05$, Figure 1). At the 672 h timepoint, the coefficients of friction of control SF returned to presynovitis baseline levels, with friction ranging from $\mu_{meas} = 0.035–0.045$ across all sliding speeds.

3.2 | Synovitis alters the lubrication modes of SF

To assess the temporal changes in the modes of lubrication of the synovitis and control SF, the Stribeck framework of tribological analysis was used to map the coefficient of friction as a function of the Sommerfeld number calculated using Equation (1) as depicted in Figure 2A,B.

On the classical Stribeck curve calculated from the measured viscosity (Figure 2A), the control SF at all time points (in blue) and synovitis SF at 0 and 672 h (in red, denoted by circle and diamond), exhibited the lowest levels of friction ranging from $\mu_{meas} = 0.035–0.045$, and at Sommerfeld numbers ranging from $S_{num} = 10^{-9} – 10^{-6}$. The SF

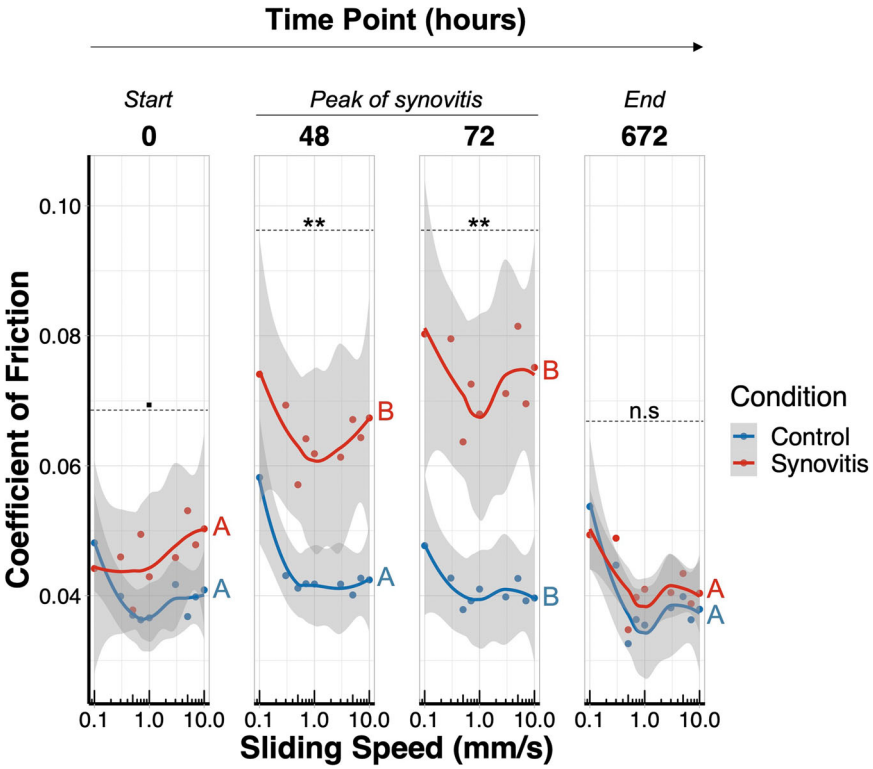

FIGURE 1 Coefficient of friction of synovitis (in red) and control SF (in blue) samples at 0, 48, 72 and 672 h as a function of sliding speed. Symbols (*) indicate comparisons between synovitis and control SF at each time point. Letters (A, B) represent the differences in coefficient of friction over the four time points within the control or synovitis groups. Solid lines represent the mean, and the shaded regions represent the standard error of the mean ($N = 6$, per time point).

samples with the highest measured coefficients of friction are the synovitis samples at 48 and 72 h (in red, square and triangle) with coefficients of friction ranging from $\mu_{meas} = 0.06–0.075$, and between Sommerfeld numbers $S_{num} = 10^{-9} - 10^{-6}$. (Figure 2A, in red). Notably, control and synovitis SF samples at all time points do not form a single continuous Stribeck curve. The samples from both groups were in the same range of Sommerfeld numbers (10^{-6}–10^{-9}) and did not show distinct modes of lubrication. This lack of stratification into distinct lubrication modes can be attributed to the fact that the average measured viscosities of all samples were within the same order of magnitude (10–90 mPa·s, Table S1). It is well established that the measured viscosity alters the Stribeck curve where higher viscosity lubricants shift the curve to the right.[28,31] Notably the use of the measured viscosity did not shift the Stribeck curve sufficiently to identify distinct modes of lubrication.

To better capture these distinct of modes of lubrication of synovitis SF, the effective viscosity framework was applied to the Stribeck analysis. The use of the effective lubricating viscosity in the Stribeck framework enabled a clearer representation of the lubricating ability of the SF samples across all conditions and timepoints with good fits to the model curve (CV(RMSE) < 20% per sample, per group). Using the effective lubricating viscosities of the SF samples revealed striking changes to the dominant modes of lubrication (Table S1). Control SF at all four timepoints, and synovitis SF at 0 and 672 h were shifted to the right towards the minimum friction mode due to their high effective lubricating viscosities (Figure 2B). In contrast, the synovitis SF at 48 and 72 h exhibited a shift to the boundary mode of lubrication with a drop in Sommerfeld number from $S_{num} = 10^{-9}$–10^{-10}, a full order of magnitude lower than the measured viscosity Stribeck curve (Figure 2A,B) due to the lower effective viscosity (Table S1). Thus, the effective viscosity framework provided a unique insight into the detrimental effect of synovitis on SF lubrication.

3.3 | Effective lubricating viscosity is the most sensitive mechanical marker of synovitis

To understand the sensitivity of mechanical outcomes like viscosity and friction to the inflammatory state of the SF, control and synovitis SF samples were compared temporally (Figure 3).

While the measured viscosity of synovitis SF was lower by fourfold, the effective lubricating viscosity was far more sensitive to the inflammatory state of the SF than the measured viscosity. Synovitis SF exhibited a dramatic decrease in effective viscosity at 48 and 72 h, nearly four orders of magnitude (~10,000 fold) lower than the effective viscosity of control SF (Figure 3 and Table S1) or before synovitis. While the minimum coefficient of friction was largely unchanged over the course of synovitis, the boundary coefficient of friction of synovitis SF at 48 and 72 h was significantly higher than control SF, returning to baseline levels by 672 h. However, these friction measurements were not as sensitive to the inflammatory state of the SF as the effective viscosity.

The effective viscosities of the control SF at all time points and the synovitis SF at 0 and 672 h were 40- to 80-fold higher than their measured viscosities ($p < 0.001$, Table S1, in bold). In contrast, the effective viscosities of the synovitis SF at 48 and 72 h were 12- to 25-fold *lower* than the measured viscosity. While the measured viscosity of the 672 h samples remained low and comparable to the viscosities at 48 and 72 h in the synovitis group, the effective

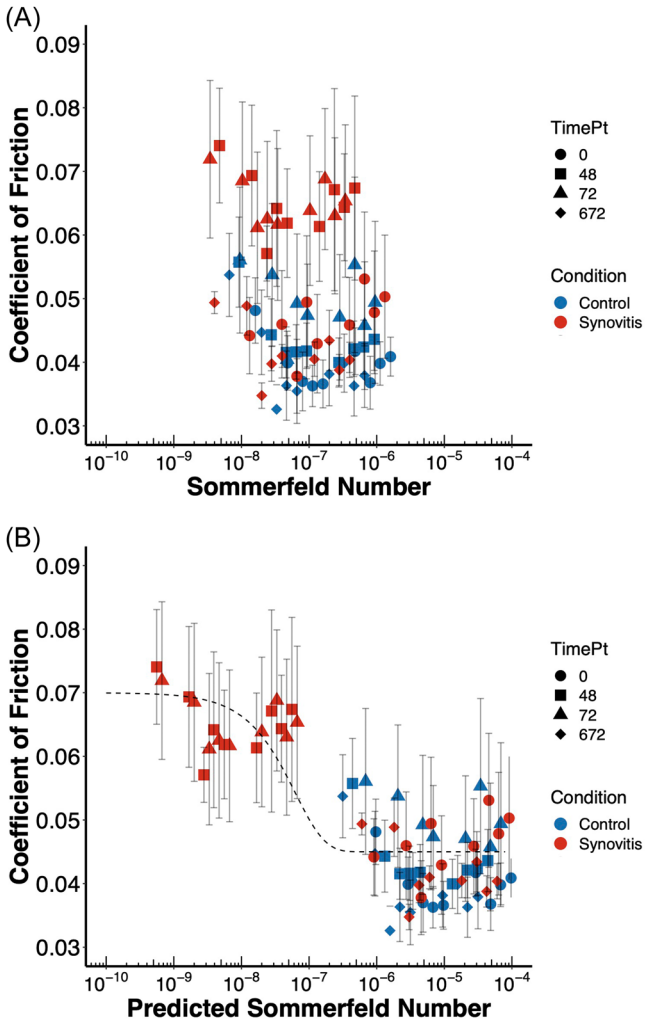

FIGURE 2 (A) Conventional Stribeck curves of all SF samples from both groups using the reported particle tracked zero-shear viscosity does not reveal differences in lubrication between synovitis and control SF. (B) Minimizing RMS error between experimental data and a model Stribeck curve (dashed line in black) for ESF enabled calculation of effective viscosities, collapsing all data sets onto one continuous Stribeck curve. The effective viscosity Stribeck curves indicate clear changes in lubricating mechanisms of synovitis SF. Markers in red are indicative of synovitis SF. Data represents the mean ± standard error ($N = 6$, per time point from both conditions).

lubricating viscosities of the 672-h synovitis group were significantly higher. This return to higher effective viscosities led to a ratio of effective viscosity to measured viscosity to be much greater than 1 for both control and synovitis samples at 672 h (Table S1).

3.4 | Inflammatory markers in SF are strongly correlated to friction, effective viscosity, and lubricin content

To understand the biochemical origins of changes in SF lubricating ability, correlations were assessed between mechanical markers such as the coefficients of friction (μ, μ_{min}, μ_b), measured viscosity (η_{meas}) and effective viscosity (η_{eff}), and previously reported biochemical and inflammatory biomarker data from these animals.[29]

Overall, synovitis SF at 48 and 72 h exhibited higher levels of friction (μ, μ_{min}, μ_b) and lower measured viscosities (η_{meas}) as demonstrated by significant moderate correlations between these variables and the condition of the SF ($r = 0.4–0.7$, $p < 0.05$, Figure 4). Of all the mechanical outcomes measured, the effective viscosity and measured coefficients of friction were strongly and significantly correlated to classic SF inflammatory markers such as the joint circumference (JC), PGE_2 concentrations, WBC count, TP content, and CC chemokine ligand 11 (CCL11) concentrations, with correlation coefficients ranging from $r = 0.8–0.95$. Interestingly, SF lubricin was positively correlated with the measured coefficients of friction and inversely correlated with the effective viscosity ($r = −0.59$, $p < 0.05$, Figure 4).

While previous work has shown that the concentration of lubricin after injury or disease is negatively correlated with the mechanical function like lubrication,[15,17,32–34] the lubricin content measured in these SF samples was positively correlated with higher coefficients of friction ($r = 0.6–0.8$, $p < 0.05$, Figure 4) and negatively correlated with the effective lubricating viscosity ($r = −0.6$, $p < 0.05$, Figure 4). The concentration of lubricin in the SF was not linearly correlated over the course of synovitis since data from the previous study indicated an increase in lubricin concentration at 48 and 72 h, followed by a return to baseline levels by the end of the study.[29] Additionally, in accordance with previously published data in the literature, the lubricin concentration in the SF was also strongly positively correlated with inflammatory biomarkers such as PGE_2, WBC count, TP, and the CCL2, CCL3 and CCL11 chemokines.[12] In contrast, the HA concentration in the SF was not significantly correlated with any mechanical or inflammatory biomarker, with the exception of the measured viscosity ($r = 0.72$, $p < 0.05$, Figure 4) and the time point. Temporally, the concentration of HA decreased from 0 to 672 h with a significant negative correlation with time ($r = −0.72$, $p < 0.01$, Figure 4) but there were no other statistically significant correlations observed between HA and other biomarkers.

The measured viscosity of the SF (η_{meas}) in this study was not correlated to mechanical markers (with the exception of the boundary coefficient of friction), SF composition biomarkers (with the exception of HA concentration), or with classical inflammatory biomarkers. In contrast, the effective lubricating viscosity of the SF (η_{eff}) was strongly and significantly negatively correlated to PGE_2 concentrations, WBC count, TP content, and CCL2 concentrations ($r = −0.7$ to $−0.8$, $p < 0.05$, Figure 4). Unsurprisingly, the effective viscosities were also found to be strongly inversely correlated to the measured coefficients of friction ($r = −0.6$ to $−0.8$, $p < 0.05$, Figure 4), and to lubricin concentrations ($r = −0.6$, $p < 0.05$, Figure 4). Cumulatively, these correlations support that cartilage tribology in conjunction with the analytical effective lubricating viscosity framework provides an extremely valuable mechanical marker to characterize the lubricating ability of diseased or inflamed SF.

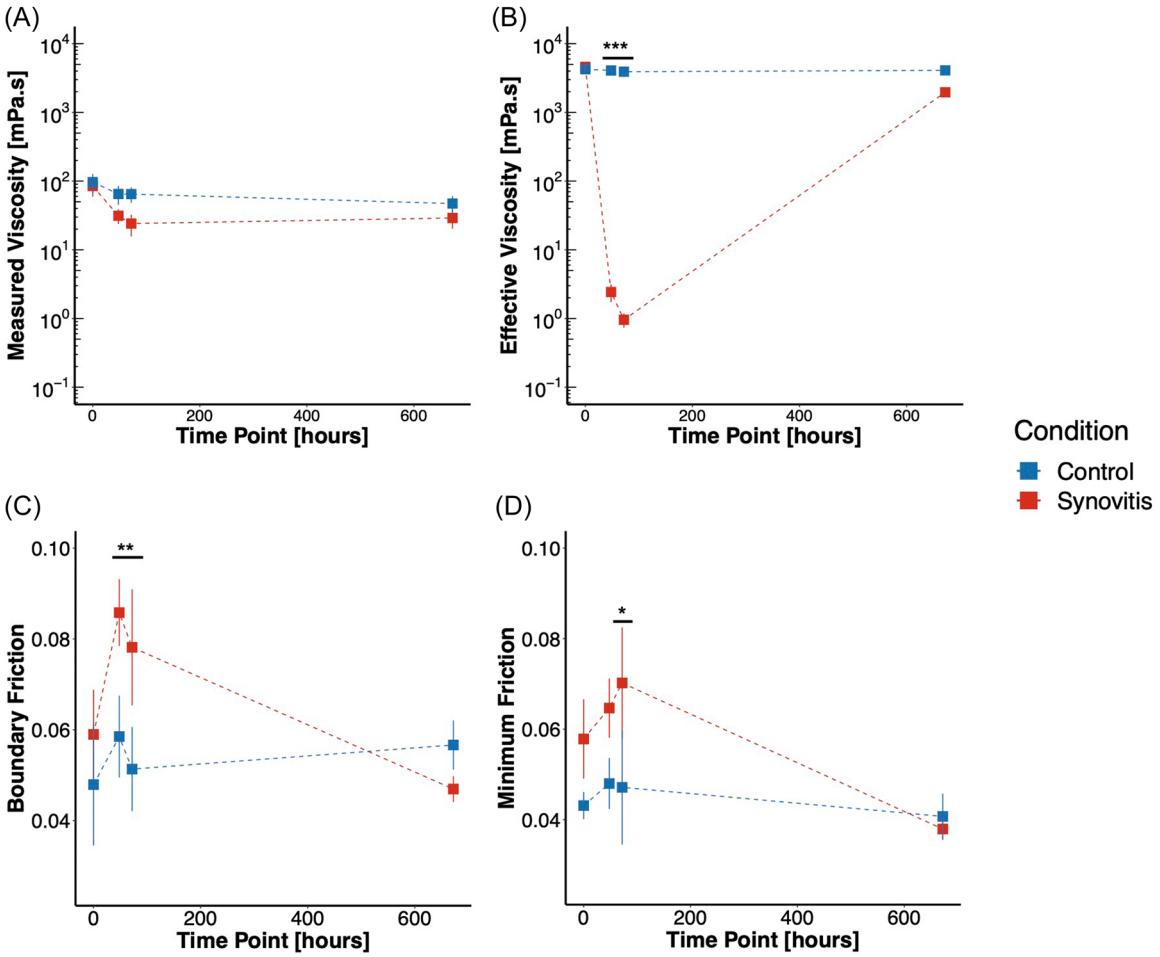

FIGURE 3 Temporal variation in mechanical markers (A) measured viscosity, (B) effective viscosity, and (C, D) boundary (at 0.1 mm/s) and minimum (at 10 mm/s) coefficients of friction over the course of the synovitis intervention. The effective viscosity was the most sensitive mechanical marker to SF inflammation. Symbols (*) indicate statistically significant differences between synovitis and control data at each time point. Significance was evaluated at $p < 0.05$. Data represents the mean ± standard error ($N = 6$ horses, per time point from synovitis and control SF).

4 | DISCUSSION

The goal of this study was to develop a novel framework that effectively couples changes in biomarker content from pathological SF and mechanical function in a large animal model of osteoarthritis-associated inflammation. Synovitis SF exhibited elevated levels of friction at the peak of inflammation (48–72 h) but returned to baseline levels by the end of the synovitis induction (Figure 1). Notably, the incorporation of an effective viscosity into the Stribeck framework revealed compelling changes to the modes of lubrication. Synovitis SF at the peak of inflammation exhibited a shift to the boundary mode of lubrication while control SF was predominantly in the mixed and minimum friction modes (Figure 2). Compared to other mechanical markers like the coefficient of friction and measured viscosity, the effective viscosity was the most sensitive marker for SF inflammation and exhibited a nearly 10,000-fold decrease at the peak of inflammation, with a return to normal levels at the end of the study period (Figure 3). Collectively, these results indicate that synovitis and the early-stage inflammation that precedes and accompanies osteoarthritis progression can dramatically alter functional outcomes like SF lubrication. Loss of effective SF lubrication is thought to be one of the factors by which synovitis can both precipitate and accelerate the progression of early osteoarthritis to more advanced changes such as cartilage degeneration. This novel effective lubricating viscosity framework provides an extremely valuable tool to characterize the lubricating ability of pathological SF and may be able to guide decision making about candidates for viscosupplementation and tribosupplementation after joint injury or inflammation.

The differences between the measured viscosity and effective viscosity in the SF over the course of synovitis is striking. The measured viscosity of the control samples decreased by half over the time frame of the experiment (Table S1) but the effective viscosity values did not. While it is possible that there is a systemic effect caused from the unilateral treatment with 10 ng/mL of IL-1β, this change in SF viscosity could be attributed to repeated arthrocentesis of the control limb. Other studies that demonstrated

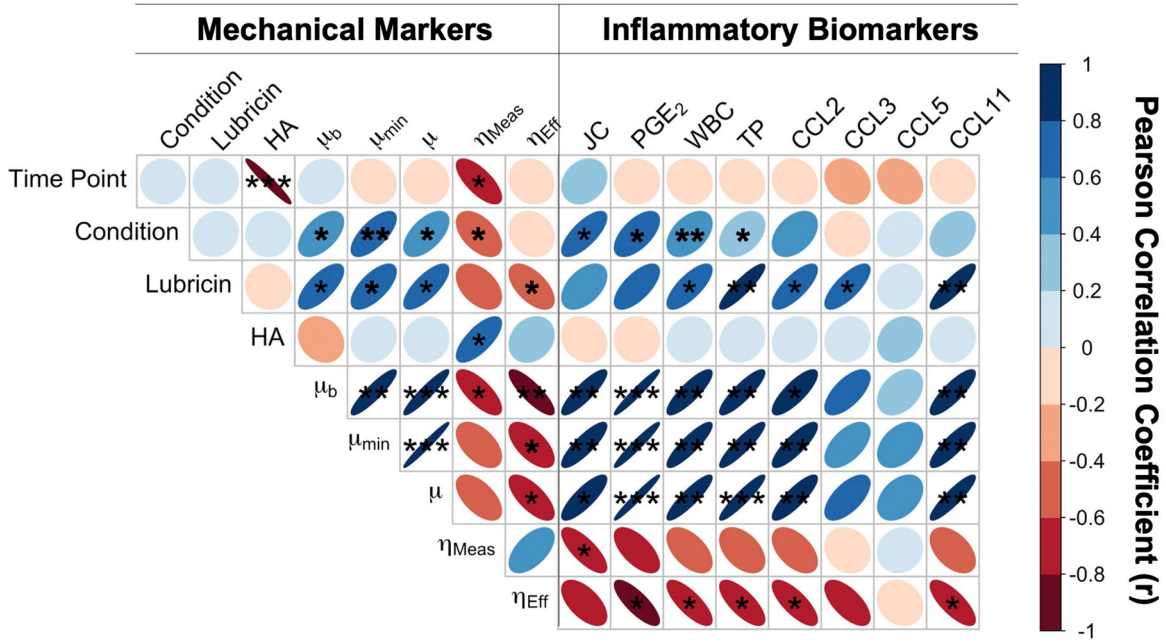

FIGURE 4 Correlation plot of pairwise comparisons between mechanical markers such as the boundary, minimum and speed averaged coefficients of friction (μ_b, μ_{min}, μ), measured viscosity (η_{meas}), effective viscosity (η_{eff}), biochemical content markers like HA and Lubricin, and other inflammatory markers in the SF as a function of time and condition. Dark blue ellipses indicate strong positive correlations and dark red ellipses indicate strong negative correlations. Correlations without an asterisk are not statistically significant ($p > 0.05$).

this same effect performed repeated arthrocentesis in the contralateral joint,[35–37] so it is possible that inflammatory mediators and blood released as a result of the sampling procedure itself caused the most significant effect on the viscosity/biochemical changes associated with the control joint. The remarkable recovery in the effective viscosity of the synovitis SF at 672 h is striking. A critical aspect of efficacious cartilage lubrication is the viscous gel-like layer that forms at the surface of the tissue.[38–41] While HA is an important contributor to this gel like layer, it is possible that there are other factors that can influence this interaction, such as the pH of the SF and the presence of other charged proteins. In the Watkins et al study, while the concentration of HA at 672 h is 50% of baseline HA levels, the concentration of high molecular weight HA in the SF (>6.1 MDa) returns to pre-synovitis levels after a drop between 24 and 72 h, without any significant differences between the synovitis and control groups at 672 h. This suggests that while the concentration of HA is important, the presence of high molecular weight HA can influence the restoration of the effective viscosity in pathological SF.

Conventional measurements of viscosity occur under conditions that do not mimic the surface chemistry of biological tissues such as cartilage. In contrast, the effective viscosity includes interactions between the surface of the cartilage and the components in SF. Previous work examining the rheological properties of protein solutions and bovine SF have revealed that there are complex, time-dependent film thickness changes at the walls of the rheometer.[42,43] These adhesion-mediated changes in film thickness are likely occurring at the surface of the cartilage as well. Consistent with this idea, our previous work has shown that the viscosity of HA is significantly altered in the presence of intact and pulverized bovine articular cartilage and depends on the gap between the articulating surfaces.[26] There may also be an increase in the viscosity at the surface of the cartilage as a result of interactions between macromolecular complexes of HA-lubricin, lubricin-lubricin dimers, and many other protein interactions that can occur at this length scale.[20,38,44–46] Moreover, the outcome of the correlation analysis in this study (Figure 4) suggests that the measured viscosity, regardless of the measurement technique, does not accurately capture the cartilage surface and lubricant interactions during articulation, nor can it fully explain mechanical or biochemical changes in the SF associated with the onset of inflammation. Future studies should evaluate both the cartilage and SF from these animals to fully characterize the equilibrium state of these interactions, and correlate the concentrations of HA and lubricin at the surface of the cartilage and the SF.

While the correlation between inflammatory markers and inferior lubrication is not surprising, the correlation between lubricin concentration in the pathological SF and friction coefficients is unexpected (Figure 4). Despite high levels of lubricin, synovitis SF at 48 and 72 h exhibited the worst lubrication outcomes. Many studies show that structurally intact lubricin may promote the localization of HA at the surface of cartilage through nonspecific physical interactions, boosting the viscosity and lubricating ability of the tissue.[20,44] While the concentration of lubricin in synovitis SF is drastically greater than the EC_{50} for lubrication and could localize HA to the surface of the cartilage, it is possible that this lubricin may be structurally altered. Additionally, the assays used to determine the

concentration of lubricin may not be specific enough to identify these alterations in the molecule. Inflammatory cytokines have been known to increase the presence of neutrophils and can elicit the release of lubricin degradation products and may prevent lubricin from interfacing with the articulating surface.[15,34,47] Lubricin may also preferentially dimerize or bind specifically to cartilage oligomeric matrix protein (COMP), and fibronectin fragments,[48] as well as can be degraded by neutrophil elastase.[15] and cathepsin G, a protease that has been found in the osteoarthritis SF.[47] While not directly measured in this study, these interactions serve to add to the growing list of biomarkers needed to fully characterize mechanical and biological outcomes associated with changes in SF pathology.

This study is not without limitations. While neonatal bovine cartilage may not be structurally representative of adult human, bovine or equine cartilage, it has been previously used in several studies involving healthy, injured and inflamed SF samples.[12,14] 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 in humans.[28] It is also vital to underscore the context of the IL-1β-induced equine synovitis model. While not a proxy for OA, several studies have shown that synovitis is a hallmark of early-stage OA. Additionally, the role of IL-1β as the primary driver of in OA is debated, with several studies suggesting it may or may not be directly implicated in the progression of the disease.[49–55] While there are multiple established methods to induce synovitis in horses, the IL-1β synovitis model is considered to be more appropriate to study the transient changes to SF pathology, owing to the role IL-1β plays in the pathogenesis of the disease. Moreover, the IL-1β synovitis model in this study has been previously used to induce inflammation and study clinical and pathological changes in early-stage OA.[55–58] We underscore the idea that the purpose of using the IL-1β model in this study was to reliably induce transient synovitis and alter SF pathology in the context of understanding changes to the mechanical function of the synovitis SF. While the use of equine SF is a limitation, equine and human articulating joints exhibit similarities in OA pathogenesis, clinical presentation, and anatomy.[59–61] Additionally, the large volume of SF aspirate from horses coupled with the ability to sample multiple times on the acute timescale make the equine system a desirable preclinical model to study transient changes in early-stage OA. Furthermore, healthy control SF is attainable from the contralateral limb in horses whereas obtaining truly normal SF in humans is challenging if not impossible.

In summary, this work demonstrated that synovitis exacerbates the decline in mechanical function of the SF in a temporal manner. The use of the Stribeck framework in conjunction with the novel effective lubricating viscosity revealed dramatic changes in the mode of lubrication of pathological SF. In particular, the effective viscosity of synovitis SF was drastically lower than the measured viscosity and most sensitive to the inflammatory state of the SF. Thus, the effective lubricating viscosity framework provides several unique insights into the complex interplay between the temporal changes in the biomarkers present in pathological SF, and the implications of these changes on mechanical and clinical outcomes. This study along with several others show that traditional measurements of rheology and tribology in the absence of the cartilage or inflamed SF do not fully capture the effects observed inside an osteoarthritic joint. Specific interactions between the various components in the SF and the cartilage are crucial to understanding the relationship between biological changes in the joint and functional mechanical outcomes. Ultimately the design of viscosupplements that can interface better with the surface of the cartilage may vastly improve mechanical and biological outcomes for these therapies.

AUTHOR CONTRIBUTIONS

Karan Vishwanath: Experimental methodology, conducted experiments, data acquisition and analysis, interpretation of results, manuscript preparation. **Erica J. Secor**: Conducted experiments, data acquisition and analysis, reviewed and edited manuscript. **Amanda Watkins**: Study design and edited manuscript. **Heidi L. Reesink**: Study conceptualization and supervision, interpretation of results, manuscript preparation and editing. **Lawrence J. Bonassar**: Study conceptualization and supervision, interpretation of results, manuscript preparation and editing.

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CONFLICTS OF INTEREST STATEMENT

Lawrence J. Bonassar is a co-founder and holds equity in 3DBio Corporation.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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