The Selective Removal of Lubricin's O-Linked Oligosaccharides Inhibits Synovial Fluid Lubrication in a Dose-Dependent Manner

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INTRODUCTION: Articular cartilage in synovial fluid (SF) yields remarkably low coefficients of friction due to the synergistic mechanical and molecular interactions between lubricin (PRG4) and hyaluronic acid (HA) [1,2,3,4]. Lubricin's ability to act as a boundary lubricant has been attributed to its bottlebrush structure with O-linked oligosaccharide side chains providing hydration [5,6]. Nonspecific degradation of the protein component of SF has been shown to greatly inhibit lubricating ability. However, the lack of specificity of enzymes used in these studies makes it difficult to understand the specific molecular mechanisms involved in lubrication [1,2]. Recently, a novel recombinant secreted protease of C1 esterase inhibitor (StcE) was shown to selectively remove O-glycosylated branches in lubricin's mucin domain without altering the core protein [7]. The selective targeting of O-linked oligosaccharides can prove to be a powerful tool in isolating the role glycosylation plays in lubricin function. We hypothesized that StcE treatment of SF will inhibit the lubricating ability of SF and increase coefficients of friction in a dose-dependent manner. The objective of this study is to understand the extent to which StcE treatment affects the lubricating ability of SF and to compare its effect with that of trypsin, a widely used non-specific enzyme. Furthermore, a Stribeck-like framework modeled the effect of different articulation speeds on lubricating ability via a cartilage-on-glass tribometer system.

METHODS: A 13.7 mg/mL StcE solution was serially diluted to obtain concentrations ranging from 0.32 ng/mL to 10 µg/mL of StcE in bovine synovial fluid (BSF) [7]. Trypsin-treated SF was prepared by digesting BSF with 50 µg/mL TPCK trypsin from bovine pancreas for two hours at 37 deg C [1]. Enzyme-free BSF and phosphate-buffered saline (PBS) were selected as control groups [5]. All treated BSF samples were incubated for 2 hours at 37°C, frozen at -20°C to stop enzyme activity, and thawed for 15 minutes prior to friction testing. Friction analysis of SF treatment groups was performed on a previously described custom tribometer [5, 6, 7]. Briefly, cartilage explants were compressed to a 30% strain, depressurized for 1 hour, and linearly reciprocated in the SF at preset sliding speeds ranging between 0.1 to 10 mm/s. For each speed, coefficients of friction were calculated as the shear-to-normal load ratio. The recorded friction data was fitted to a 4-parameter VSCR Model to validate dose-dependent behavior via logarithmic regression.

RESULTS: Overall, increasing concentrations of StcE increased coefficients of friction. **(Figure 1A)**. Lower concentrations of StcE (under 0.32 ng/mL) had no effect on the lubricating ability of SF, while higher concentrations (more than 10 ng/mL) yielded friction values on par with levels of trypsin-treated BSF **(Figure 1A)**. The minimal effective concentration of StcE was found to be between 1 and 3.2 ng/mL, at which point friction increased suddenly. The maximal effective concentration of StcE in SF was 10 ng/mL, at which point friction values plateaued around an average µ=0.18, consistent with trypsin treatments. StcE exhibited a dose-dependent relationship with friction across all articulation speeds (**p-value<0.01** at n=3 per speed per concentration) **(Figure 1B)**. The Stribeck-like surface revealed a distinct speed-dependent trend: while no speed dependence was observed at higher concentrations of StcE, friction decreased significantly with increasing speed at low concentrations. Speed dependence was greatest at intermediate concentrations of StcE **(Figure 1C)**.

DISCUSSION: The results of the study demonstrate that recombinantly produced StcE impaired SF lubrication in a dose-dependent manner. Notably, maximal effective concentrations of StcE inhibited SF lubrication at similar levels to trypsin **(Figure 1A)**. This is striking because it suggests that the absence of lubricin O-glycosylation is sufficient in completely degrading BSF's lubricating function, further affirming the critical role of O-linked oligosaccharides in lubrication. Additionally, the Stribeck-like surface plot undergoes an elastoviscous transition from boundary mode (high friction) to hydrodynamic mode (low friction) as O-glycosylation increases. This suggests that O-linked oligosaccharide degradation seems to interfere with the formation of the Lubricin-HA gel layer previously attributed to decreasing friction [1]. Particularly, because speed dependence is greatest at the ED50 concentration of StcE, the friction-reducing layer forms only at higher articulation speeds in intermediate doses or at lower doses entirely **(Figure 1C)**. Future studies should include confocal imaging of Lubricin-HA interactions across different articulation speeds at the ED50 dose of StcE. Further, the reintroduction of recombinant lubricin to enzymatically degraded SF may demonstrate its restorative effects on pathological SF.

SIGNIFICANCE: This is the first study that effectively employs a lubricin-targeted enzyme to study friction. The removal of lubricin's O-linked oligosaccharide branches yields an effect on par with total non-specific degradation by trypsin demonstrating that SF without functional O-glycosylated lubricin has inferior lubricating properties and elevated coefficients of friction.

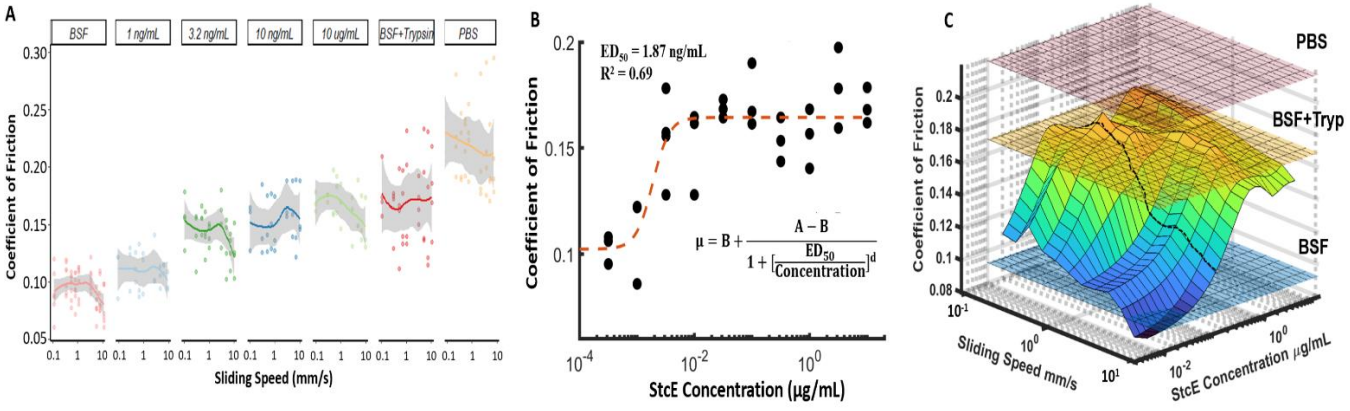

Figure 1: (A) Coefficient of Friction for various doses of StcE across three orders of magnitude of articulation speed (n=3 per conc. per speed). **(B)** Friction Data fitted to a 4-Parameter VSCR Model for 1mm/sec (representative data at all speeds). **(C)** Surface plot of friction as a function of sliding speed and StcE concentration in context of PBS, BSF, and BSF treated with trypsin. Bold contour line depicts the influence of sliding speed on the ED50 concentration.

REFERENCES: [1] Bonnevie et al. *Plos One* (2015); [2] Das et al. *Biomacromoleules* (2013); [3] Jay GD et al. *Mat. Biol* (2014); [4] Gleghorn JG et al. *J. Orthop Res.* (2008); [5] Bayer et al. *Lubricants* (2018); [6] Swann D *Biochem J* (1985); [7] Malaker et al. *Pnas* (2019)