The Role of Lubricant Composition on Cartilage Synergistic Lubrication

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INTRODUCTION: Articular cartilage facilitates phenomenally low friction coefficients *in vivo* (μ <0.005).[1] Only recently have benchtop studies replicated cartilage's unmatched *in vivo* lubricity, enabled by redeployment of the convergent stationary contact area (cSCA) testing configuration.[2,3] In the cSCA, and in the presence of PBS, sliding promotes speed-dependent, hydrodynamically-mediated recovery and long-term maintenance of high fluid load support (FLS), interstitial lubrication (ISL), and low friction values (μ~0.03), an observation we've termed 'tribological rehydration'.[2] When synovial fluid (SF) is introduced into high-speed cSCA studies, stable, truly biofidelic equilibrium friction coefficients (*i.e.*, μ<0.004) are generated on the benchtop.[3] We've termed this frictional benefit, accompanying the presence of SF and hydrodynamically driven recovery of ISL—by tribological rehydration, 'synergistic lubrication'.

The unanticipated discovery of SF-mediated 'synergistic lubrication' in rapidly articulated cSCA contacts raises two critical questions: 1) what is the mechanism underlying synergistic lubrication in cartilage? And 2) what aspect(s) of SF mediate this synergy? SF is composed of numerous macromolecules thought to influence cartilage lubrication in ways ranging from viscous to boundary lubrication.[3-9] One such molecule is hyaluronic acid (HA), which is a non-sulfated, high molecular weight glycosaminoglycan (typically >7MDa) found in SF at concentrations of ~3mg/mL.[5]

HA contributes to SF's viscosity[5-7] and has been traditionally thought to provide boundary[5,8,9] lubricating activities in articular cartilage, and thus has seen use as the primary component of viscosupplementation strategies.[4] However, the role that HA plays in promoting/regulating synergistic lubrication—which cSCA studies hint at—requires greater study. Here, we explored friction mitigating and synergistic lubrication interactions among hydrodynamics and ISL recovery (titrated through sliding speed-dependent behaviors), lubricant presence, and bath viscosity (mediated through the presence of HA or polyethylene glycol [PEG] of varying molecular weight [MW]) in benchtop cSCA explant tribology tests.

METHODS: 19mmΦ osteochondral explants were extracted from mature bovine femoral condyles.[2,3] Explants were rinsed and stored in 1X PBS + sucrose (added to approximate the osmolarity of synovial fluid, 400mOsm[10]; hereon referred to as PBS). Studies were performed to examine the effects of 1) HA and 2) PEG MW on cSCA explant lubrication in a custom reciprocating tribometer.[2] All explants were preconditioned by static compression against a glass counterface for 30min at 9N, followed by a load reduction to 5N for 15min, then sliding at 10, 80, and 10mm/s for 15mins each (under 5N).

In the first study, after preconditioning, sliding "speed sweep" tests (80 – 1mm/s) were conducted (at 5N) to investigate the effect of HA MW on speed-dependent friction coefficients. 3mg/mL HA dissolved in PBS was tested at 0, ~30k, ~375k, and ~1.4MDa MW. In the second study, "speed sweep" tests were conducted for 3mg/mL PEG dissolved in PBS at 0, 8k, 400k, 1M, and 4MDa MW. For all tests, sliding speeds were only changed when deformation and friction rates achieved equilibria (defined as <1um/min & <0.01μ/min [for μ>0.1] or <0.001μ/min [for μ<0.1]). Equilibrium compression magnitudes and kinetic friction coefficients were recorded across all cycles for each sliding speed and lubricant combination using LABVIEW and then analyzed in MATLAB.

RESULTS: In a manner unique to the cSCA configuration, the lowest observed μ_{Eq} always occurred at 80mm/s sliding speeds, regardless of HA/PEG presence (at 3mg/mL) or MW (**Fig 1A**). Both max and minimum μ_{Eq} were suppressed with increasing HA MW, with the lubrication benefit associated with high-speed sliding (80mm/s) increasing from 9.2-fold in PBS to 43.8-fold in ~1.4MDa HA in the cSCA (relative to max μ_{Eq}) (**Fig 1B**). The max μ_{Eq} always occurred at our slowest sliding speeds (1mm/s) (**Fig 1B**).

Intriguingly, similar μ_{Eq} trends were observed for the synthetic polymer PEG (at 3mg/mL). At 80mm/s, where minimum μ_{Eq} was always observed, the lubrication benefit increased from 8.8-fold in PBS to 22.3-fold in 4MDa PEG in the cSCA (relative to max μ_{Eq}) (**Fig 1A,C**). At the slowest sliding speeds (1mm/s), μ_{Eq} decreased relative to max μ_{Eq} (observed at 10mm/s), with a frictional reduction of ~1.9-fold found for 4MDa PEG (relative to max μ_{Eq}) (**Fig 1C**).

DISCUSSION: The cSCA testing configuration, in being able to replicate a range of cartilage tribology conditions on the benchtop, represents a novel tool for studying the role of various putative SF or synthetic lubricants on cartilage lubrication.[2] Furthermore, facile titration of lubricant concentration and composition during tests provides a powerful tool to further our mechanistic understanding of the role of SF constituents (like HA) on synergistic lubrication.

In the absence of hydrodynamics and TR (*e.g.*, at slow sliding speeds), both HA and PEG appear to function as modest, classically understood boundary lubricants—ones marginally impacted by MW. Intriguingly, 4MDa PEG appears to behave more similarly to SF than 1.4MDa HA at slow sliding speeds, promoting decreases in friction at slow sliding speeds (*e.g.*, 1mm/s) relative to max friction (*e.g.*, 10mm/s). This might support a MW effect on boundary lubrication. However, as hydrodynamic forces are generated by increasing cSCA sliding speeds (*e.g.*, ≥40mm/s), the effects of HA and PEG on friction evolves dramatically. As sliding-induced TR drives the recovery of interstitial pressure and FLS, both lubricants transition to having powerful synergism with ISL.

Because lubricant viscosity also increases as HA and PEG MW[5-7] increases, the sliding-speed and lubricant-dependent behaviors of the cSCA contact, suggest—indirectly—the renewed possibility of interfacial fluid films mediating cartilage's phenomenal lubrication. Intriguingly, such films would appear to be able to be generated by both biological (HA) and synthetic (PEG) polymeric macromolecules, however, only in conjunction with recovery of interstitial pressure as observed via TR. Considering mass/fluid-balance, a 'porous' cartilage interface could be made 'effectively' impermeable to fluid influx by large interstitial pressures; presenting a possible solution to the nagging paradox of how a 'porous' cartilage surface could support fluid film-based lubrication.[4,11,12] Studies characterizing the effect of lubricant viscosity, in the presence and absence of HA and PEG, are continuing to explore the role of viscosity in articular cartilage (synergistic) lubrication—and possible fluid film generation.

SIGNIFICANCE: The present study highlights a crucial synergy between interstitial hydration recovery and HA/PEG-presence in regulating *in vivo*-like cartilage lubrication behaviors under biofidelic conditions of high FLS and high sliding speeds. Rather than HA being crucial for lubrication, *per se*, it appears that lubricant viscosity plays a critical role in cartilage's synergistic lubrication. Insights from tribological rehydration and synergistic lubrication will continue to advance our understanding of cartilage's unmatched *in vivo* lubricity and its function in both health and disease.

REFERENCES: [1] Linn, *J Biomech*, 1968; [2] Moore, *OA&C*, 2017; [3] Farnham, *Trib Let*, 2021; [4] Bonnevie, *J Biomech Eng*, 2020; [5] Fam, *Biorheol*, 2007; [6] Krause, *Biomacromol*, 2001; [7] Miyazaki, *J Appl Poly Sci*, 1998; [8] Ogsten, *J Physiol*, 1953; [9] Bonnevie, *PloS One*, 2015; [10] Baumgarten, *J Bo Join Surg*, 1985; [11] McCutchen, *Wear*, 1961; [12] Dowson, *Proc Inst Mech Engrs*, 1967.

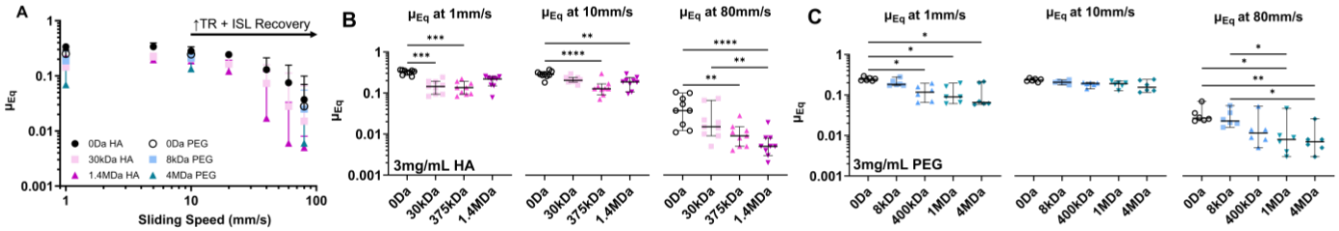

Figure 1: (A) Equilibrium friction vs sliding speed curves for cartilage in PBS (black), 2 HA MW (pinks), and 2 PEG MW (blues). (B-C) Equilibrium friction coefficients at increasing MW of HA (B) and PEG (C) at 1, 10, and 80mm/s. (*p<0.05, **<0.01, ***<0.001, ****<0.0001, Friedman Test).