

Articular Cartilage Lubrication by HYADD4 Reduces Tissue Strains, Chondrocyte Death, and Apoptosis

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Introduction: Viscosupplementation with hyaluronic acid (HA) has been used for decades to treat patients with mild to moderate osteoarthritis (OA). HA functions to increase the viscosity of synovial fluid and improve articular cartilage lubrication. Several studies also have reported positive biological consequences of HA administration including anti-inflammatory, analgesic, and chondroprotective effects¹. However, it remains unclear how the lubricating properties of HA mediate biological changes in cartilage tissue. Recently, our lab has developed methods to measure cellular changes in response to cyclic sliding motion². The objective of this study was to examine the relationship between the lubricating properties of HYADD4, an HA viscosupplement, and chondrocyte health after sliding articular cartilage in HYADD4.

Methods: Cartilage was explanted from the femoral condyles of 7 neonatal bovids. Samples were articulated against glass under 15% normal strain for 30 cycles at 1 mm/s for 1 hour while bathed in either HYADD4 or PBS as friction coefficients were measured as described previously². Samples were subsequently cultured for 24 hours in DMEM, and then bisected and stained with calcein AM and ethidium homodimer (for live and dead cells, respectively) or Caspase 3/7 to detect apoptotic cells. Images were collected on a confocal microscope, and cells were quantified as a function of depth from the cartilage surface using MATLAB. In parallel, cartilage samples were bisected, fluorescently stained, and mounted onto a tissue deformation imaging stage³. The sample was bathed in either PBS or HYADD4, and the tissue surface was articulated against glass at speeds ranging from 0.1 to 1 mm/s. Depth-dependent shear deformations were tracked by analyzing displacements of photobleached lines oriented perpendicular to the articular surface throughout the depth of the sample. The local shear strains were calculated as previously described⁴. A two-way ANOVA and Tukey HSD was used for comparing strain between lubricants, and Pearson's correlation coefficient was calculated to describe the relationship between strain and cell death.

Results: The coefficient of friction for PBS-lubricated samples was 0.36 ± 0.001 while HYADD4-lubricated samples showed a seven-fold decrease in friction coefficient at 0.05 ± 0.001 . For PBS-lubricated cartilage, shear strain reached a maximum of $53.7 \pm 6.0\%$ at the tissue surface, while shear strains in HYADD4-lubricated cartilage reached $17.5 \pm 2.2\%$ at the surface (Fig. 1A). Over a range of sliding speeds, PBS-lubricated samples showed significantly higher surface strains compared to HYADD4-lubricated samples for all speeds (Fig. 1B, $p < 0.001$). HYADD4-lubricated cartilage showed lower cell death and fewer caspase-activated cells throughout the depth of the tissue compared to PBS-lubricated samples (Fig. 1DEFG). The strain resulting from lubrication by PBS or HYADD4 showed a strong positive correlation with cell death ($\rho = 0.77, p < 0.001$, Fig. 1C).

Discussion: This study demonstrated that HYADD4 lowers friction, tissue surface strains, chondrocyte death, and apoptosis in cartilage explants that experienced sliding at 1 mm/s for 1 hour. Notably, the coefficient of friction for cartilage lubricated in PBS was seven times greater than that lubricated with HYADD4, while the maximum shear strains at the surface of cartilage lubricated in PBS were only three times greater than HYADD4-lubricated cartilage. Such differences are consistent with the known non-linearity and heterogeneity of shear properties at the cartilage surface^{3,4} and may also suggest that friction coefficients may not be constant throughout the sliding cycle. Cell death was sustained for 24 hours after 30 cycles of sliding in either lubricant, with PBS showing higher cell death compared to HYADD4 and controls. Both PBS and HYADD4 groups showed a positive correlation between cell death and strain, demonstrating that the local mechanics experienced by the tissue are related to the cellular response. Both lubricants produced local shear strains of less than 1% strain 400 μ m from the surface, and similarly both lubricants exhibited comparably low levels of cell death (< 7%) at this depth. These results are qualitatively similar to those reported previously for a comparison between lubrication by PBS and synovial fluid². In both studies effective lubrication by synovial fluid or HYADD4 lower strains at the tissue surface and prevented cell death and apoptosis. As such, the current study is consistent with the idea that hyaluronic acid therapies for arthritis may act in part through a mechanical pathway that preserves viability of chondrocytes at the cartilage surface.

Significance: This work describes a mechanobiological effect of lubrication by HA, whereby decreasing friction lowers strains at the cartilage surface, which in turn prevents cell death and apoptosis.

References: 1. Strauss *Am. J Sports Med* 2009 2. Bonnevie *ORS Annual Meeting* 2017 3. Buckley *J. Biomech* 2008 4. Buckley *J. Biomech* 2010

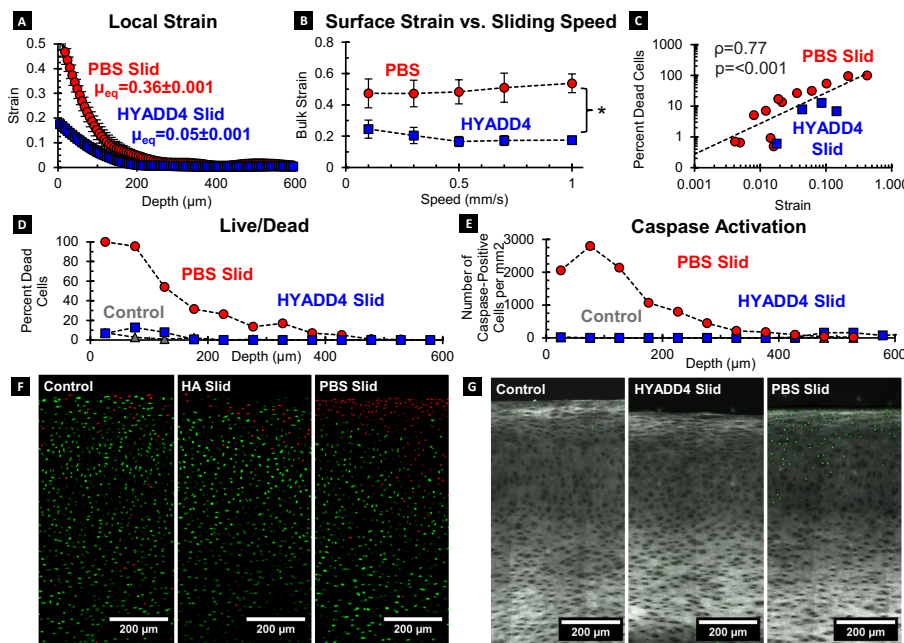


Figure 1: (A) Local strains as a function of depth are lower in HYADD4, and (B) surface strains are significantly lower in HYADD4 across different sliding speeds compared to PBS (n=4-5). Cell death (D,F) and caspase activation (E,G) was lower in HYADD4-lubricated samples and similar to control tissue. Cell death and tissue strains are significantly correlated (C).