

Improved Lubrication of Natural Tissues by Hyaluronic Acid (HA) Penetration

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Statement of Purpose: Hyaluronic Acid (HA) is usually considered to be the main natural lubricant for articulating joints. Yet, frictional testing of HA or HA-rich synovial fluid generally has not yielded a very low coefficient of friction in laboratory trials. Similarly, synthetic eye drops giving excellent clinical performance did not correlate with laboratory data from standard friction tests. This challenge was met with a tissue-on-tissue bench testing regimen that matched clinical results (Meyer et al. 2007). Reliability of the in-vitro protocol greatly improved by using standardized pericardium tissue, allowing testing to extend to oral lubricants such as natural saliva and ingredients utilized in commercial products claiming relief of dry mouth. The work reported here set out to determine the underlying mechanism for results specifically demonstrating long-term improvements in tissue lubrication with HA in desiccating environments.

Methods: Commercially available glutaraldehyde-tanned bovine pericardium (Peri-Guard®, Synovis Surgical Innovations, St. Paul, MN, USA) was used as the tissue model. Tissues stored at room temperature in their packaged storage solution were soaked in and rinsed with physiologic saline to remove the storage solution prior to use. Three groups of tissue segments were used for subsequent treatment and testing: [1] control tissue (physiologic saline group), [2] tissue soaked 7 d in 35X supersaturated mineralization solution, and [3] tissue soaked 7 d in 25% glutaraldehyde solution. After soaking, all tissues were rinsed thoroughly in fresh saline and then used in tissue-on-tissue friction tests with HA solutions and comparison solutions. HA (Sigma Product H1751) solutions were prepared from five separate aqueous solvents: physiologic saline, distilled water, 0.15M CaCl₂, physiologic electrolyte, and 6 M urea. Control of solution pH was confirmed by measurement. Differential interference contrast light microscopy was used to image the surfaces of the tissues after the friction experiments. Surface textures of non-articulated control tissues and articulated experimental tissues were determined with a stylus profilometer within a Class 100 clean room. Readings were taken in the “test track” of the tissue, first parallel to the sliding motion, and then perpendicular to the sliding motion. Scanning electron microscopy was also used for resolving each sample’s topographical features at high magnifications. With IACUC approval, fresh porcine pericardium was retrieved, preserved in 4% paraformaldehyde, and used for confirming friction experiments with 1:10 mixtures of unlabeled:labeled HA in aqueous solution. These tissue samples were inspected by confocal fluorescence microscopy.

Results: The duration of tissue lubricity of saliva and commercial oral lubricants was just many minutes, while a subset of HA solutions retained minimum frictional values beyond 8 hours in physiologic saline, calcium chloride, and distilled water solutions (Figure 1); a wide range of minimum friction values was observed for the

HA in 6M urea. Chemically modifying the tissues with 25% glutaraldehyde or mineralization solution diminished the maximum achievable lubricity with HA. These solutions had made the tissue less compliant. After articulating the tissues with 0.5% HA solutions, differential interference contrast light microscopy showed a superficial smooth layer in the test tracks, even after 4 hours of continuous articulation with no added hydration. These results are most consistent with elasto-hydrodynamic lubrication, where the sliding materials deform under pressure (Moghani et al. 2007). In contrast, after articulating tissues with 0.5% concentrations of other tested polymers (xanthan gums, hydroxypropyl guar), the very viscous formulations were seen to have been “plowed” or displaced from the tissue surfaces to form a non-lubricating ridge around the tissue friction track. Surface profilometry, confirmed by SEM results, showed statistically lower roughness values for the no-friction tissues and HA-lubricated articulated tissues (2.1 micrometers), in comparison with hydroxypropyl guar-lubricated (2.8 micrometers) and physiologic saline-lubricated articulated tissues (3.7 micrometers). SEM analysis showed that the physiologic saline-lubricated tissues were damaged, as severed collagen fibers and holes were apparent. Confocal imagery demonstrated that HA entered the tissue to a depth exceeding 20 micrometers. Subsequent experimentation showed that the imbibed HA was removed from the tissue by continued tissue-on-tissue friction using a series of HA-free saline aliquots as the lubricant.

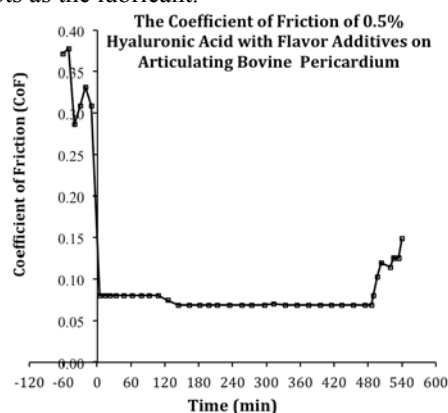


Figure 1. Tissue-on-Tissue Friction Results of HA Solution, Persistence of Lubricity Exceeds 8 Hours

Conclusions: It is concluded from these studies that a new modality of lubrication of collagenous tissues has been recognized: reversible penetration of fibrotic connective tissues by Nature’s lubricant, hyaluronic acid.

References: Meyer AE et al. *J Biomed Mater Res* **82B**:74-87, 2007. Moghani T et al. *Computers and Structures* **85**:1114-1120, 2007.

Disclosure: The authors filed a New Technology Disclosure on these findings in 2008. The University at Buffalo has since licensed the technology to YouFirst Services, Inc.