**Heterogenous distribution of viscosupplements in vivo is correlated to ex vivo frictional properties of equine cartilage**

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**INTRODUCTION:** Intra-articular injections of hyaluronic acid (HA), commonly known as viscosupplementation, are the cornerstone of arthritis treatments.¹⁻² Viscousellmentation hinges on the idea that restoration of viscosity of pathological synovial fluid (SF) improves its lubricating ability. However, due to HA’s short clearance time in the joint (16-48 hours) and the wide range of commercially available HA formulations, the efficacy and mechanism of action of HA viscosupplementation is debated, with several clinical societies offering varying recommendations for its use.³⁻⁴ Unlike HA, synthetic viscosupplements like polyacrylamide (pAAm) can be chemically modified to resist enzymatic degradation,⁵ effectively lubricate cartilage,⁶ and substantially lower lameness in horses.⁷ While the distribution of HA inside cadaveric joints has been assessed qualitatively⁸, the ability of synthetic viscosupplements to interface with cartilage in a live articulating joint is complex, unclear, and vastly understudied. Understanding the quantitative distribution of these injections within a joint would provide vital insight into the potential mechanism of action and could inform the design of viscosupplements that preferentially localize to damaged articulating surfaces in a joint. The goal of this study was to measure the distribution of a fluorescently labeled pAAm hydrogel 48 hours after being injected into a live equine fetlock joint and determine whether preferential localization of pAAm on the cartilage surface could influence the tribological properties of the tissue.

**METHODS:** **Study Design:** Following IACUC approval, 2.5 mL of a Cy5.5-NHS ester labeled polyacrylamide (pAAm-Cy5.5), a control unlabeled polyacrylamide (pAAm), and a dye only injection (Cy5.5) were administered laterally into the fetlock joints of four adult horses 48 hours prior to sacrifice. Intact fetlocks were dissected after 24 hours to image the cartilage and surrounding soft tissue regions lining the cannon bone (MCII), the short patern bone (P1), and the posterior synovial membrane of the PSB. **Fluorescence imaging:** All fetlock regions were imaged using the IVIS Spectrum to determine the planar fluorescence distribution as a proxy for surface coverage, measured as fluorescent intensity of each treatment group on the cartilage and soft tissue regions with the fluorescence filter pair for Cy5.5. All tissue sections were imaged beside the respective control section to remove tissue autofluorescence. **Tissue Harvest:** After IVIS imaging, osteochondral cores (6 mm diameter) were extracted from the cartilage regions lining the MCII-Ch, P1 and PSB bones and frozen at -20°C prior to tribological evaluation. **Tribology:** Tribological characterization was performed using a previously described⁹ custom cartilage-on-glass tribometer. Briefly, osteochondral cores from thawed in PBS with protease inhibitors prior to friction tests. Samples were glued to brass posts (6 mm diameter), mated against a polished glass surface and first lubricated in PBS followed by bovine synovial fluid (BSF). The cores were then compressed to 30% axial strain and reciprocated at sliding speeds ranging from 1.1-10 mm/s using a DC motor. These strains and sliding speeds were selected due to the strong correlation between measured friction data and clinical outcomes.¹⁰ **Statistics:** A linear mixed effects regression model was used to fit log transformed fluorescent intensity as a function of the treatment and region. Random effects in the model included the fetlock and limb, nested within each horse. Post-hoc pairwise comparisons (Student’s t-test) were conducted to assess the effect of lubricant treatment on coefficient of friction between all regions. Pearson correlation analysis was used to assess the relationship between the measured fluorescent radiant efficiency and friction of the cores in BSF (at 0.1 mm/s).

**RESULTS:** The pAAm-Cy5.5 conjugate was present on all 4 sections of each fetlock and had the highest area normalized fluorescent intensity of all the treatment groups (Fig. 1B). Notably, the viscosupplement was not uniformly distributed on all regions of the fetlock. Fluorescence of pAAm-Cy5.5 was consistently 3- to 5-fold higher than the jointed control and vehicle control group in each region (Fig. 1B, p ≤ 0.05). The percent localization of the pAAm-Cy5.5 was in the PSB region, approximately 4-fold higher than the MCII-Ch and P1 regions. Additionally, pAAm and pAAm-Cy5.5 treated fetlocks had a significantly lower coefficient of friction in BSF than the uninjected control and Cy5.5 treated fetlocks (Fig. 1C, p = 0.018, n=3-2 per region, per treatment). Across all regions of the fetlock, reduction in friction was modest but significantly negatively correlated with the presence of pAAm-Cy5.5, as indicated by fluorescent intensity (Fig. 1D, R = -0.61, p < 0.05).

**DISCUSSION:** This study showed that synthetic viscosupplements like pAAm are not evenly distributed within the fetlock joint, even 48 hours after injection. Notably, fetlocks treated with pAAm had lower coefficients of friction than control fetlocks, supporting the idea that viscosupplements that have an affinity to the cartilage surface can improve its frictional properties. Additionally, the correlation between friction and radiant efficiency indicates that the amount of lubricant on the cartilage can also improve its frictional behavior, which may have beneficial clinical outcomes. Consistent with previous studies, a greater proportion of pAAm was present in surrounding soft tissues and synovium.¹² The conjugate was between the PSB and P1 bones. This asymmetry in distribution may be attributed to (1) the limited space in the fetlock, particularly at the narrow MCIII/P1 interface, (2) the high viscosity of the pAAm gel, and (3) physical or chemical interactions between the pAAm and the cartilage surface.

**SIGNIFICANCE:** Viscosupplements are not evenly distributed in joints even days after administration, and this uneven distribution has consequences for therapeutic efficacy. This study is the first to establish a method to quantify in vivo distribution inside a live large animal joint.