Characterization of Cartilage Boundary Lubricant Composition and Function of Ovine Synovial Fluid Following Knee Surgery

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INTRODUCTION:
Osteoarthritis (OA) is a degenerative joint disease involving the breakdown of articular cartilage1, which is common after injury or with aging2. Cartilage lubrication is a vital mechanism for the protection and maintenance of joints3. Proteoglycan 4 (PRG4), a glycoprotein present in synovial fluid (SF) that is synthesized and secreted by cells that line the joint4, contributes to the boundary lubrication of cartilage and maintenance of the joint5. PRG4 also acts synergistically with hyaluronan (HA), another molecule present in SF, as cartilage boundary lubricants6. Acute injury to the anterior cruciate ligament (ACL) shows a decrease in PRG4 and HA concentration in human SF and ovine SF (oSF), respectively, and a decrease in cartilage lubricating ability in human SF6. These alterations in SF lubricant composition and function have been shown to return to normal within a year6. In an ovine knee injury model7, altered gait mechanics and degradation of the cartilage has been observed 20 weeks post surgery; however, potential alterations in SF lubricant composition and function remain to be determined. Therefore, the objective of this study was to determine changes in 1) HA and PRG4 composition and 2) cartilage lubricating ability, of oSF from surgical sham (SHAM), ACL/medial collateral ligament (MCL) transection, and meniscectomy (MEN) 20 weeks post operatively.

METHODS: oSF Samples. In previous studies8,9,10, 15 mature 3-year-old female Suffolk-cross sheep were subject to one of three surgical procedures: SHAM (n=5), ACL/MCL transection (n=6), or MEN (n=4), with the contralateral left joint serving as the non-operative control (CTRL). Biochemical Characterization. PRG4 Concentration. A sandwich ELISA11 was used to measure, in triplicate, PRG4 concentration in oSF, using anti-epitope capture antibody LPN and detection antibody peanut agglutinin lectin (PNA); oSF samples were digested with neuraminidase (Prozyme) and S. Hyaluronidase (Seikagaku). PRG4 prepared from bovine cartilage discs, purified via anion exchange chromatography and size exclusion chromatography, served as a standard12. HA Concentration. An ELISA kit (R&D Systems) was used to measure, in triplicate, HA concentration in oSF13. HA MW Distribution. Agarose gel electrophoresis followed by densitometric analysis was used to quantify HA MW distribution for oSF, as described previously14. Cartilage Lubricating Ability. Cartilage lubricating ability of oSF was assessed using normal bovine osteochondral cores with a previously described in vitro cartilage-cartilage friction test under boundary lubrication conditions15. Briefly, cartilage surfaces were compressed to 18%, allowed to stress relax, then articulated against each other at an effective velocity of 0.3 mm/s with pre-sliding durations (Tps) of 1200, 120, 12, and 1.2 s. Static (μStatic) and kinetic (μKinetic) friction coefficients were calculated. The following sequential test sequence was used (n=4 for each oSF surgical group): phosphate buffered saline (PBS), operated oSF, non-operative (CTRL) oSF, bovine SF (bSF). Statistical Analysis. Data are presented as mean±SEM. T-tests were used to assess changes in PRG4 and HA composition and MAY MW distribution within each surgical ovine model. ANOVA was used to determine the effects of lubricant and Tps, as a repeated factor, on μKinetic and μKinetic, with Tukey post-hoc testing on μKinetic, at Tps = 1.2s.

RESULTS: Biochemical Characterization. PRG4 Concentration. PRG4 concentration in operated oSF samples was not statistically different than respective CTRL samples in all groups (SHAM p=0.10, ACL/MCL p=0.20, MEN p=0.27) (Table 1), with values ranging from −404 to −566 μg/mL. HA Concentration. Similarly, HA concentration in operated oSF samples was not statistically different than respective CTRL samples in all groups (SHAM p=0.86, ACL/MCL p=0.69, MEN p=0.90) (Table 1), with values ranging from −0.82 to −1.02 mg/mL. HA MW Distribution. HA MW distribution within operated oSF was also similar to respective CTRL samples in all experimental groups (Fig. 1A), with the majority of the HA being >1.1MDa. Cartilage Lubricating Ability. Lubricants and Tps modulated friction. μKinetic increased with increasing Tps for all lubricants, with an interaction (p<0.001). Values of μKinetic were greatest in PBS, while operated oSF, respective CTRL oSF, and bSF were all similar and lower then PBS. μHyaluronan also varied with lubricant, only slightly with Tps (values at 1.2s were within 15 ± 8% of those at 1200s), with an interaction (all p<0.001). μHyaluronan at Tps=1.2s was significantly higher than all experimental groups, both operated and CTRL oSF, and bSF in all cases (p<0.05) (Fig. 2). μHyaluronan at Tps=1.2s in operated oSF (SHAM=0.045±0.008, Fig. 2A), ACL/MCL=0.045±0.011, Fig. 2B, MEN=0.039±0.004, Fig. 2C) were similar to CTRL oSF (0.040±0.013, 0.034±0.004, 0.041±0.005, respectively, p=0.93-1.0) and bSF (0.031±0.004, 0.035±0.002, 0.037±0.005, respectively, p=0.85-1.0) in all experimental groups. In all cases, CTRL oSF was also not statistically different than bSF (p=0.85-1.0), indicating bovine cartilage is an acceptable substrate for evaluating the cartilage lubricating ability of oSF.

Table 1: HA and PRG4 concentration in SHAM, ACL/MCL transection, and MEN oSF, and respective non-operated (CTRL) oSF.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>SHAM</th>
<th>ACL/MCL</th>
<th>MEN</th>
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<tbody>
<tr>
<td>PRG4 (µg/mL)</td>
<td>0.0625 ± 0.016</td>
<td>0.0812 ± 0.022</td>
<td>0.0812 ± 0.016</td>
</tr>
<tr>
<td>HA (µg/mL)</td>
<td>0.0520 ± 0.005</td>
<td>0.0520 ± 0.005</td>
<td>0.0520 ± 0.005</td>
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Figure 1: HA MW distribution for SHAM (A), ACL/MCL transection (B), and MEN (C) oSF.

Figure 2: Kinetic μKinetic at Tps=1.2s friction coefficients in SHAM (A), ACL/MCL transection (B), and MEN (C) oSF, respective non-operated (CTRL) oSF, and bSF.

DISCUSSION: These data indicate normal HA and PRG4 composition, and cartilage boundary lubricating function, are present in oSF 20 weeks post meniscectomy and ACL/MCL transection. These data are consistent with previous studies demonstrating that while SF lubricant composition can be significantly altered immediately after an acute injury16, levels can return to normal at later stages17, though still potentially at a 'semi-acute' stage. The specific time course of such alterations, if any, remains to be fully elucidated in these models, as PRG4 expression/localization has been shown to be decreased as late as 12 weeks post operatively in a similar ovine meniscectomy model18, which may have potential implications on windows for biotherapeutic intervention following injury, such as PRG4 supplementation19. Furthermore, the relationship between SF lubricant composition-function and cartilage surface integrity at various time points remains to be elucidated as well, such as 2 weeks post operatively, since MEN at 20 weeks has been shown to result in significant cartilage damage compared to CTRL19.

SIGNIFICANCE: This study contributes to the understanding of alterations in lubricant composition and function in SF at the early stages of OA, and may ultimately contribute to biotherapeutic strategies aimed at restoring lubricant function and protecting the joint after acute injury or trauma.


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