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Cartilage boundary lubrication of ovine synovial fluid following anterior cruciate ligament transection: a longitudinal study

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SUMMARY

Objective: To assess ovine synovial fluid (oSF) from different post-injury time points for (1) proteoglycan-4 (PRG4) and hyaluronan (HA) concentration, (2) HA molecular weight (MW) distribution, (3) cartilage boundary lubrication function, and (4) lubricant composition-function relationships. The association between cartilage boundary lubrication and gross cartilage changes after injury was also examined.
Methods: oSF was collected 2, 4, 10, and 20 weeks post anterior cruciate ligament (ACL) transection in five skeletally mature sheep. PRG4 and HA concentrations were measured using sandwich enzyme-linked immunosorbent assay, and HA MW distribution by agarose gel electrophoresis. Cartilage boundary lubrication of oSF was assessed using a cartilage–cartilage friction test. Gross damage to articular cartilage was also quantified at 20 weeks using modified Drez scoring protocol.
Results: Early (2–4 weeks) after ACL injury, PRG4 concentrations were significantly higher ($P = 0.045$, $P = 0.037$), and HA concentrations were substantially lower ($P = 0.005$, $P = 0.005$) compared to 20 weeks. The HA MW distribution also shifted towards lower ranges in the early post-injury stage. The kinetic friction coefficients were significantly higher 2–4 weeks post injury ($P = 0.008$ and $P = 0.049$) compared to 20 weeks. Poor cartilage boundary lubricating ability early after injury was associated with cartilage damage at 20 weeks.
Conclusion: Altered composition and diminished boundary lubrication of oSF early after ACL transection may pre-dispose the articular cartilage to degenerative changes and initiate osteoarthritis (OA). These observations also provide potential motivation for biotherapeutic interventions at earlier time points post injury.

Introduction

Articular cartilage is the lubricious, low-friction, wear resistant, load bearing tissue at the end of long bones in synovial joints[1]. Severe injuries to the knee joint such as anterior cruciate ligament (ACL) rupture may lead to failure of articular cartilage homeostasis and increased risk of osteoarthritis (OA) development in the long term[2,3].

There are a number of biophysical mechanisms that contribute to the lubrication of articulating cartilage surfaces and maintenance of cartilage homeostasis, mainly classified as fluid film and boundary lubrication modes[4,5]. In the fluid film mechanism, the interstitial fluid within cartilage becomes pressurized, or forced between articular surface asperities, and contributes substantially to the bearing of normal load with little resistance to shear force, leading to a considerably low friction coefficient[4]. On the other hand, in the boundary mode of lubrication, load is supported by surface-to-surface contact, and the associated frictional properties are determined by lubricant surface molecules[4]. This mode of lubrication has been suggested to be vital for the protection and maintenance of articular surfaces since the opposing cartilage layers make contact over ~10% of the total area, and this may be where most of the friction occurs[4,5].

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Synovial fluid (SF) has been demonstrated to function as an effective friction-lowering boundary lubricant for opposing articular cartilage surfaces *in vitro*[6,7]. There are two primary macromolecules that contribute significantly to the boundary lubricating ability of SF: proteoglycan-4 (PRG4), also known as lubricin[8] or superficial zone protein (SZP)[9], and hyaluronan (HA). These molecules are secreted by chondrocytes in the superficial layer of articular cartilage[10], synoviocytes in synovium[11] or cells within menisci[12], and mediate boundary lubrication of articular cartilage by adsorbing to the articular surfaces and reducing the interaction of opposing tissues[4,13].

Boundary lubricating ability of SF may be altered in joint disease or acute joint injury, potentially due to alterations in PRG4 and HA concentrations[8,14,15]. Poor lubrication function of human SF has been reported following inflammatory and traumatic insults including rheumatoid arthritis[16] and post-traumatic knee effusions[17]. Furthermore, animal models of meniscectomy[18], posterior cruciate ligament and ACL disruption[19], and unilateral ACL transection[20] have all indicated altered compositions of PRG4 or HA acutely post injury, associated with articular cartilage damage. Collectively, these studies suggest that normal HA and PRG4 composition is essential for SF lubricating function and normal cartilage homeostasis[4,5].

A recent study in an ovine injury model suggested that ovine SF (oSF) boundary lubricant composition and function 20 weeks following joint injury were similar to normal levels[21]. PRG4 concentrations at 20 weeks in sham, ACL/MCL (Medial Collateral Ligament) transection, and meniscectomy oSF (131 ± 26, 225 ± 7, 244 ± 62 μg/ml, respectively) were not significantly different from their respective contralateral controls (193 ± 49, 296 ± 58, 173 ± 54 μg/ml, respectively). Similarly, HA concentrations (0.82 ± 0.11, 0.83 ± 0.29, 1.02 ± 0.08 mg/ml, respectively) were not significantly different from the respective contralateral controls (0.86 ± 0.18, 0.95 ± 0.05, 0.97 ± 0.09 mg/ml, respectively)[21]. While oSF composition and function at 20 weeks post injury were restored to normal ranges, significant cartilage damage was demonstrated by the ACL/MCL transection and meniscectomy sheep compared to the shams[21], suggesting that any potential changes in PRG4 and HA concentration of oSF following injury might have occurred at earlier time points in these ovine models and warrant further investigation.

The time course of alteration of lubricant composition and function following joint injury has not been investigated in a large animal model, where gross joint changes could also be evaluated. Thus, the main objective of the present study was to evaluate composition-function alterations in the oSF over time following ACL injury. We also aimed to examine the association between cartilage boundary lubrication changes after joint injury and gross cartilage changes in the longer term. The governing hypothesis was that oSF would exhibit diminished boundary lubricating ability short term (2–4 weeks) post ACL transection, associated with gross cartilage damage at 20 weeks. This objective was tested by the following specific aims: determine (1) PRG4 and HA composition of oSF using enzyme-linked immunosorbent assay (ELISA), (2) HA molecular weight (MW) distribution of oSF using agarose gel electrophoresis, (3) cartilage lubricating ability using a cartilage–cartilage *in vitro* boundary mode friction test of post injury oSF at 2, 4, and 20 weeks, and (4) oSF composition-function relationships. The association between cartilage boundary lubrication and gross cartilage changes post injury was also examined.

Materials and methods

Sample preparation

Five skeletally mature (3-year-old) female Suffolk-cross sheep underwent arthroscopic unilateral ACL transection in their right hind stifle joint. oSF samples were aspirated from the operated joint of each subject early post injury (2 and 4 weeks), as well as medium term (10 weeks) and long term (20 weeks) following ACL transection. Prior to SF collection at each time point, the operated limbs underwent 20 cycles of passive flexion-extension, to provide a consistent procedure for SF aspirations. oSF samples were clarified by centrifugation (3000g for 30 min at 4°C) prior to storage at −80°C with protease inhibitors (PIs) and without PIs for HA MW analysis[22]. The total volume of oSF aspirated at 2, 4, and 20 weeks post injury for each sheep was approximately 1.5 ml, where 90 μl was used for PRG4 ELISA, 1 μl for HA ELISA, 35 μl for HA MW assessments, 1.2 ml for cartilage boundary lubrication tests, and the remaining portion for any further analyses. The amount of SF that could be collected from the joint at the 10 week time point for each sheep was not sufficient (400–600 μl) to allow both the SF composition and the cartilage boundary lubrication tests. Little or no SF could be collected from the pre-injury state or from the contralateral limbs, due to practical difficulties. All procedures were reviewed and approved by the University of Calgary Animal Care Committee and comply with Canadian Council on Animal Care guidelines.

PRG4 composition

PRG4 concentration in oSF samples was measured using a previously described custom sandwich ELISA[22], with anti-PRG4 antibody LPN (Lubricin Polyclonal) (recognizing the C-terminal of PRG4[23]) for capture and peanut agglutinin lectin horse radish peroxidase (PNA-HRP; Sigma–Aldrich, St. Louis, MO) for detection.

HA composition

A commercially available sandwich ELISA (R&D Systems, Minneapolis, MN) was used to measure HA concentration in oSF[22]. The diluted oSF samples (1:40,000 in 5% Tween 20 in PBS (Phosphate Buffered Saline)) were assessed in triplicate, and a standard HA control curve was used for calculation of HA concentrations[22].

HA MW distribution

The MW distribution of HA in oSF was quantified in duplicate using agarose gel electrophoresis[1]. The oSF samples, stored without PIs and treated with proteinase K, were diluted to 0.5 mg/ml and Hi-Ladder (0.5–1.5 MDa) and Mega-Ladder (1.5–6.1 MDa; Oklahoma City, OK) MW markers were used as HA MW standards. One blank lane was left between samples for background measurement. After electrophoresis for 3 h at 50 V in a horizontal gel apparatus (Bio-Rad, Mississauga, ON), the gels were stained with 0.005% Stains-All in 50% ethanol and de-stained in 10% ethanol. The migration of HA was evaluated by densitometric analysis with Image J (NIH, Bethesda, MD)[22].

Cartilage boundary lubricating ability

Cartilage boundary lubricating ability of oSF from different post-injury time points (2, 4, and 20 weeks) was evaluated using normal bovine osteochondral cores with a previously described *in vitro* cartilage–cartilage friction test under the boundary lubrication regimen[4,21]. Briefly, annulus and core-shaped osteochondral samples were prepared from the patellofemoral groove of skeletally mature bovine stifle joints. Samples were nutated vigorously overnight at 4°C in 40 ml of PBS to rid the articular surface of residual SF[22]. Samples were then stored at −80°C in PBS with PIs until the day before testing, when they were defrosted and again nutated vigorously overnight at 4°C in 40 ml of PBS. Samples were bathed in