Arthroscopic Irrigation of the Bovine Stifle Joint Increases Cartilage Surface Friction and Superficial Zone Chondrocyte Apoptosis

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Introduction: Arthroscopic knee surgery is among the most commonly performed orthopedic procedures in the United States (1). While arthroscopic partial meniscectomy addresses acute mechanical symptoms, rates of osteoarthritis (OA) progression following this procedure are high. In one study, 48% of patients had developed radiographic OA at 15 to 22 year follow up (2). The causes of OA progression after this procedure most likely include a combination of direct cartilage damage, altered joint contact mechanics, and inflammatory damage. There are no current treatments known to reduce the risk of degenerative joint disease following arthroscopic partial meniscectomy. One potentially damaging consequence of arthroscopic irrigation that has not been well-studied is the dilution and removal of protective synovial fluid lubricants, specifically the mucinous glycoprotein lubricin and hyaluronic acid. The contribution of inadequate surface lubrication to progressive cartilage damage following arthroscopic irrigation is unknown, but a recent study by Waller et al. using cultured articular cartilage plugs found that mechanically tested cartilage samples lubricated by solutions lacking lubricin demonstrated a greater percentage of apoptotic cells compared to cartilage samples lubricated with either human synovial fluid or purified human synoviocyte lubricin (3). The purpose of this study was to investigate the effects of arthroscopic irrigation on cartilage surface friction and chondrocyte viability using fresh intact bovine stifle joints. The hypotheses of this study were that (1) arthroscopic joint irrigation would increase articular surface friction, and (2) elevated articular surface friction following arthroscopic irrigation would result in increased chondrocyte apoptosis as measured by expression of the apoptosis marker activated caspase-3.

Methods: Fresh bovine stifle joints with the capsule intact were obtained on the day of slaughter from a local abattoir. For the Control specimens (n=4), a capsulotomy was performed and synovial fluid was collected from the joint for use as a lubricant during mechanical testing. For the Arthroscopy specimens (n=4), arthroscopic irrigation was performed as follows: an arthrotomy was made in the superior capsule and an arthroscopy cannula was introduced. The joint was irrigated with 6L of lactated ringer solution at room temperature at a fluid pressure of 55 mm Hg. The irrigant fluid was collected after 3L had been cycled through the joint and saved for use as a lubricant during mechanical testing. For both Control and Arthroscopy knees, paired, full-thickness osteochondral plug bearings of 6 mm and 12 mm diameter were cored out from the anterior, middle, and posterior load-bearing regions of the medial femoral condyle. Static and dynamic coefficients of friction (COF) were measured using a Bose 3230 - AT Series II material testing system (Bose, Framingham, MA). The plugs were kept moist with test lubricant prior to testing and additional lubricant was applied to the cartilage surfaces prior to testing. During friction testing, a 12 N compressive load was applied across the plug surfaces followed by an 8 minute dwell period to allow for stress relaxation. The large plug was then rotated relative to the small plug for 12 rotations of 720° while torque was recorded. Coefficients of friction were calculated as $COF = \tau / ((2/3)*(r)*(load))$, where $r$ = measured radius of the small plug (3,4). Static COF was calculated from the maximal torque measured during the first 20° of rotation and the equilibrium load. Dynamic COF was calculated using the equilibrium load and the average torque measured during the last 720° rotation. Immediately following testing, the 12 mm plugs were immersed in formalin for a minimum of 72 hours prior to decalcification and paraffin embedding for histologic analysis. Sections from the central contact area of each plug were stained for activated caspase-3 using DAPI. Chondrocyte apoptosis was quantified as the percent of nuclei costaining for DAPI and red fluorescence divided by the total number of nuclei at the following depths from the cartilage surface: (A) 0-100 µm; (B) 100-200 µm; and (C) 200-300 µm. Three 10X views were used from each section to obtain an average score for the plug unless artifact prevented the visualization of three separate surface areas. Results for static COF, dynamic COF, and mean percent expression of activated caspase-3 were compared between groups using unpaired, two-tailed t-tests for each depth and surface location.

Results: Compared to Control specimens, the articular cartilage surfaces from the medial femoral condyle of the Arthroscopic specimens demonstrated a significantly elevated Static COF in the Middle condylar region ($p=0.021$) (Figure 1A) and significantly elevated Dynamic COF in the Middle ($p=0.007$) and Posterior condylar regions ($p=0.002$) (Figure 1B). Caspase-3 activation was found to be significantly greater in cartilage sections from the superficial zone (Zone A) in the middle medial femoral condyle of Arthroscopy specimens compared to Controls ($p=0.044$), but not in the deeper layers (Figure 2).

Discussion: The results of this pilot study support our hypotheses that arthroscopic irrigation of the bovine stifle joint results in elevated cartilage surface friction and increased chondrocyte apoptosis. These findings are consistent with results found by Waller et al. for cultured articular plugs lubricated by PBS versus synovial fluid or purified human lubricin. Our results suggest that arthroscopic irrigation compromises articular surface lubrication at the time immediately following surgery. Modifying the
lubricating environment of the joint may provide an opportunity to reduce OA progression following this common procedure. Of note, a recent study using a mouse meniscal injury model found that OA progression was significantly reduced in animals treated with a viral vector to induce lubricin overexpression. It should also be noted that this study used intact joints from otherwise healthy animals, so synovial fluid and articular surface lubricants were likely at physiologic concentrations prior to irrigation and mechanical testing. In the setting of acute joint injury, lubricin levels have been found to be diminished and synovial fluid lubricating ability reduced. Therefore, the vulnerability of chondrocytes to secondary injury post-procedure may be greater clinically than what has been found in this study.

Significance: This is the first study to investigate the consequences of arthroscopy on cartilage surface lubrication and chondrocyte viability. Further investigation is needed to understand cartilage surface mechanics following arthroscopy at post-surgical time points in a living animal model.

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Figure 1: Coefficients of Friction (COF) by region (A) Static and (B) Dynamic.

Figure 2: Chondrocyte apoptosis by region and depth zone.