Journal of Biomechanics 49 (2016) 3106–3110

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

Short communication

# Arthroscopic irrigation of the bovine stifle joint increases cartilage surface friction and decreases superficial zone lubricin

Erin Teeple [a,b], Naga Padmini Karamchedu [f], Katherine M. Larson [c], Ling Zhang [d], Gary J. Badger [e], Braden C. Fleming [c,f], Gregory D. Jay [c,d,*]

[a] Department of Occupational and Environmental Medicine, Harvard School of Public Health, Boston, MA, USA
[b] Department of Orthopedic Surgery, Brigham and Women's Hospital, Boston, MA, USA
[c] School of Engineering, Brown University, Providence, RI, USA
[d] Department of Emergency Medicine, Brown University/Rhode Island Hospital, Providence, RI, USA
[e] Department of Medical Biostatistics, University of Vermont, Burlington, VT, USA
[f] Bioengineering Laboratory, Department of Orthopaedics, Warren Alpert Medical School, Brown University/Rhode Island Hospital, Providence, RI, USA

ARTICLE INFO

*Article history:*
Accepted 22 July 2016

*Keywords:*
Arthroscopy
Lubricin
Osteoarthritis
Osteoarthrosis
Tribosupplementation

ABSTRACT

The purpose of this study was to determine the effects of arthroscopic irrigation on cartilage superficial zone lubricin and surface friction. Arthroscopic partial meniscectomy is one of the most commonly performed orthopedic surgeries in the United States, but rates of osteoarthritis progression following this procedure are high. The effect of arthroscopic irrigation on articular surface lubrication has not been previously considered as a contributing factor in outcomes after arthroscopy. Fourteen bovine stifle joints were randomized to receive arthroscopic irrigation ($n=7$) or no treatment ($n=7$). Full-thickness osteochondral explants from these joints underwent friction testing to measure static and dynamic coefficients of friction. Following mechanical testing, samples were fixed and stained for lubricin. Percent integrated density, a measure of the amount of lubricin in the superficial zone (0–100 μm depth), was determined. Static and dynamic coefficients of friction were found to be significantly greater in arthroscopy specimens compared to controls ($p=0.02$ and $p<0.001$, respectively). Percent integrated density of lubricin in the superficial zone was significantly lower in arthroscopy specimens compared to controls ($p<0.001$).



## 1. Introduction

Arthroscopic partial meniscectomy is one of the most common orthopaedic surgeries in the United States (Molina et al., 2015). This is concerning, as Englund and Lohmander have reported radiographic osteoarthritis in 48% of subjects 15 to 22 years after partial meniscectomy (Englund and Lohmander, 2004) Additionally, increased rates of cartilage loss have been detected after partial meniscectomy compared to healthy controls via magnetic resonance imaging (MRI) at a mean follow up of 28.6 months (Cicuttini et al., 2002), and a retrospective review of repeat knee MRIs during a seven year interval in patients with meniscal tears found significantly greater cartilage loss in patients post-meniscectomy versus those who did not undergo meniscectomy (Cohen et al., 2012). While selection bias toward surgical intervention might result in patients with more active, symptomatic disease electing to undergo arthroscopic surgery, other factors that might accelerate cartilage loss include iatrogenic injury due to removal of functional meniscal tissue or inadvertent direct cartilage injury. Another potential mechanism accelerating cartilage loss after knee arthroscopy is the effect of arthroscopic irrigation on cartilage health, via its impact on cartilage lubrication. During arthroscopy, pressurized fluid is used to distend and lavage the joint. Current post-arthroscopy protocols do not replenish synovial fluid lubricants post-procedure. Patients are generally allowed to bear weight on the knee immediately after arthroscopic partial meniscectomy. However, this may result in mechanical damage to the cartilage surface if lubrication is impaired.

The mucinous glycoprotein lubricin (PRG4) has been recognized as an essential boundary lubricant that protects articular cartilage from damage (Jay et al., 2012; Rhee et al., 2005; Waller et al., 2013). Lubricin is found on cartilage and meniscal surfaces (Musumeci et al., 2014) within the superficial zone (Elsaid et al., 2012), and within the synovial fluid (Jay, 1992; Schmidt et al., 2007). It is expressed embryologicaly shortly after diarthrodial

* Correspondence to: Department of Emergency Medicine, 1 Hoppin Street, Coro West, Suite 106, Providence, RI 02903. Fax: +1 401 444 5456.
*E-mail address:* gregory_jay_md@brown.edu (G.D. Jay).

http://dx.doi.org/10.1016/j.jbiomech.2016.07.024
0021-9290/

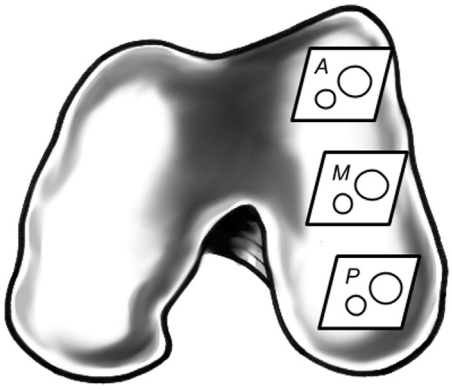


**Fig. 1.** Graphic showing anterior (A), middle (M), and posterior (P) cartilage harvest sites from the medial femoral condyle of a bovine stifle joint.

joint nucleation (Rhee et al., 2005), when joint surfaces become demarcated. Lubricin provides both anti-adhesive and lubricating activity (Chang et al., 2008) as an end-grafted brush molecular configuration (Zappone et al., 2007). Lack of lubricin gene function results in Camptodactyly Arthropathy Coxa Vara Pericarditis (CACP) Syndrome (Marcelino et al., 1999), where advanced multifocal joint degeneration develops by the second to third decades of life (Faivre et al., 2000). Lubricin null mice show an increased coefficient of friction (COF) ex vivo (Jay et al., 2007) and superficial and upper intermediate zone chondrocyte apoptosis (Waller et al., 2013) resulting in cellular loss (Karamchedu et al., 2016). Cartilage under pressure and shear stimulates lubricin expression *in vitro* (Grad et al., 2006; Nugent et al., 2006), which is impeded by additive IL-1α which also raised COF (Larson et al., 2016).

The present study was undertaken to determine the effects of arthroscopic irrigation on the frictional properties of the cartilage surface post-arthroscopic irrigation. Using live cartilage explants from fresh bovine stifle joints, our hypothesis was that arthroscopic irrigation of the cartilage surface would be associated with elevated static and dynamic COF and with decreased lubricin within the superficial cartilage zone compared to control samples lubricated with synovial fluid.

## 2. Materials and methods

Fourteen individual bovine stifle joints with intact capsules were obtained from a local abattoir. Samples for each specimen were obtained; experiments were completed, and fixed for histology within eight hours of euthanasia. For control specimens ($n=7$), a superior capsulotomy was performed and whole synovial fluid aspirated using a 10 ml syringe and 18-gauge needle. For arthroscopy specimens ($n=7$), a superior capsulotomy was performed and an arthroscopic cannula introduced into the joint. Arthroscopic irrigation was performed using 6 L of lactated ringers (LR) solution at 55 mmHg fluid pressure. During irrigation, joints were passively ranged through flexion and extension to distribute the fluid and simulate arthroscopy. Approximately 10 ml of fluid was collected from arthroscopy joints after 3 L of total irrigation and saved for use as a lubricant during mechanical testing.

Following synovial fluid collection (Controls) or irrigation (Arthroscopy), joint capsules were opened and full thickness large (12 mm) and small (6 mm) osteochondral explant pairs were harvested from three regions in the weight-bearing portion of the medial femoral condyle: anterior, middle, and posterior (Fig. 1). Explants were harvested using custom drill bits cooled with phosphate buffered saline (PBS). Following harvest, explants were rinsed in PBS to remove debris and then incubated in the test lubricant; either synovial fluid (Controls) or LR irrigant (Arthroscopy).

Static and dynamic COF were measured for explant pairs using a Bose Electroforce 3200 Series III Material Testing System (Bose, Framingham, MA). Each explant pair was mounted in a custom fixture with the cartilage surfaces apposed. Explant surfaces were kept moist with regular applications of test lubricant. Additional lubricant was applied to cartilage surfaces just before mechanical testing was initiated. The mechanical testing protocol was based upon that described by Waller et al. (2012). During mechanical testing, a 12 N compressive load was applied across the explant surfaces followed by an 8 min dwell to ensure stress relaxation and surface engagement. The large explant was then rotated relative to the small explant for 12 rotations of $\pm 720^{\circ}$ while axial torque ($\tau$) was recorded. COFs were calculated as $\mathrm{COF} = \tau/((2/3)^{*}(r)^{*}(\mathrm{load})$, where $r$ = measured radius of the small explant and load is the equilibrium force following the 8 min of cartilage decompression (Schmidt and Sah, 2007; Waller et al., 2013). Static COF was calculated from maximal torque measured during the first 20° of rotation, while dynamic COF was calculated using average torque measured during the last 720° rotation.

Immediately following testing, the 12 mm explants were immersed in formalin for a minimum of 72 h prior to decalcification and paraffin embedding for histology. Sections from the central contact area of each explant were stained for lubricin. Briefly, sections were heated to 60 °C for 30 min, deparaffinized and hydrated in three changes xylene and serial alcohol and antigen retrieval using pepsin solution (Thermo scientific). After two PBS washes, 9g3 monoclonal antibody at 1:200 dilution was added to the slides and incubated at 4 °C overnight. After washing with PBS three times, the sections were incubated with fluorescein goat anti-mouse igG (F2761, Thermo fisher scientific, USA) at 1:100 dilution for one hour at room temperature in darkness. The sections were washed five times using PBS and slipcovered with Vectashield mounting medium with 4′,6-diamidino-2-phenylindole (DAPI) (Vector Laboratories Inc, Burlingame, CA). Images acquired using a fluorescence microscope (Nikon, ECLIPSE90i) were imported to ImageJ (Jensen, 2013) and the mean intensity and % area occupied were calculated. Mean intensity values were corrected for background and normalized (Model and Burkhardt, 2001) using a 10% solution of standard fluorescein dye (Catalog#F1300, Thermo Fisher Scientific). Percent integrated density, defined as the corrected mean intensity times the % area occupied by lubricin in the superficial zone (0–100 μm), was reported.

Comparisons between treatments (arthroscopic versus control) and regions (anterior, middle, versus posterior) were performed using two-way analysis of variance. The model consisted of two-fixed factors, treatment (an across-subject

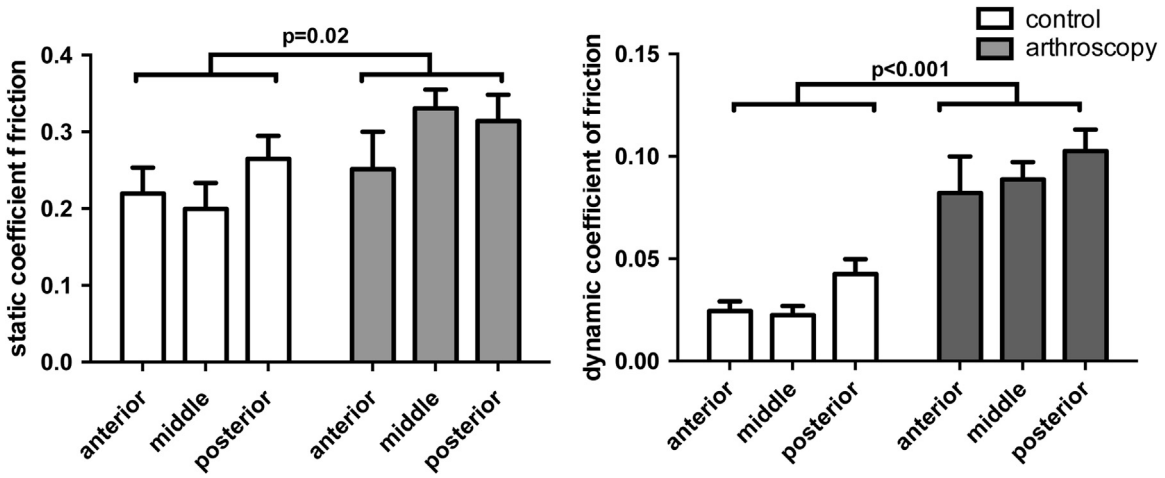


**Fig. 2.** Mean static and dynamic coefficients of friction for control and arthroscopically irrigated cartilage explants. Static and dynamic friction was significantly higher in the arthroscopy explants compared to controls.