Lubricin Distribution in the Menisci and Labra of Human Osteoarthritic Joints

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Abstract

Objective: Lubricin is the principal boundary lubricant on articular cartilage. We aimed to describe the distribution of lubricin in the other articulating structures in the human knee and hip—menisci and labra—and to relate this distribution to the degree of tissue degeneration. Methods: Eighteen menisci and 6 labra were obtained from patients with osteoarthritis undergoing total knee and total hip replacements, respectively. Macroscopically intact specimens were fixed in formalin and processed for H&E staining and immunohistochemical evaluation with an antilubricin monoclonal antibody. Results: Lubricin was found in all tissues as a discrete layer on the tissue surface, within the extracellular matrix, and intracellularly, indicating that it plays a role in the tribology of these tissues in human subjects, and can be synthesized by cells within the tissues. While none of the samples displayed macroscopic tears, approximately 40% of the surface of the menisci and 80% of the surface of the labra displayed microscopic fibrillations and slight fraying. There was no effect of the degenerative changes on the distribution of lubricin. Conclusions: Lubricin coats nearly the entirety of the surfaces of menisci and labra, including microfibrillations and tears, with possible implications towards the tribology of the tissues and healing of tissue damage.

Keywords

lubricin, fibrocartilage, meniscus, labrum

Introduction

The fibrocartilaginous meniscus of the knee joint and the acetabular labrum of the hip joint, henceforth referred to simply as the menisci and labra, play critical roles in the function of the respective joints. The meniscus assists load bearing by decreasing stress on the articular cartilage and subchondral bone and aids in stability,¹ and the labrum deepens the acetabulum and contributes to the stability of the joint.² Breakdown of the tissues by wear or rupture can jeopardize the health of the entire joint and thus focuses attention on the molecules responsible for their tribology (i.e., lubrication, friction, and wear). Prior studies, which have demonstrated the presence of lubricin, a principal lubricating mucinous glycoprotein, in bovine³ and canine⁴ menisci prompted the current study of the distribution of lubricin in the human meniscus and labrum.

Initially isolated from synovial fluid,⁵ lubricin was found to be produced by synovial cells.⁶ In normal and arthritic⁷ human articular cartilage and in normal bovine meniscus,³ lubricin was identified as a discrete layer on the surface of the tissue and in the extracellular matrix (ECM) and in cells in certain regions of the tissues. The presence of lubricin as a layer on the tissues reflected its role as a boundary lubricant for facilitating the articulating function of the tissues, and its distribution in the interior suggested that it may play a role in lubricating the movement among collagen bundles. The fact that lubricin could be found intracellularly demonstrated the chondrocytic and fibrochondrocytic expression of the protein.

The objective of the present study was to determine the distribution of lubricin in human menisci and labra using immunohistochemistry. The only source of samples available to us was joint replacement for osteoarthritis. While we only selected for study meniscus and labrum samples that

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were not torn and that did not display macroscopic signs of degeneration, we histologically documented the degenerative condition of the tissues for the ultimate correlation with the levels of lubricin recorded in the samples. The principal compelling reason for determining the distribution of lubricin in human menisci and labra was that the presence of lubricin on the surface and in the body of the tissues would indicate that this lubricating molecule may be playing a role in the tribology of these tissues in human subjects. The source of the samples provided the opportunity to begin to assess whether there may be sufficient lubricin in the osteoarthritic joint to obviate the need to consider injection of exogenous lubricin in the future as a therapeutic modality for osteoarthritis. Finally, the presence of the lubricating and antiadhesion lubricin on microfibrillations in the tissues may underlie an absence of an integrative reparative response to such damage, as has been shown in articular cartilage. Even though the samples were obtained from osteoarthritic joints, it was not the intent of this work, however, to attempt to determine the role of lubricin in the osteoarthritic process.

Methods

Eighteen menisci and 6 labra, which were intact (i.e., were not torn and did not display macroscopic signs of degeneration), were collected from 18 total knee arthroplasty (TKA) patients and 6 total hip arthroplasty (THA) patients, respectively, with Institutional Review Board approval. The clinical indication for total joint arthroplasty was osteoarthritis. Cases involving trauma or infection were excluded. Ages of TKA patients ranged from 50 to 81 years; ages of THA patients ranged from 51 to 66 years.

After the menisci and labra were excised, the tissues were fixed, processed, sectioned, and embedded using a previously described protocol. Tissues were stained with either a primary antilubricin monoclonal antibody (S6.79, provided by the Rush University Medical Center, Chicago, IL) or with a negative mouse immunoglobulin-2b (IgG2b) control.

Light microscopy was used to visualize stained samples. Digitized micrographs were acquired using a MicroFire camera (model S99809, Meyer Instruments, Houston, TX) mounted on an Olympus microscope (BX51, Olympus, Tokyo, Japan). Meniscal and labral surfaces were analyzed histologically for degenerative changes using criteria adapted from previously published work. Surfaces were assigned to 1 of 3 categories of fibrillation and tearing (0, +, and +++) based on the following microscopic criteria:

• 0: smooth surface free of acute-angled fraying
• +: presence of small, acute-angled fibrillations; tearing resulting in free tissue at one end and exposing an undercut surface underneath
• ++: extensive tearing with overt splitting of the extracellular matrix

Degeneration was assessed on both the femoral and tibial sides of menisci and on both the femoral and acetabular sides of labra. On each side of the meniscus and labrum, the analysis was divided along circumferential lines into the inner third, middle third, and outer third of the tissue. Within each third, the percentage of surface exemplifying each of the 3 categories of degeneration was reported.

Lubricin expression in menisci and labra was evaluated 1) as a discrete layer on the tissue surface, 2) within the ECM, and 3) intracellularly. Surface lubricin staining was assessed on both the femoral and tibial sides of menisci and both the femoral and acetabular sides of labra. Within each side, analysis was again divided among the inner third, middle third, and outer third of the tissue. Within each third, the percentage of the surface staining for lubricin was reported.

ECM staining was assessed within the inner third, middle third, and outer third of each sample. Matrix staining was graded on a scale from 0 to ++++ with the following cutoffs:

• 0: no ECM lubricin staining observed
• +: positive lubricin staining in 1% to 25% of ECM
• ++: positive lubricin staining in 26% to 50% of ECM
• +++: positive lubricin staining in 51% to 75% of ECM
• ++++: positive lubricin staining in 76% to 100% of ECM

Intracellular lubricin staining was defined as the presence of red detection chromogen at the border of the hematoxylin-stained cell nucleus or distributed within the cytoplasm. To evaluate the percentage of cells displaying intracellular staining for lubricin, 5 random fields of view (FOVs) were selected from one microscope slide from each tissue with a 20x objective lens magnification and a 10x magnification eyepiece, corresponding to 0.25 mm². The number of lubricin-positive cells and the total number of cells in each FOV were recorded. The total cell count per FOV ranged from 20 to 2,169, with a mean of 262.

All measurements were recorded by one evaluator (D.Z.), with selected concurrences by 2 subsequent observers (T.C., M.S.). Because no assessment of interobserver variability was made for any of the measurements, we did not base differences among groups on fine discriminations of the data. Three-factor analysis of variance was employed to determine the significance of effects of the tissue (meniscus v. labrum), the side (femoral v. tibial for the menisci, and femoral v. acetabular for the labra), and location (inner,
middle, and outer thirds) on the percentage of the surface displaying a grade of 0, +, and ++ for degeneration. Fisher’s exact test was performed for comparisons of degrees of degeneration between anatomic locations. The standard significance criterion of $\alpha = 0.05$ was employed for all statistical tests.

Results
The meniscal and labral samples exhibited varying cellularity, reflected in the variation in the distribution and number density of cells (Fig. 1). Some regions displayed a low cell number density (Fig. 1A), while others showed pockets of high cell density (Fig. 1B). While many cells were of fibroblast morphology, rounded cells in lacunae, characteristic of chondrocytes, were frequently observed (Fig. 1B and 1D), especially near the tissue surface. Vascularity was low in the inner circumferential portions of the tissues (white zones) but increased in outer circumferential areas (red zones).

Degenerative Condition of the Menisci and Labra

All 3 histological categories of degeneration (graded 0, +, and ++) were observed in the menisci (Figs. 1A-C and 2A) and labra (Figs. 1D-F and 2B). Whereas the majority of the surface of the menisci displayed no microscopic signs of degeneration (grade 0) (Fig. 2A), most of the surface of the labral samples demonstrated a grade of + for degeneration (Fig. 2B). Three-factor ANOVA revealed that there was a statistically significant effect of tissue type (i.e., meniscus v. labrum; $P < 0.0001$; power = 1) but no statistically significant effects of side (i.e., femoral or opposing side) or location (i.e., inner, middle, or outer third segment) on the percentage of the surface displaying a degenerative grade (+ or ++) or a more severe degenerative change (grade ++). After averaging the values for each surface of each segment for each sample, the range of the percentage of the total surface of the menisci that demonstrated some degree of fibrillation (+ or ++) was 8% to 92% and for the

Figure 1. Representative micrographs showing the degeneration of the menisci and labra graded 0, +, and ++. Degenerative changes were analyzed by light microscopy using sections stained with H&E. (A) Menisci with intact surfaces free of fibrillations were observed. (B) Degenerative surfaces with sharp fibrillations, continuous on one side and torn on the other side, exposing an undercut surface beneath the tear were also seen. (C) The most dramatic manifestation of degeneration was frank splitting of the matrix. Note the extensive calcification seen on this micrograph. (D) Labra with intact surfaces free of fibrillations were observed. (E) Degenerative labra exhibited acute, jagged surfaces. Note the chondrocytic proliferation and hyalinization seen on this micrograph. (F) Most dramatically, degenerative labra demonstrated frank tearing.
labra was 53% to 100%. The percentage of the surface displaying the most severe degeneration (i.e., grade ++) ranged from 0% to 52% for the menisci and 0% to 30% for the labra.

By averaging the above grades across the surfaces of all samples, it was found that for this population of menisci, about 60% of the meniscal surface was smooth (grade 0), approximately 30% was microfibrillated (grade +), and 10% showed frank tearing (grade ++). However, of note was that the coefficient of variation was high. In contrast, degenerative labra predominantly showed small fibrillations (grade +) along the surface of both the femoral and acetabular sides, although all 3 categories were observed. Three of the 6 labrum samples displayed a grade of ++++, and 2 had grade ++ in at least one of the zones.

Calcifications were found in both degenerative menisci and labra (Fig. 1C). Extensive chondrocyte proliferation and hyalinization were found in labra (Fig. 1E). There was no significant difference in the degenerative pattern among the 3 circumferential zones. Neither meniscal nor labral degeneration correlated with age of the patient.

**Distribution of Lubricin**

Lubricin was consistently seen as a discrete layer on articular cartilage positive control samples in accordance with a previous study; none of the immunohistochemical negative control sections showed the red chromogen (data not shown). Lubricin-positive immunohistochemical staining was observed in menisci and labra in the following locations (Fig. 3):

1. as a discrete layer on the surface of the tissue, on both the femoral and tibial sides of menisci and both the femoral and acetabular sides of labra;
2. within the ECM; and
3. intracellularly.

Similar distributions of lubricin have been reported in a number of previous studies of other tissues. The discrete surface lubricin-positive layer, approximately 5 μm thick, was observed overwhelmingly on all surface segments of the menisci and labra (Fig. 3 and Table 1). Lubricin was seen on almost 100% of the surface (Table 1) as a discrete layer coating the fibrillations and tears. Only 1 of the 18 menisci (#14) and 2 of the 6 labra (#1 and #5) displayed 3 of the 6 segments, with 20% or less of the surface displaying a lubricin layer. Lack of lubricin staining on fresh-cut surfaces of the tissues, produced during trimming of the samples in preparation for paraffin embedment, suggests that the presence of lubricin on tissue borders was not the result of edge-artifact staining. Surface lubricin staining was observed in areas of menisci and labra showing hypercellularity (Fig. 3F and 3J) as well as areas showing hypocellularity (Fig. 3C, 3D, 3G, and 3K).

Diffuse lubricin staining of the ECM was observed in inner, middle, and outer circumferential portions of all meniscal and labral samples; however, the degree of ECM staining varied widely (Table 1 and Fig. 3A, 3B, 3E, 3F, 3G, 3L). The chromogen staining the matrix was categorically less intense than the chromogen staining the surface layer. While ECM lubricin staining was sometimes seen to permeate the tissue, matrix staining was often most intense immediately beneath the surface layer, decreasing in intensity in deeper tissue (Fig. 3B and 3F). Matrix staining was seen to follow the crimp pattern of collagen fibrils (Fig. 3B) as well as the trabecular pattern of collagen fibers (Fig. 3K and 3L, black arrows). Discrete granular depositions of lubricin within the ECM were also observed (Fig. 3G-I).

Intracellular lubricin staining was found in both meniscus and labra (Table 1) both within fibroblast-like and chondrocyte-like cells (Fig. 3). The mean values of the percentage of lubricin-positive cells, determined from the analysis.

![Figure 2](image-url). Graphs showing the percentage of the surface of the (A) menisci (n = 18) and (B) labra (n = 6) displaying degeneration graded 0, + (1), and ++ (2) in the inner, middle, and outer segments on the sides of the samples facing the femur and tibia or acetabulum. Mean ± standard error of the mean.

**Figure 2.** Graphs showing the percentage of the surface of the (A) menisci (n = 18) and (B) labra (n = 6) displaying degeneration graded 0, + (1), and ++ (2) in the inner, middle, and outer segments on the sides of the samples facing the femur and tibia or acetabulum. Mean ± standard error of the mean.
Figure 3. Micrographs showing representative positive immunohistochemical staining for lubricin (red chromogen) at the surface of the tissues, in the extracellular matrix, and intracellularly in the meniscus and labra. Micrographs are taken from the inner, middle, and outer thirds (segments) of the femoral and tibial sides of menisci and of the femoral and acetabular sides of labra. All micrographs are shown on the same scale. (A) Sample shows extensive matrix and intracellular staining for lubricin beneath a more pronounced surface-staining layer. (B) Matrix staining often follows the crimp pattern of collagen fibrils and fades with increasing depth into the tissue. (C) Lubricin staining is observed intracellularly in some cells (black arrows) but not in others (white arrows). (D) Lubricin staining is seen mainly as a surface coating in certain samples. (E) In some samples, lubricin in the matrix envelops the lacunae of chondrocyte-like cells, while the cell itself lacks intracellular staining (black arrows). (F) Lubricin is drastically seen here in a hypercellular region, fading out in deeper tissue. (G) Deposition of lubricin is seen in discrete granules within the extracellular matrix. (H) Lubricin is observed in granules in the matrix and intracellularly. (I) Granular deposition of lubricin in the matrix is most pronounced near the labral surface. (J) Intracellular lubricin is also more often observed near the labral surface. (K) Lubricin appears to diffuse into deeper tissue along the trabecular network of collagen fibers (black arrows). (L) Lubricin recapitulates the trabecular network of collagen fibers (black arrow).
Table 1. Evaluation of the Distribution of Lubricin in the Menisci and Labra

<table>
<thead>
<tr>
<th>No.</th>
<th>Femoral surface</th>
<th>Tibial/acetabular surface</th>
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lubricin-containing cells in the samples with the highly
staining ECM was 3-fold higher than the mean value for the
samples with the lower levels of ECM staining (24% ± 13% compared to 9% ± 7%), but the difference was not statistically
significant (P = 0.076, 1-tailed Student t test) owing to the
low sample size and variance.

There was no association of the lubricin expression with
the degree of degeneration of the menisci. For example, the
menisci were divided into groups with relatively high and
low degrees of degeneration based on whether the surface
developed more or less than 50% of the surface with a grade
of + or ++ fibrillation. There was no statistically significant
difference in the percentage of lubricin-containing cells in
the high and low degeneration groups (10% ± 7% v. 11% ± 8%, respectively). In addition, there was no meaningful cor-
relation between the percentage of the surface of the menisci
with a degenerative change (i.e., grade + or ++) versus the
percentage of cells containing lubricin by linear regression
analysis. Moreover, only 5 of the 18 menisci that displayed
relatively high degrees of degeneration also displayed rela-
tively high ECM staining for lubricin, and only 2 of the 18
menisci with high degrees of degeneration also demonstrated
high levels of matrix lubricin staining.

Discussion

This is the first demonstration of lubricin in the human
acetabular labrum and meniscus; it has been previously
shown in canine and bovine menisci. A notable finding of
the study was the presence of a discrete lubricin layer,
approximately 5 μm thick, on most of the surface of the
human meniscus and labrum. That the lubricin layer was
found equally on the meniscal surfaces articulating with the
femoral condyles and tibia and on the labral surfaces articu-
lating with the femoral head and acetabulum reflects the
relative mobility of these structures, respectively, in their
joints. While the lubricin layer adherent to the surface of
the tissues was likely derived from the joint fluid, the lubri-
cin that was found intracellularly within the meniscus and
labrum provides evidence of endogenous production of the
protein within the tissues.

The presence of lubricin in the ECM was correlated
with the percentage of cells expressing the protein, support-
ing the supposition that the lubricin distributed through the
tissues was due in part to meniscus and labrum cell synthe-
sis. While intracellular and ECM staining was found in all
locations, the cells and matrix immediately below the tis-
sue surface stained most intensely, and the intensity of
staining progressively decreased deeper into the tissue.
This distribution pattern of lubricin staining, with a
greater percentage of cells near the articulating surface
expressing lubricin, is consistent with articular cartilage and
the finding that surface motion stimulates the chondrocytic
production of lubricin. The majority of the surface of the menisci in our study
developed no fraying or fibrillation, and the majority of the
surface of the labrum demonstrated a grade of +. The degen-
erative changes seen histologically in our population of
menisci were consistent with those previously reported for
menisci obtained from a random series of 94 autopsies of
patients (aged 21–94 years) whose knee joints were never
operated on and whose joints did not display signs of inflam-
atory disease. Thus, while our menisci were obtained
from osteoarthritic joints, they generally displayed no more
degeneration than the menisci from joints without osteoa-
thritis. These findings raise questions about the causal rela-
tionships between degenerative changes within select joint
tissues such as menisci and labra and osteoarthritis, which
were outside the scope of the present work. There was no
association of the percentage of cells expressing lubricin or
of the lubricin contained within the ECM with the histologi-
cal degree of degeneration of the tissue.

In the majority of cases, the degeneration of the menis-
cus and labrum was limited to microscopic surface fibrilla-
tions, and the degree of degeneration was similar in the
inner, middle, and outer thirds of the tissues, suggesting that
vascularity has little effect on degenerative change. Moreover,
the degree of degeneration in both tissues was not found to
be correlated with patient age. At the molecular level,
meniscal degeneration has been linked with an upregula-
tion of the p38–NF-κB axis elements and COX-2, both of which
are well known to play regulatory roles in inflammatory
diseases. Future work remains to be done to elucidate the
mechanisms of labral degeneration.

The limitations of this study include, first, a limited sam-
ple size, precluding further stratification of the data accord-
ing to other factors such as age and gender. Second, there
was an absence of a “normal,” contemporaneous control
population of menisci and labra from nonosteoarthritic
joints. The fact, however, that changes in the menisci includ-
ing fibrillation, fraying, and splitting are common features in
the nonosteoarthritic population underscores the challenge
in identifying “normal” meniscus controls. Third, no pheno-
typic assessment of cells, besides their morphology, was
made, and no characterization of ECM molecules was made.
Fourth, a single monoclonal antibody to lubricin was
employed, and future work would benefit from the use of
other lubricin antibodies for comparison. And last, in regards
to our supposition that lubricin impairs integrative repair, it
may be the case that lubricin plays roles yet unknown that
may in fact promote the healing process.

Discrete layers of lubricin were found on the surfaces of
menisci and labra that displayed degenerative changes,
suggesting that the degenerative process was not due to its
absence. It is possible, however, as previously proposed for
articular cartilage, that in the initial stages of degeneration of
the meniscus and labrum, there is a temporary enzymatic
breakdown of the protective lubricin layer due to an
inflammatory process, permitting wear and fibrillation. Subsequent compensatory processes, perhaps including TGF-β1–induced upregulation of lubricin expression,17-19 may result in re-establishment of lubricin expression and its reappearance at the meniscus and labrum surface, a process of loss and reacquisition.

The fact that lubricin is present on the surfaces of menisci and labra in the osteoarthritic joint questions the necessity and long-term benefit of intra-articular injections of exogenous lubricin. Given the propensity of lubricin to impair integrative healing,8 our data are consistent with the hypothesis that a surface lubricin coating impairs normal healing of the microfibrillations induced by the inflammatory consequences of osteoarthritis. Over time, microfibrillations collect on the tissue surface, resulting in a degenerative meniscus and predisposing to frank tearing. If this hypothesis proves correct, lubricin would be best described as having conflicting roles in osteoarthritis: While lubricin is necessary for normal healing,8 our data are consistent with the hypothesis that a surface lubricin coating impairs normal healing of the microfibrillations induced by the inflammatory consequences of osteoarthritis. Over time, microfibrillations collect on the tissue surface, resulting in a degenerative meniscus and predisposing to frank tearing. If this hypothesis proves correct, lubricin would be best described as having conflicting roles in osteoarthritis: While lubricin is protective against osteoarthritis by serving as a boundary lubricant and reducing frictional wear-induced damage,20 it is also deleterious by inhibiting proper healing of surface microfibrillations (from mechanical stress, shearing, inflammation, etc.). In developing targeted long-term treatment for osteoarthritis, a multipronged approach taking into account the degenerative changes of menisci and labra should be considered. Future work should focus on the capacity for lubricin to inhibit microfibrillation repair in vitro and in vivo.

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